School of Medicine
Marmara Med J

MARMARA
MEDICAL JOURNAL

VOLUME 35 • ISSUE 3 • OCTOBER • 2022

Correspondence and Communications
Seza Arbay
Marmara Üniversitesi Tıp Fakültesi Dekanlığı,
Temel Tıp Bilimleri Binası, 3. Kat, Başıbüyük Mahallesi,
Başıbüyük, Maltepe, İstanbul, Turkey
Tel: +90 216 4144734, Faks: +90 216 4144731
E-mail: mmj@marmara.edu.tr

Publisher
Marmara University Press
Göztepe Kampüsü, Kadıköy 34722 İstanbul, Turkey
Tel. +90 216 777 1400, Faks +90 216 777 1401
E-mail: yayinevi@marmara.edu.tr
Typesetting: Elif TUFAN KIRKIL, Burcu DİKER

VOLUME 35 • ISSUE 3 • OCTOBER • 20222

Instructions to Authors

About Journal

The Marmara Medical Journal, Marmara Med J, is a multidisciplinary, academic publication of Marmara University, School of Medicine. It is an open access, double blind peer-reviewed journal. It publishes manuscripts that focus on clinical and laboratory medicine, health care policy and medical education, ethics, and related topics. It includes original research papers, case reports, reviews, articles about clinical and practical applications and editorials, short reports, letters to the editor and occcasionally a photo-quiz.

The Marmara Medical Journal is continuously published since 1988 and its archive with full-text manuscripts can be reached under www.dergipark.org.tr/marumj/archive.

Frequency: Three times a year (January, May, October)

Year of first print issue: 1988

Year of first online issue: 2004 (Between 2004 and 2011 the Journal was published solely in an electronic format.)

Language: English
Print ISSN: 1019-1941 **eISSN:** 1309-9469

The manuscripts published in the Marmara Medical Journal are indexed and abstracted in: Thomson Reuters/ Emerging Sources Citation Index (ESCI), EBSCO, SCOPUS, EMBASE/Excerpta Medica, DOAJ (Directory of Open Access Journals), CrossRef, ULRICH'S Database, Google Scholar, The British Library, Turkish Academic Network and Information Center (ULAKBİM)-Turkish Medical Database, TURK MEDLINE–Türk Sağlık Bilimleri (Index of Turkish Health Sciences), Türkiye Makaleler Bibliyografyası (Bibliography of Articles in Turkish Periodicals), Türkiye Klinikleri Tip Dizini (Turkish Citation Index).

Aims and Scope

The Marmara Medical Journal, Marmara Med J, is a peer-reviewed, multidisciplinary academic publication of Marmara University, School of Medicine, which is authored by physicians both nationally and internationally.

The journal aims to publish papers of general interest relating to advances in medical practice and novel treatments that will be of interest to general practitioners, medical students, and senior practitioners and specialists. Marmara Medical Journal also aims to publish all types of research conducted by medical students.

The Marmara Medical Journal is among the most widely read and cited scientific publications for physicians among journals of its kind nationally and increasingly gaining new readers and authors internationally with its English only format since 2016.

The journal consists of manuscripts on recent developments in general and internal medicine and new methods of treatment based on original research. We greatly welcome research papers, case reports, reviews and occasionally a photo-quiz of an interesting medical encounter in English, only.

Each manuscript is strictly assessed by a select Editorial Board. and refereed critically by two or more reviewers, at least one from another institution. The editor reserves the right to reject or to return the manuscript to the author(s) for additional changes.

Special review issues with invited editors are published since 2015 to focus on specific areas of medicine to bring recent data into attention covering multiple aspects of the chosen topic. Marmara Medical Journal welcomes and encourages physicians from all over the world to publish a special review issue on the topic of their preference as an "Invited editor" to collaborate with authors on the same focus area with the aim of increasing scientific collaboration via publishing.

The Marmara Medical Journal has an open access policy. All articles in the journal are permanently available online for all to read.

Author Guidelines

The Marmara Medical Journal publishes original scientific research papers, case reports, manuscripts about clinical and practical applications and editorials, short reports, letters and occasionally a photo-quiz.

Manuscripts submitted under multiple authorship are reviewed on the assumption that all listed authors concur with the submission and that a copy of the final manuscript has been approved by all authors and tacitly or explicitly by the responsible authorities in the laboratories where the work was carried out.

Manuscripts are accepted for review with the understanding that no substantial portion of the study has been published or is under consideration for publication elsewhere.

VOLUME 35 • ISSUE 3 • OCTOBER • 2022

The Marmara Medical Journal is in compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals created by International Committee for Medical Editors (ICMEJ link), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE) and the European Association of Science Editors (EASE).

Preparation of the Manuscript

1. Manuscript files must be prepared in Word, WordPerfect, EPS, LaTeX, text, Postscript, or RTF format. Figures/Images should be embedded in the manuscript file or sent as external files in TIFF, GIF, JPG, BMP, Postscript, or EPS format.

2. Manuscripts should be approximately 20-25 pages double-spaced, including references, with margins of 2.5 cm.

Pages should be numbered consecutively and organized as follows:

1. Title Page
2. Abstract
3. Keywords
4. Introduction
5. Materials and Methods
6. Results
7. Conclusion
8. References

1. Title Page

The title page should contain the article title, authors' names and academic or professional affiliations, and the address for manuscript correspondence (including e-mail address, Open Researcher and Contributer ID (ORCID) identifier, telephone and fax numbers).

2. Abstract

Abstract of not more than 200 words must be included. The abstract should be divided into the following sections: Objective, Materials and Methods, Results and Conclusion,

3. Keywords

Three to six keywords should be supplied below the Abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH).

http://www.nlm.nih.gov/mesh/meshhome.html

4. Introduction

State why the investigation was carried out, note any relevant published work, and delineate the objective of the investigation.

5. Materials and Methods

New methods or significant improvements of methods or changes in old methods must be described. Methods for which an adequate reference can be cited are not to be described, except for providing information about the aims of the method. Details regarding animal housing conditions should be given. All clinical studies must contain :

1. A statement that all experimental protocols have been approved by the Ethical Committee of the Institution prior to the commencement of the studies,

2. A statement that all participants gave informed consent.

6. Results

Duplication between the text of this section and material presented in tables and figures should be avoided. Tabular presentation of masses of negative data must be avoided and replaced with a statement in the text whenever possible. The results must be presented clearly, concisely and without comment.

7. Discussion

The discussion should begin with a brief summary of the findings, followed by the following: how this study is similar or different from prior studies with regards to methods and results and limitations of this study. This section must also relate the significance of the work to existing knowledge in the field and indicate the importance of the contribution of this study.

8. References

The style of references is that of the Index Medicus. List all authors when there are six or fewer, when there are seven or more list the first three, then add "et al.". Unpublished results or personal communications should be cited as such in the text. Where a doi number is available it must be included at the end of the citation. Please note the following examples:

i. Yazici D, Taş S, Emir H, Sunar H. Comparison of premeal mixed insulin three times daily and basal – bolus insulin therapy started post-operatively on patients having coronary artery bypass graft surgery. Marmara Med J 2011; 25:16-9.doi: 10.5472/

ii. Walker M, Hull A. Preterm labor and birth. In: Taeusch HW, Ballard RA, eds. Avery's Diseases of the Newborn. Philadelphia: WB Saunders, 1998: 144,153.

iii. Hagström H, Nasr P, Ekstedt M, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol 2017; 67: 1265-73. doi: 10.1016/j.jhep.2017.07.027.

iv. WONCA Ad Hoc Task Force on Tobacco Cessation.

http://globalfamilydoctor.com/publications/new/november/09.htm
(Accessed on …….)

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1-3] or [1,3]. References must be numbered consecutively in the order they are first mentioned.

Figures, Tables, Units

Diagrams and illustrations should be given Arabic numerals. All figure legends should be grouped and written on a separate page. Each Figure should be in one of the following preferred formats: Tiff, JPEG, PDF, and EPS.Tables should be numbered consecutively with Roman numerals in order of appearance in the text. Type each table double-spaced on a separate page with a short descriptive title directly above and with essential footnotes below.

Units will be in general accordance with the International System (SI) as adopted by the 11th General Conference on Weights and Measures.

Ethical Issues

Compliance with the principles of the last version of the Declaration of Helsinki for humans and the European Community guidelines for the use of animals in experiments is accepted as a policy by the Marmara Medical Journal. Studies involving human or animal subjects should conform to national, local and institutional laws and requirements. Manuscripts which do not properly consider ethical issues for humans or animals will not be accepted for publication.

http://www.wma.net/e/policy/b3.htm

Double-blind Review

This journal uses double-blind review, which means that both the reviewer and author identities are concealed from the reviewers, and vice versa, throughout the review process. To facilitate this, authors need to ensure that their manuscripts are prepared in a way that does not give away their identity.

Plagiarism

Manuscripts are investigated for possible plagiarism once they are accepted for possible publication. If an author receives a plagiarism notice regarding his/her manuscript, the corrections should be made within one month. If the Editorial Board detects any plagiarism on the second check after correction of the manuscript by the authors, the chief editor can reject the manuscript. your article will be checked by the plagiarism detection software iThenticate.

Funding Source

All sources of funding should be declared as an acknowledgment at the end of the text.

Following Documents are Required Prior Publication

Approval of the Institutional Ethics Committee

a) Marmara Medical Journal requires that investigations performed on human subjects have the prior approval of the Institutional Ethics Committee on Human Experimentation. Authors are required to submit a signed statement as to the date and details of the appropriate review. The authors must state that the investigation conforms with the principles of Declaration of Helsinki.

b) When studies involve the use of experimental animals, manuscripts should briefly describe the procedures employed for animal care and handling. Where drugs are used at particular concentrations in intact animal systems, the author should indicate some rationale for selection of the particular concentration.

Copyright Release Form

Copyright Release Form must be read and signed by all authors.

Copyright Release Form pdf

Authorship

It is the responsibility of every researcher listed as an author of a manuscript in Marmara Medical Journal to have contributed in a meaningful and identifiable way to the design, performance, analysis, and reporting of the work and to agree to be accountable for all aspects of the work.

Before publication, each author must sign a statement attesting that he or she fulfills the authorship criteria of the

ICMJE Recommendations.

http://www.icmje.org/recommendations/

Financial Associations/Conflicts of Interest

All participants – not only the corresponding author – must consider their conflicts of interest when fulfilling their roles in the process of article preparation and must disclose all relationships that could be viewed as potential conflicts of interest according to the Committee on Publication Ethics (COPE) Guidelines and/ or Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (ICMJE) Recommendations. Disclosure forms filed by all authors alongside the full text of each article is mandatory.

https://publicationethics.org/guidance/Guidelines

http://www.icmje.org/recommendations/

We encourage the authors on using the ICMJE Form for Disclosure of Conflicts of Interest to standardize authors' disclosures.

Conflict of Interest Form.pdf

Statement of Human Rights and Statement ofAnimal Rights

Statement of human rights and statement of animal rights, when necessary, must be signed by all authors prior publication.

Statement of human and animal rights form.pdf

Patient Consent for Publication

Patients have a right to privacy. Identifying information, including patients' names, initials, or hospital numbers, should not be published in written descriptions, photographs or in any kind of patient-related materials. In circumstances where this information is essential for scientific purposes, authors should obtain the patient's (or the legal guardian's) written informed consent prior to the publication.

Patient Consent for Publication pdf

VOLUME 35 • ISSUE 3 • OCTOBER • 20222

Statement of Human Rights

Title:

This is to certify that the procedures and the experiments followed for the manuscript were in accordance with the ethical standards of the …… Ethics Committee on human experimentation and with the ethical standards in the Declaration of Helsinki 2013, as well as the national law.

Author's Name	Signature	Date
…….	……...	…….
…….	……...	……...
……...	…….	…….

Statement of Animal Rights

Title:

This is to certify that the procedures and the experiments were conducted in accord with the highest scientific, humane and ethical principles of the Institutional and National Guide for the Care and Use of Laboratory Animals.

Author's Name	Signature	Date
…….	……...	…….
…….	……...	……...
……...	…….	…….

School of Medicine
Marmara Med J

MARMARA
MEDICAL JOURNAL

VOLUME 35 • ISSUE 3 • OCTOBER • 2022

Contents

Original Articles

Case Reports

VOLUME 35 • ISSUE 3 • OCTOBER • 20222

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Thyroid diseases in patients with active endogenous Cushing's syndrome

Tayfur TOPTAS[1], Kubra Bercem KAHRAMAN[2], Zilan TOPCU[2], Hayri BOSTAN[3], Pinar KADIOGLU[4]

[1] Divison of Hematology, Department of Internal Medicine, School of Medicine, Marmara University Pendik Training and Research Hospital, Istanbul, Turkey.
[2] Department of Internal Medicine, School of Medicine, Marmara University Pendik Training and Research Hospital, Istanbul, Turkey.
[3] Endocrinology Clinic, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey.
[4] Department of Internal Medicine, Istanbul University Cerrahpasa Research and Training Hospital, Istanbul, Turkey.

Corresponding Author: Tayfur TOPTAS
E-mail: toptast@gmail.com

Submitted: 30.03.2022 **Accepted:** 12.07.2022

ABSTRACT

Objective: Data about the impact of Cushing's syndrome (CS) on thyroid is scarce. We aimed to identify the prevalence of thyroid diseases in patients with CS.

Patients and Methods: Nineteen patients with CS and 40 healthy participants were included in the study. All patients were tested for free tri-iodothyronine (fT3), free thyroxine (fT4), thyroid-stimulating hormone (TSH), anti-thyroglobulin (anti-Tg), and anti-thyroid peroxidase (anti-TPO) levels, and thyroid ultrasonography (US).

Results: Overt hypothyroidism, subclinical hypothyroidism, and subclinical hyperthyroidism was evident in 5.3%, 5.3%, and 21.1% of patients with CS; and 2.5%, 7.5%, and 15% of healthy subjects, respectively. fT3 and fT4 levels were lower in patients with CS. None of the patients with CS and 27.5% of the control group had autoimmune thyroid disease (AITD), which was demonstrated by both US findings and anti-TPO positivity (P=0.01). Frequency of thyroid nodule was 52.6% and 52.5% in patients with CS and controls, respectively (P=0.99).

Conclusion: The prevalence of thyroid dysfunction, nodular thyroid disease, and goiter is comparable to healthy population. However, AITD is less prevalent among patients with CS. Although, hypercortisolism has an impact on hypothalamic-hypophyseal-thyroid axis, its clinical implication does not seem to be significant.

Keywords: Cushing's syndrome, Thyroid diseases, Thyroid nodule, Hypercortisolism, Autoimmune thyroid disease

1. INTRODUCTION

Adrenal glucocorticoids can influence hypothalamic-hypophyseal-thyroid axis at several steps including prothyrotropin-releasing hormone (pro-TRH) mRNA, thyrotropin-releasing hormone, thyroid-stimulating hormone (TSH) and TSH-beta mRNA, 5'-monodeiodination of T3 to T4, and T4 to reverse-T3 conversion [1-5]. When compared to healthy volunteers, low levels of TSH in hypercortisolism is accompanied by lower circulating levels of triiodothyronine (T3), thyroxine (T4) and free T3 in these individuals. By contrast, free T4 (FT4) has generally been described as normal. [6].

Hypercortisolism induces a state of immunosuppression by diminishing proinflammatory cytokines, interacting with other transcription factors related with T-cell survival. Autoimmune diseases frequently improve during active Cushing's syndrome (CS). There are several case series reporting overt immune dysfunction and exacerbation of autoimmune thyroid diseases (AITD) upon remission [7-10]. Lower frequency of AITD is expected in patients with CS. However, Onal et al., reported that there was not a difference in the frequency of anti-thyroid autoantibody positivity between the patients with endogenous hypercortisolism and the healthy individuals in contrast to previous studies [11].

Previous studies investigating primary thyroid diseases in patients with CS reported that nodular and diffuse goiter is more prevalent in patients with CS [11-14]. However, these studies have certain limitations. Data are scarce in order to draw a definite conclusion regarding to goiter prevalence within this population.

We aimed to investigate the frequency of thyroid dysfunction, AITD, nodular, and diffuse goiter in patients with CS.

How to cite this article: Toptas T, Bercem Kahraman K, Topcu Z, Bostan H, Kadioglu P. Thyroid diseases in patients with active endogenous Cushing's syndrome. Marmara Med J 2022 ;35 (3):257-262. doi: 10.5472/marumj.1186788

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1186788
Marmara Med J 2022;35(3): 257-262

2. PATIENTS and METHODS

Study Design and the Patients

This study was both retrospective and observational case-control study (Figure 1). All subjects were aged ≥18 years in this study. In retrospective part of the study, data about a total of 74 patients with persistent/recurrent or de novo CS were screened from the patients' medical charts and hospital's medical network. Since we planned to perform this study in patients with active CS, prerequisite for the study inclusion was determined to have thyroid tests performed after the diagnosis and before the treatment of CS, including at least free tri-iodothyronine (fT3), free thyroxine (fT4), TSH, anti-thyroglobulin (anti-Tg), and anti-thyroid peroxidase (anti-TPO) levels, and thyroid ultrasonography (US). Only eleven out of 74 patients were eligible for the study inclusion.

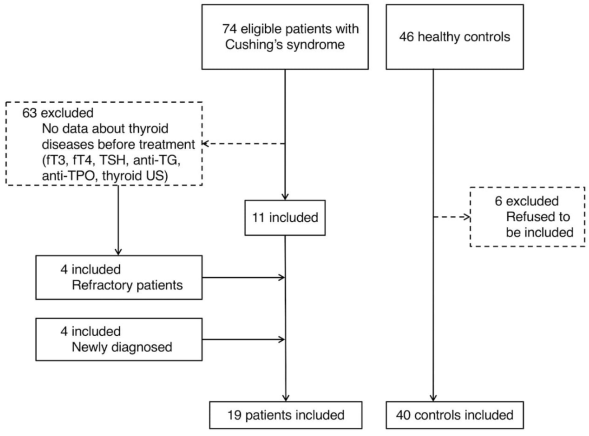

Figure 1. *Study design*

Eight patients, four with newly diagnosed and four with refractory active CS, were recruited for the required study tests. Finally, a total of 19 (17 women and 2 men; median age, 43 [interquartile range: 28 to 54]) patients with CS were included in the study. All patients presented clinical features of CS. Fourteen out of 19 patients had Cushing's disease. Five patients were diagnosed with adrenal adenoma. All patients with refractory disease had Cushing's disease. Median time-to-study testing after the diagnosis was one month (Table 1).

Table I. *Characteristics of the patients with Cushing's syndrome*

Variables	Cushing's syndrome (n=19)	Control (n=40)
Female gender, n (%)	17 (89.5)†	34 (85.0)†
Age, years, median (IQR)	43 (28-54)‡	48 (37-56)‡
Cause of Cushing's syndrome		N/A
Cushing's disease	14 (73.7)	
Microadenoma	12 (85.7)	
Macroadenoma	2 (14.3)	
Adrenal adenoma	5 (26.3)	
Time-to-study testing, months, median (95% CI)	1 (0.78-24.15)	N/A

**NA denotes not applicable; IQR, interquartile range; 95% CI, 95% confidence interval.*

All subjects were informed about the study and a written and signed consent was obtained from each participant. The study was conducted in accordance with the Good Clinical Practice Guidelines [15] according to the principles of declaration of Helsinki and Istanbul University, Cerrahpasa Medical, Surgical and Pharmaceutical Research Ethics Committee approved the study protocol (approval number 2226, date 20.09.2005) [15].

Power and Sample Size Analysis

Power and sample size estimation revealed that at least 40 subjects in control were needed in a 1:2 study design to show a 40% difference with the assumptions that overall type I error of 5%, type II error of 20 percent, number of patients with CS of 20, and frequency of thyroid nodules in CS and healthy people of 60% and 20%, respectively in chi-square test [12]. According to this estimation, at least 17 and 34 patients should be included in the experimental and control arms, respectively.

Controls

Pseudo-Cushing states, including alcoholism, anxiety, depression, poorly controlled diabetes, and morbid obesity; hirsutism, unusual features for the age (e.g., non – traumatic fracture, hypertension, or cutaneous atrophy in young individuals), as well as Cushingoid features and past history of CS were defined as the exclusion criteria for the healthy controls. Pregnant or puerperal women, people who take steroids, antiepileptic drugs, amiodarone, or iodine containing drugs, past history of any severe systemic disease including chronic renal failure were excluded as well.

A total of 46 subjects, who were family members of the patients in Endocrinology clinic, were recruited for the study inclusion. However, later, six withdrew their consents due to lack of their time for the study tests. Finally, 40 (34 women and 6 men; median age, 48 [interquartile range: 37 to 56]) participants were included in the study (Figure 1, Table I).

Definitions

The diagnosis of CS was made by means of several standardized biochemical tests and imaging techniques depending on the availability. A low-dose dexamethasone suppression test, 24-h urinary free cortisol, midnight plasma cortisol, and plasma cortisol circadian rhythm were used to confirm hypercortisolism. The cause of CS was established by additional tests including CRH stimulation, pituitary imaging by magnetic resonance imaging (MRI) with contrast, and bilateral inferior petrosal sinus sampling [16].

The diagnosis of thyroid diseases was made based on commonly accepted clinical and laboratory criteria, including thyroid US, serum TSH and free thyroid hormone levels, anti-TPO antibodies, and anti-Tg receptor antibodies. Overt hyperthyroidism was defined as a TSH< 0.4 mU/L and an FT4 >1.9 pmol/L, and/or a TSH <0.4 mU/L and an fT3 > 4.2 pmol/L; subclinical hyperthyroidism, a normal FT4 and FT3, and a TSH <0.4 mU/L; overt hypothyroidism, a TSH >4.0 mU/L and FT4 <0.8 pmol/L; subclinical hypothyroidism, a normal FT4 and TSH >4.0 mU/L.

Goiter was defined as a thyroid volume exceeding 30.2 mL for males and 20 mL for females. The cut-off levels were extrapolated from the mean (+2 SD) thyroid volume in 251 healthy Turkish subjects (105 males and 146 females) without thyroid dysfunction and/or a previous thyroid disease on ultrasonography [17].

We defined autoimmune thyroid disease (AITD) at two levels: *i.* anti-TPO antibody positivity (anti-TPO$^+$) *ii.* anti-TPO antibody and thyroid US positivity (anti-TPO$^+$/US$^+$) (16). A nonhomogeneous and diffuse hypoechogenic pattern has been accepted as the determinant of AITD on thyroid US [18].

Thyroid nodule was defined as discrete lesions within the thyroid gland which were discriminated from hypoechoic areas by darker appearance in comparison to the surrounding thyroid tissue [19]. Presence of at least one nodule sizing at least two to three mm were defined as nodular thyroid disease.

Measurements

Serum fT3, fT4, and TSH measurements were performed by competitive analog-based immunoassay; solid phase chemiluminescence competitive analog-based immunoassay; and solid phase chemiluminescence immunometric assay, respectively (DPC kits, Los Angeles, USA). Anti-Tg, and anti-TPO was measured by solid phase enzyme-labeled chemiluminescence immunometric assay (DPC kits, Los Angeles, USA). Intra – and inter-assay variation coefficients were less than 10% in all analyses. Reference ranges were 1.8-4.2 pg/L for fT3; 0.8-1.9 pg/L for fT4; 0.4-4.0 mU/L for TSH; <20 U/mL for anti-Tg; and <10 U/mL for anti-TPO.

All those tests were performed by the same kits in all patients irrespective to date of diagnosis. Because, the patients included into the study had test results those were performed within the previous five years of the study year. Our institution used the same kits during this period and we provided the same kits for the analyses of newly included patients.

Siemens Sonoline Sienna (Siemens, Germany) US device and 40 mm, 7.5 MHz linear probe (7.5L40, Q2000, Siemens, Germany) was used for ultrasound imaging.

Two-dimensional ultrasound estimation of thyroid volume was calculated by the ellipsoid volume formula. Width (w), length (l), and depth (d) of each thyroid lobe measured by longitudinal and transverse scans. The volume of the lobe was calculated by the formula: V (ml) = 0.479 x d x w x l (cm), as recommended by the World Health Organization (WHO) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD). Thyroid volumes were added in order to get total thyroid volume. Isthmus volume was not included into the calculation [20].

Statistical Analysis and Study Endpoints

Primary endpoint of this study was the frequency of nodular thyroid disease. Secondary endpoints were frequency of AITD, goiter, and thyroid dysfunction; and levels of fT3, fT4, TSH, and thyroid volumes.

Descriptive data were expressed as median and interquartile range (IQR), if skewed, or mean ± standard deviation (SD), if normally distributed. Log transformed levels of fT3, fT4, TSH, and thyroid volume were used to test differences between groups of patients and in healthy donors. Continuous variables were compared by Student's t-test if normally distributed. Comparison of skewed data was made by means of Wilcoxon rank-sum test. Statistical analysis of categorical variables was performed by chi-square or Fisher's exact test, when appropriate. Two-tailed p-value of less than 0.05 was defined as statistically significance level.

3. RESULTS

Thyroid Functions

Patients with CS had similar rate of thyroid dysfunctions in comparison to healthy subjects (Figure 2). Overt hypothyroidism, subclinical hypothyroidism, and subclinical hyperthyroidism was evident in 5.3% (n=1), 5.3% (n=1), and 21.1% (n=4) of patients with CS; and 2.5% (n=1), 7.5% (n=3), and 15% (n=6) of healthy subjects, respectively. 68.3% (n=13) of CS group and 75% (n=30) of control group was euthyroid. Overt hyperthyroidism was not detected in any participant.

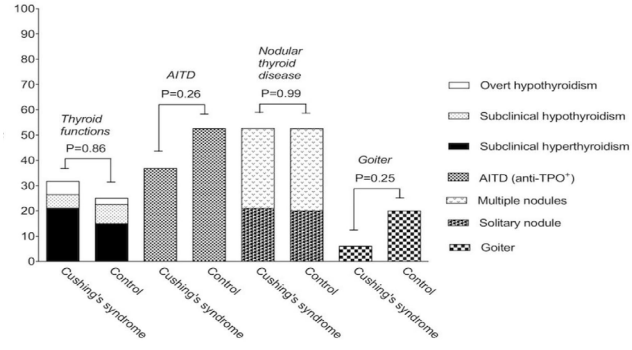

Figure 2. *Comparison of frequencies of thyroid dysfunction, autoimmune thyroid disease, nodular thy-roid disease, and diffuse goiter in patients with Cushing's syndrome and healthy subjects*

fT3 and fT4 levels were lower in patients with CS (2.41±0.79 vs 2.97±0.67 pg/L for fT3; and 1.18±0.29 vs 1.48±0.79 pg/L for fT4). There was no difference in terms of TSH between the groups (Figure 3). When only euthyroid subjects were included into the TSH comparison, groups remained comparable (P=0.63).

ained comparable (P=0.63).

http://doi.org/10.5472/marumj.1186788
Marmara Med J 2022;35(3): 257-262

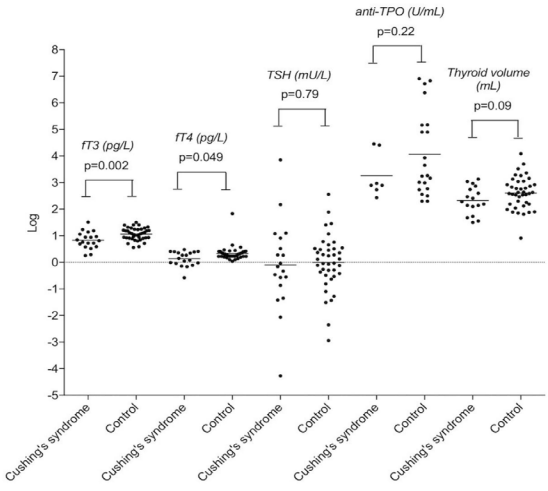

Figure 3. *Free T3 (fT3), free T4 (fT4), thyroid-stimulating hormone (TSH), anti-thyroid peroxidase (an-ti-TPO) levels and thyroid volumes in patients with Cushing's syndrome and healthy subjects have been shown. All values were log transformed*

Autoimmune Thyroid Disease

Prevalence of AITD, defined as anti-TPO positivity alone, tended to be lower (36.8% (n=7) vs 52.5% (n=21) in patients with CS (Figure 2). However, when the definition of anti-TPO$^+$/US$^+$ was used to denote AITD, none of the patients with CS had AITD. On the other hand, AITD was demonstrated in 27.5% (n=11) of the control group (P=0.01).

Among subjects with AITD (anti-TPO$^+$), having CS had no impact on serum anti-TPO titers (Figure 3). Even though we did not incorporate into the AITD definition, anti-Tg, as an autoimmune antibody, was observed 15.8% (n=3) of CS patients and 32.5% (n=13) of healthy subjects (P=0.22).

Nodular Thyroid Disease

A frequency of thyroid nodule was 52.6% (n=10) and 52.5% (n=21) in patients with CS and controls, respectively. More than half of the subjects with a thyroid nodule in each group had multiple nodules [60% (n=6) vs 61.9% (n=13), CS vs control, respectively] (Figure 2).

Goiter

Although not significant, goiter seemed to be less prevalent among patients with CS [5.9% (n=1) vs 20% (n=8)] (Figure 2). Concordantly, thyroid volumes had a tendency to be smaller in CS (11.58±5.83 vs 16.26±10.75) (Figure 3).

4. DISCUSSION

We demonstrated that the thyroid diseases including thyroid dysfunctions, AITD, nodular thyroid disease, and goiter could be detected in similar frequencies in patients with CS and healthy population. CS is associated with lower fT3 and fT4 levels, less ultrasonographic manifestations of AITD, and a tendency to have smaller thyroid sizes.

Exogenous glucocorticoids can inhibit pro-TRH mRNA in a cell-specific manner in rats. Hypothetically, these can lead to reduction in the biosynthesis and release of TRH in hypophysiotropic neurons of the paraventricular nucleus [1]. According to Tasker et al., glucocorticoids activate membrane glucocorticoid receptors in order to stimulate endocannabinoid synthesis in the hypothalamic paraventricular nucleus (PVN). Subsequently it give rise to retrograde cannabinoid type I receptor-mediated suppression of the excitatory synaptic drive to PVN neuroendocrine cells. Rapid corticosteroid actions in the hippocampus, amygdala, and pituitary are mediated by various cellular mechanisms and may also contribute to the rapid negative feedback regulation of the HPA neuroendocrine axis as well as to the stress regulation of emotional and spatial memory formation [21].

In patients with CS, pulsatile nocturnal TSH secretion is decreased due to blunting of TSH response to TRH which leads to decreased levels of thyroid hormone levels.

In CS, plasma TSH or T4 levels may be affected regardless of etiology. However, it is found that no correlation was found between the baseline cortisol and fT4 levels [1,6]. We failed to show lower levels of TSH in either whole CS cohort or in euthyroid patients with CS. Interestingly in a previous study, Onal el all., who performed a study exploring primary thyroid diseases in 38 patients with CS, also reported no difference in terms of TSH in euthyroid CS patients before and after the treatment [11].

Actually in healthy people, mean nocturnal TSH levels are 51% higher than afternoon values One of the reasons of discrepancy between the results of various studies might be affected by blood withdrawal time. Sample size differences across the studies may also give rise different results [6]. It is reported that, in CS patients, the levels of T4, T3, and FT3 are reduced and reverse T3 levels are increased. The decreased ratio of T3:T4 can be attributed to glucocorticoid-related inhibition of peripheral deiodination [6,22,23]. The nocturnal serum thyrotropin surge is abolished in patients with adrenocorticotropin (ACTH)-dependent or ACTH-independent Cushing's syndrome [18]. Our findings are consistent within this context.

We found that AITD, defined as anti-TPO$^+$, seems to be more prevalent in control group but this association is not significant. Accordingly, anti-TPO titers had a trend to be lower in patients with CS. In previous studies, AITD, defined as the autoantibody positivity, was reported in patients with CS and controls with no significant association [12,22,24,25].

Typical appearance in US imaging is also mainly due to lymphocytic infiltration and increased blood flow [26]. Hypercortisolism reduces T-cell mediated cellular immunity within the thyroid gland. However, B-cell mediated immunity is less affected and autoantibody secretion will be maintained even if the secreted thyroid antigen levels decrease. Accordingly, we detected less AITD, defined as anti-TPO$^+$/US$^+$, in CS group.

But anti-TPO positivity and titers were similar. Nevertheless, it should be considered that our study was not powered to detect AITD. The induction of autoimmunity is associated with the normalization of CS. It appears to be linked to an improvement in immunological activity, which is inhibited throughout the active phase of the disease by endogenous hypercortisolism. According to some studies, high TSH with/without low FT4 has been linked to an exacerbation of underlying autoimmune disease, as well as a decline in serum cortisol levels, which is occasionally followed by an increase in antithyroid antibody titer [14,24,27].

We found that prevalence of nodular thyroid disease is comparable to that in healthy population. In previous studies, thyroid nodules detected by US were reported to be more prevalent in patients with CS than that in healthy population [12,14]. However, Niepomniszcze *et al.* screened thyroid nodules by palpation and reported that nodule prevalence is comparable between CS and control groups (8.4% vs 2.5%, respectively, p=0.20) [13]. It is well known that nodule prevalence increases with age [28].

Another important limitation for all studies including the current study is lack of information about the iodine status of study participants. Even small differences in iodine intake within population may lead to significant differences in thyroid volume, frequency of diffuse or nodular goiter, and nodule sizes [18]. It is emphasized that thyroid size in the community may not return to normal for months or years after correction of iodine deficiency [29]. So, it is probable that the conclusions about the nodular thyroid disease in CS are biased and incidental. Even it is assumed that the prevalence of nodular thyroid disease is higher in patients with CS, perhaps patients with CS have now less time, which may not be enough for developing thyroid nodules, under hypercortisolism due to increased awareness about CS than before. Onal et al. demonstrated that the frequency of nodular goiter is 42.5% in CS patients, whereas 30% in control group. This data signifies that there is no remarkable difference between the CS patients and control subjects with respect to the frequency of nodular goiter, which is not in agreement with earlier findings [11]. These findings support our results.

Niepomniszcze *et al.* reported that 22.4% of patients with CS had diffuse goiter by palpation. In the control group, only 6.0% had goiter (P=0.008) [13]. However, our findings indicate quite the opposite (5.9% in CS vs 20% in controls, P=0.25). Two other studies performed in patients with CS do not give details about the frequency of diffuse goiter [12,14]. The traditional method for determining thyroid size is inspection and palpation. Ultrasonography provides a more precise and objective measurement of thyroid volume compared with palpation. This becomes especially significant when the prevalence of visible goiters is small [19]. Hypothetically, decreases in basal secretion, pulsatile secretion, mean pulse mass and total secretion of TSH in 24-hour measurements are supposed to result with smaller thyroid volumes and less goiter frequency [3].

The most important limitation of the current study was the limited sample size which was calculated considering the primary end-point of the study as the presence of nodular thyroid disease. That is why it should be interpreted cautiously that all other conclusions except nodular thyroid disease may be underpowered.

In conclusion, the prevalence of thyroid dysfunction, nodular thyroid disease, and goiter is comparable to healthy population. However, AITD was less prevalent in patients with CS. Although hypercortisolism has an impact on hypothalamic-hypophyseal-thyroid axis, its clinical implication does not seem to be significant.

Acknowledgment

We thank Nurse Kaniye Karababa for their precious help in screening the patients' medical charts and hospital's medical network.

Compliance with Ethical Standards

Ethical Approval: Ethical approval for this study was obtained from Istanbul University, Cerrahpasa Medical, Surgical and Pharmaceutical Research Ethics Committee approved the study protocol (approval number 2226, date 20.09.2005)

Financial Support: The authors received no financial support for the research and/or authorship of this article.

Conflict of Interest: The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

Author Contribution: TT and PK: designed the study, TT, KK, and ZT: performed the literature search and data gathering; TT, KK, ZT, HB, SA, and PK: performed the quality assessment, TT, KK, and ZT: performed the statistical analysis, and TT, KK, ZT, HB, SA, and PK: wrote the manuscript. All authors approved the final manuscript.

REFERENCES

[1] Dogansen SC, Yalin GY, Canbaz B, Tanrikulu S, Yarman S. Dynamic changes of central thyroid functions in the management of Cushing's syndrome. Arch Endocrinol Metab 2018 ;62:164-71. doi: 10.20945/2359.399.7000000019. PMID: 29641732.

[2] Nicoloff JT, Fisher DA, Appleman MD Jr. The role of glucocorticoids in the regulation of thyroid function in man. J Clin Invest 1970;49:1922-9. doi: 10.1172/JCI106411.

[3] Roelfsema F, Pereira AM, Biermasz NR, et al. Diminished and irregular TSH secretion with delayed acrophase in patients with Cushing's syndrome. Eur J Endocrinol 2009 ;161:695-703. doi: 10.1530/EJE-09-0580.

[4] Shimokaze T, Toyoshima K, Shibasaki J, et al. TSH suppression after intravenous glucocorticosteroid administration in preterm infants. J Pediatr Endocrinol Metab 2012;25:853-7. doi: 10.1515/jpem-2012-0075.

[5] Bános C, Takó J, Salamon F, Györgyi S, Czikkely R. Effect of ACTH-stimulated glucocorticoid hypersecretion on the serum concentrations of thyroxine-binding globulin, thyroxine, triiodothyronine, reverse triiodothyronine and on

the TSH-response to TRH. Acta Med Acad Sci Hung 1979; 36:381-94.

[6] Shekhar S, McGlotten R, Auh S, Rother KI, Nieman LK. The hypothalamic-pituitary-thyroid axis in Cushing syndrome before and after curative surgery. J Clin Endocrinol Metab 2021; 106:e1316-e1331. doi: 10.1210/clinem/dgaa858.

[7] da Mota F, Murray C, Ezzat S. Overt immune dysfunction after Cushing's syndrome remission: a consecutive case series and review of the literature. J Clin Endocrinol Metab; 96(10):E1670-4 2011. doi: 10.1210/jc.2011-1317.

[8] Russo L, Vitti P, Pinchera A, Marinò M. Exacerbation of autoimmune thyroiditis following bilateral adrenalectomy for Cushing's syndrome. Thyroid 2010; 20:669-70. doi: 10.1089/thy.2010.0040.

[9] Arikan E, Guldiken S, Altun BU, Kara M, Tugrul A. Exacerbations of Graves' disease after unilateral adrenalectomy for Cushing's syndrome. J Endocrinol Invest 2004;27:574-6. doi: 10.1007/BF03347482.

[10] Takasu N, Komiya I, Nagasawa Y, Asawa T, Yamada T. Exacerbation of autoimmune thyroid dysfunction after unilateral adrenalectomy in patients with Cushing's syndrome due to an adrenocortical adenoma. N Engl J Med 1990; 322:1708-12. doi: 10.1056/NEJM199.006.143222404.

[11] Onal ED, Sacikara M, Saglam F, Ersoy R, Cakir B. Primary thyroid disorders in patients with endogenous hypercortisolism: an observational study. Int J Endocrinol 2014:732736 . doi: 10.1155/2014/732736.

[12] Invitti C, Manfrini R, Romanini BM, Cavagnini F. High prevalence of nodular thyroid disease in patients with Cushing's disease. Clin Endocrinol (Oxf) 1995; 43(3):359-63. doi: 10.1111/j.1365-2265.1995.tb02044.x.

[13] Niepomniszcze H, Pitoia F, Katz SB, Chervin R, Bruno OD. Primary thyroid disorders in endogenous Cushing's syndrome. Eur J Endocrinol 2002; 147:305-11, 2002. doi: 10.1530/eje.0.1470305.

[14] Colao A, Pivonello R, Faggiano A, et al. Increased prevalence of thyroid autoimmunity in patients successfully treated for Cushing's disease. Clin Endocrinol (Oxf) 2000;53:13-9. doi: 10.1046/j.1365-2265.2000.01018.x.

[15] ICH Topic E 6 (R1). Guideline for Good Clinical Practice. ICH Harmonised Tripartite Guideline. Version of July 1996 including post step errata of July 2002 [Internet]. London:Emea;2002. Available from: http://www.emea.europa.eu/pdfs/human/ich/013595en.pdf. Accessed on 07.05.2019

[16] Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. Lancet 2006;367:1605-17. doi: 10.1016/S0140-6736(06)68699-6.

[17] Seker S, Tas I. Determination of thyroid volume and its relation with isthmus thickness. Eur J Gen Med 2010;7:125-9. doi.org/10.29333/ejgm/82838.

[18] Schott M, Scherbaum WA. Autoimmune Schilddrüsenerkrankungen. Dtsch Arztebl 2006;103:A3023-A3032.

[19] American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper, DS, Doherty, GM, Haugen, BR et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid : Official Journal of the American Thyroid Association; 2009;19:1167-214. doi: 10.1089/thy.2009.0110.

[20] World Health Organization. Indicators for assessing iodine deficiency disorders and their control through salt iodization. Geneva, Switzerland: World Health Organization,1994 [Document No. WHO/NUT94.6]

[21] Tasker JG, Herman JP. Mechanisms of rapid glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis. Stress 2011;14:398-406. doi: 10.3109/10253.890.2011.586446.

[22] Paragliola RM, Corsello A, Papi G, Pontecorvi A, Corsello SM. Cushing's syndrome effects on the thyroid. Int J Mol Sci 2021;22:3131. doi: 10.3390/ijms22063131.

[23] Bartalena L, Martino E, Petrini L, et al. The nocturnal serum thyrotropin surge is abolished in patients with adrenocorticotropin (ACTH)-dependent or ACTH-independent Cushing's syndrome. J Clin Endocrinol Metab 1991;72:1195-9. doi: 10.1210/jcem-72-6-1195.

[24] Xiang B, Tao R, Liu X, et al. A study of thyroid functions in patients with Cushing's syndrome: a single-center experience. Endocr Connect 2019; 8:1176-85. doi: 10.1530/EC-19-0309.

[25] Laurberg P, Pedersen KM, Hreidarsson A, Sigfusson N, Iversen E, Knudsen PR. Iodine intake and the pattern of thyroid disorders: a comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. J Clin Endocrinol Metab 1998;83:765-9. doi: 10.1210/jcem.83.3.4624.

[26] Nishiyama S, Takada K, Tada H, Takara T, Amino N. Effect of interleukin-6 on cell proliferation of FRTL-5 cells. Biochem Biophys Res Commun 1993;92: 319-23. doi: 10.1006/bbrc.1993.1416.

[27] Jones TH. Interleukin 6 and endocrine cytokine. Clin Endocrinol (Oxf) 1994;40: 703-13, . doi: 10.1111/j.1365-2265.1994.tb02502.x.

[28] Hegedüs L. Clinical practice. The thyroid nodule. N Engl J Med 2004;351:1764-71. doi: 0.1056/NEJMcp031436.

[29] Knudsen N, Bulow I, Jorgensen T, Laurberg P, Ovesen L, Perrild H. Goitre prevalence and thyroid abnormalities at ultrasonography: a comparative epidemiological study in two regions with slightly different iodine status. Clin Endocrinol (Oxf) 2000;53: 479-85. doi: 10.1046/j.1365-2265.2000.01121.x.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

The effects of alpha-lipoic acid (ALA) on the urinary bladder injury in rats exposed to chronic stress: A histochemical study

Necati YURDAKUL[1,2], Esra CIKLER[1,3], Hale Zerrin TOKLU[4,5], Serap SIRVANCI[1]

[1] Department of Histology and Embryology, School of Medicine, Marmara University, Istanbul, Turkey.
[2] Current Address: Department of Immunology, School of Medicine, Marmara University, Istanbul, Turkey.
[3] Current Address: Department of Histology and Embryology, School of Medicine, Health Sciences University, Istanbul, Turkey.
[4] Department of Pharmacology, School of Pharmacy, Marmara University, Istanbul, Turkey.
[5] Current Address: Department of Clinical Sciences, College of Medicine, University of Central Florida, Orlando, Florida, United States of America.

Corresponding Author: Serap SIRVANCI
E-mail: ssirvanci@marmara.edu.tr

Submitted: 23.02.2022 **Accepted:** 01.04.2022

ABSTRACT

Objective: In the present study, we aimed to investigate the morphological and biochemical effects of alpha-lipoic acid (ALA) on bladder injury caused by water avoidance stress (WAS) and to show its effect on the number of degranulated mast cells, which increase after stress.

Materials and Methods: Wistar albino rats were subjected to WAS and the animals in the treatment group were injected ALA. After the urinary bladder tissues were subjected to routine tissue processing, hematoxylin-eosin staining and periodic acid-Schiff reaction were applied to observe general morphology and acidic toluidine blue method to investigate mast cells. Biochemical assessments of malondialdehyde (MDA) and glutathione (GSH) were also obtained. Transmission electron microscope was used for the ultrastructural, and scanning electron microscope for the topographical analyses.

Results: The experiments showed that chronic stress caused injury in the bladder, increased degranulated and total number of mast cells and decreased GSH and increased MDA levels. ALA treatment after WAS ameliorated bladder injury in most areas, decreased degranulated and total mast cell number and increased GSH and decreased MDA levels.

Conclusion: It was concluded that ALA can be a useful agent in the treatment of interstitial cystitis.

Keywords: Alpha-lipoic acid (ALA), Interstitial cystitis, Mast cell, Stress, Urinary bladder

1. INTRODUCTION

Interstitial cystitis (IC) is a symptom syndrome complex characterized by persistent pain and urinary frequency and/or urgency [1, 2]. Bacterial and viral infections, hormonal and neuropsychologic disorders, allergy and immune disorders related to collagen diseases may cause IC, however, exact reasons are not well understood [3, 4].

Physical and psychological stress affect negatively urinary bladder epithelium [5]. One of the mechanisms related to stress is mast cell activation, which is a characteristic of IC [6]. Studies have reported that mast cells were significantly increased and associated with bladder pain and inflammation in patients with IC and in animal models [7]. Stress causes release of corticotropin-releasing hormone, which is a potent activator of mast cell degranulation. The inflammation effect of stress shows itself in the urinary bladder as damage. Being exposed to various stress conditions causes urothelial damage and increase in the number of mast cells [8, 9]. Mast cells in the urinary bladder and intestines are localized very close to the neurons including neuropeptides and neurotransmitters. In the presence of urothelial damage and factors causing bladder inflammation, nerves release various neuropeptides when stimulated. These neuropeptides cause inflammation. In the pathophysiology of IC, mechanisms such as release of neuropeptides from mast cells play a role [10, 11].

Mast cells are located in the connective tissue in humans and rats [12]. The etiology of IC is not well understood; however, mast cells were reported to play a role in inflammation and pain. Mast cells act as modulators of nociceptive neurons via release of histamine, serotonin, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6 [13].

Electron microscopic studies showed that mast cell activation was present and they were localized around neuronal

How to cite this article: Yurdakul N, Cikler E, Toklu HZ, Sirvanci S. The effects of alpha-lipoic acid (ALA) on the urinary bladder injury in rats exposed to chronic stress: A histochemical study. Marmara Med J 2022: 35(3):263-269. doi: 10.5472/marumj.1191211

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1191211
Marmara Med J 2022;35(3): 263-269

projections in IC [11]. The characteristic of mast cells is that they contain dense granules in their cytoplasm. Granules show metachromasia when stained with toluidine blue. Mast cells are primarily localized around the blood vessels, nerves, gland ducts, and epithelial serous and synovial membranes surrounded by loose connective tissue. Mucosal mast cells are found in the urinary bladder and gastrointestinal system, and connective tissue mast cells in the lung and skin [14]. Being exposed to various stress conditions causes urothelial damage, leading to increase in the number of mast cells. Increased mast cell activation causes release of inflammatory mediators such as histamine, proteases, prostaglandins, leukotrienes, and cytokines. Mast cells play a role in inflammatory diseases and allergic reactions [15].

Alpha-lipoic acid (ALA) or thioctic acid is a natural organic sulphur compound and a ketoacid dehydrogenase mitochondrial complex that can be produced by plants, animals and humans [16]. ALA and its metabolites function as antioxidants [10, 17]. It was reported that ALA was used as a therapeutic agent in diseases such as diabetic neuropathy and stroke [18]. It was also reported that it played a neuroprotective role in Parkinson's disease [19]. ALA and dihydrolipoic acid (DHLA) was shown to prevent oxidative stress by repressing reactive oxygen species [20]. ALA and DHLA play a role in regeneration of endogen and exogen antioxidants such as vitamin C and E, and glutathione (GSH), chelation of metal ions, and in repair of oxidized proteins [21, 22].

In the present study, we aimed to investigate the effects of ALA on bladder damage caused by chronic water avoidance stress (WAS), morphologically and biochemically, and to show the effect of WAS on mast cell number and morphology.

2. MATERIALS and METHODS

Female Wistar albino rats (250-300 g) were used in the present study. Animals were obtained from Marmara University, The Experimental Animal Implementation and Research Center. All experiments were approved by the Marmara University Local Ethical Committee for Experimental Animals (approval number 03.2009.mar). The animals were housed with free access to water and food in a 12-h light/dark cycle and humidity controlled room (21±2°C and 65-70% humidity). For WAS, a platform having a size of 8x8 cm was placed in a water container with a diameter of 90 cm and a depth of 50 cm. The platform was placed 1 cm above the water and the animals were placed on that platform.

Experimental groups were as follows:

1. Control group (n=10): Tissues were obtained from this group of animals without any procedure.

ALA group (n=10): This group of animals were given 25 mg/kg ALA (i.p.) for 5 days, once a day, on the same hours with the other groups.

2. WAS group (n=10): This group of animals were exposed to WAS for 5 days on the same hours with the other groups for the same duration (2 h).

3. WAS+ALA group (n=10): This group of animals were exposed to WAS and immediately, 25 mg/kg ALA (i.p.) was injected once a day.

4. Animals were decapitated after 5 days and bladder tissues were obtained and fixed in 10% formaldehyde for 24 h for light microscopic observation. For malondialdehyde (MDA) and GSH measurements, tissues were kept at – 20°C until biochemical evaluations were performed.

Light Microscopy

After fixation, tissues were dehydrated in increasing series of ethanol and cleared in toluene. The tissues were incubated in 60°C incubator in liquid paraffin and then embedded. Five-micron-thick sections were obtained on a rotary microtome. Sections were stained with hematoxylin and eosin for demonstrating bladder morphology and with periodic acid Schiff (PAS) reaction for observing glycosaminoglycan (GAG) layer.

Serial sections were obtained and stained with toluidine blue to examine mast cell morphology and number. Granulated and degranulated mast cells were counted separately in randomly selected 10 areas under x40 objective of an Olympus BX51 photomicroscope (Olympus, Tokyo, Japan) with the aid of a metric ocular.

Transmission Electron Microscopy

Bladdder tissues were fixed in 2.5% glutaraldehyde and then postfixed in 1% osmium tetroxide for 1 h. After dehydrating in increasing series of ethanol, tissues were cleared in propylene oxide and incubated in propylene oxide/epon (1:1) mixture overnight. On the next day, tissues were incubated in pure epon, embedded in epon, and then polymerized in 60°C incubator overnight. Semi-thin sections were obtained by an ultramicrotome (Leica Ultracut R) and stained with toluidine blue for proper orientation. Thin sections were obtained on grids coated with Coat-Quick 'G' pen. Sections were contrasted with uranyl acetate and lead citrate, and examined and photographed under an SIS Morada CCD camera (Olympus, Tokyo, Japan) attached JEOL-JEM-1200EX II transmission electron microscope (JEOL, Tokyo, Japan).

Scanning Electron Microscopy

Bladdder tissues were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide for 1 h, dehydrated in increasing series of ethanol, incubated in amyl acetate, and then dried in critical point dryer (BIO-RAD E3000). Tissues were coated with gold in a gold coating instrument (BIO-RAD-SC502) and examined and photographed under a JEOL JSM-5200 scanning electron microscope (JEOL, Tokyo, Japan).

http://doi.org/10.5472/marumj.1191211
Marmara Med J 2022;35(3): 263-269

Biochemical Evaluations

Malondialdehyde (MDA) Measurement

Amount of MDA, an indicator of lipid peroxidation, was determined by Beuge method [23]. After decapitation, bladder tissues were washed in saline, dried and weighed. 10% bladder homogenate was prepared on ice in Ika Werk homogenizator with 150 mM KCl solution. Thiobarbituric acid (1 ml, 0.375%) was added onto the homogenate, left in boiled water bath, and the tubes were cooled at room temperature. After centrifugating (Hettich Universal) at 3000 cycles/min, top phase were taken and absorbance of the resulting color was determined in a spectrophotometer at 532 nm.

Glutathione (GSH) Measurement

Glutathione measurement was done according to Ellman method [24]. 10% homogenates, which were prepared for MDA measurement, were used in GSH measurement. 0.4 ml 10% homogenate was mixed with 0.2 ml 20% thiobarbituric acid homogenate, and centrifuged (Hettich Universal) at 3000 cycles/min. GSH was examined at top phase, and the precipitate was thrown away. 1 ml 0.3 M Na_2HPO_4+0.05 ml Ellman solution was added and mixed, and incubated for 5 min. Absorbance of the resulting color was determined in a spectrophotometer at 412 nm.

Statistical Analysis

Data were interpreted as mean±S.E.M. and evaluated with One-Way ANOVA and Tukey-Kramer multiple comparison tests. Significance level was determined as $p<0.05$.

3. RESULTS

General Morphology

Mucosa was in normal appearance in control (Figure 1a) and ALA (Figure 1b) groups, and epithelium was intact. There was severe epithelial damage and it was observed that epithelial cells shed into the lumen in WAS group (Figure 1c). Although epithelial damage was evident in some areas in WAS+ALA group, the epithelium was generally intact (Figure 1d).

GAG layer was intact in control (Figure 1e) and ALA (Figure 1f) groups. Disorganized GAG layer and epithelial damage was observed in WAS group and leukocytes which migrated to this region were seen (Figure 1g). Although there was disorganization in GAG layer in some regions, this layer was generally intact in WAS+ALA group (Figure 1h).

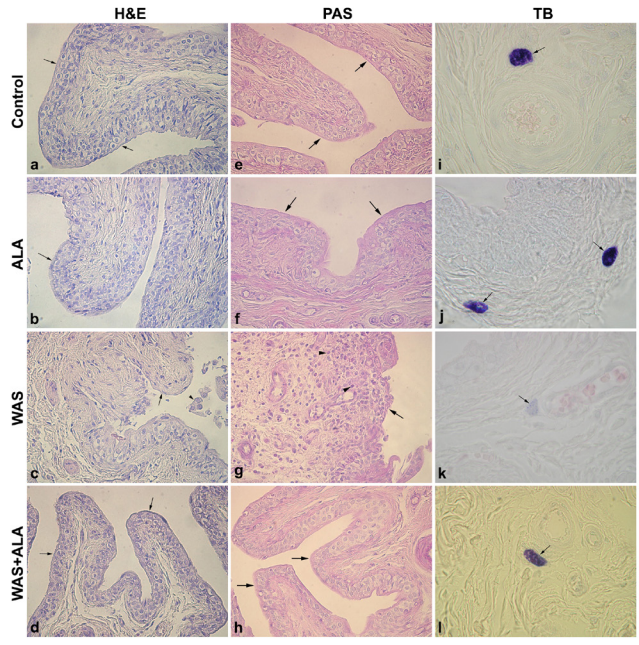

Figure 1. *Normal appearance of epithelium (arrows) in control (a) and ALA (b) groups. (c) Shed epithelium (arrow) and residual cells (arrowhead) in the lumen in WAS group. (d) Normal appearance of epithelium in WAS+ALA group (arrows). Normal appearing GAG layer (arrows) in control (e) and ALA (f) groups. (g) Shed epithelium in the lumen and damaged GAG layer (arrow), and inflammatory cell infiltration (arrowhead) in WAS group. (h) Normal appearance of GAG layer (arrows) in WAS+ALA group. (i) Granulated mast cell (arrow) around a blood vessel in control group. (j) Granulated mast cells (arrows) in the mucosal layer in ALA group. (k) Regranulated mast cell (arrow) around a blood vessel in the mucosal layer in WAS group. (l) Granulated mast cell (arrow) around a blood vessel in WAS+ALA group. (a-d) Hematoxylin and eosin (H&E) staining. (e-h) Periodic acid-Schiff (PAS) reaction. (i-l) Toluidine blue staining. Original magnification: x400.*

Mast cell Morphology

Although, some granulated mast cells were observed in mucosal regions of bladder sections in control and ALA groups, mast cells were generally granulated in these groups (Figure 1i, 1j). Besides granulated and degranulated mast cells in mucosa and near the smooth muscle in WAS group, regranulated mast cells were also observed (Figure 1k). Granulated mast cells and a few degranulated mast cells were seen in WAS+ALA group (Figure 1l).

Mast cells were generally granulated in semi-thin sections in mucosal regions of control (Figure 2a) and ALA (Figure 2b) groups. Granulated and degranulated mast cells were seen both in mucosa and near the smooth muscle in WAS group (Figure 2c). Mast cells were generally located near the blood vessels. Many mast cells were observed to migrate to the epithelium and degranulated in this region in WAS group. Besides granulated mast cells in mucosal region, a few degranulated cells were also observed in WAS+ALA group (Figure 2d).

http://doi.org/10.5472/marumj.1191211
Marmara Med J 2022;35(3): 263-269

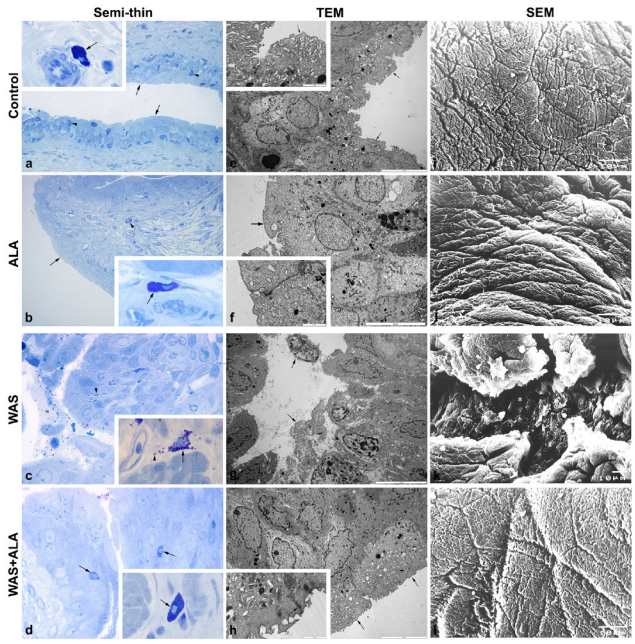

Figure 2. *(a) Normal epithelial layer (arrows) and granulated mast cells (arrowhead) in the epithelium in control group. Inset: Granulated mast cell (arrow). (b) Normal epithelial layer (arrow) and granulated mast cell (arrow) in ALA group. Inset: Granulated mast cell (arrow). (c) Epithelial cell remnants (asterisk) in the lumen and released mast cell granules (arrowhead) in WAS group. Inset: Released mast cell granules (arrowhead) from a degranulated mast cell (arrow) in the mucosa. (d) Granulated mast cells (arrows) in the mucosa in WAS+ALA group. Inset: Granulated mast cell (arrow). (e) Normal urothelium (arrows) in control group. Inset: Normal urothelium (arrow) and normal appearing tight junction (arrowhead). (f) Normal urothelium (arrow) and granulated mast cell (m) in the epithelium in ALA group. Inset: Normal appearing tight junction (arrow). (g) Epithelial cells shed into the lumen (arrows) in WAS group. (h) Normal urothelium (arrows) in WAS+ALA group. Inset: Normal appearing tight junction (arrow). Normal appearing urothelium in control (i) and ALA (j) groups. (k) Epithelial cells were shed into the lumen (asterisk) in WAS group. (l) Normal appearing urothelium in WAS+ALA group. (a-d) Toluidine blue stained semi-thin sections. Original magnification: x400, insets: x1000. (e-h) TEM imaging. Bars: 10 µm, insets: 2 µm. (i-l) SEM micrographs. Bars: 10 µm.*

Transmission Electron Microscopy

In semi-thin sections of control (Figure 2e) and ALA (Figure 2f) groups, epithelium was normal and intact. Tight junctions were also intact and fusiform vesicles were normal in appearance in both goups. A few mast cells were present near the blood vessels. Severe epithelial degeneration was seen in WAS group (Figure 2g). Epithelial cells shed into lumen, intercellular junctions were opened, and there were vacuoles in the cells. The number of fusiform vesicles was decreased. Besides epithelial damage in some regions, intercellular openings, and degranulated mast cells in the epithelium, there was generally an amelioration of these findings in WAS+ALA group (Figure 2h). Tight junctions were intact in this group.

Scanning Electron Microscopy

Urothelial surface was normal in control (Figure 2i) and ALA (Figure 2j) groups. There was severe epithelial shedding in WAS group (Figure 2k). Besides epithelial shedding in some regions, most regions appeared normal in WAS+ALA group (Figure 2l) compared to WAS group.

Mast Cell Number

The number of granulated and degranulated mast cells in the mucosal layer of control and ALA groups was similar (Figure 3a). The number of granulated ($p<0.05$) and degranulated ($p<0.01$) mast cells was increased compared to control group. The number of granulated and degranulated mast cells was decreased in WAS+ALA group compared to WAS group ($p<0.001$).

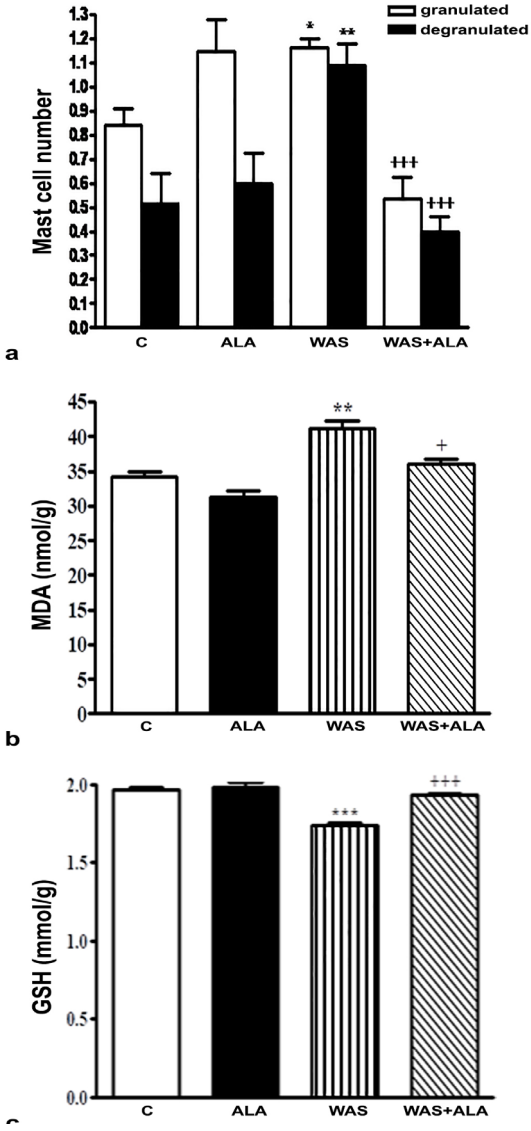

Figure 3. *Statistical analysis. (a) Mast cell number. *p<0.05, **p<0.01; compared to control group. +++p<0.001; compared to WAS group. (b) MDA analysis. **p<0.01; compared to control groups. +p<0.05; compared to WAS group. (c) GSH analysis. ***p<0.001; compared to control groups. +++p<0.001, compared to WAS group.*

http://doi.org/10.5472/marumj.1191211
Marmara Med J 2022;35(3): 263-269

MDA Measurement

In the present study, WAS caused lipid peroxidation in the bladder tissue and MDA levels, which is an indicator of lipid peroxidation, was increased compared to the control groups (Figure 3b, $p<0.01$). MDA levels in WAS+ALA group was decreased compared to the WAS group ($p<0.05$).

GSH Measurement

Glutathione was decreased in WAS group compared to control groups (Figure 3c, $p<0.001$). GSH level was increased in WAS+ALA group compared to WAS group ($p<0.001$). Values of control and ALA groups were similar.

4. DISCUSSION

In the present study, ALA, a potent antioxidant, was used in rats exposed to WAS, and was observed to have ameliorating effects on bladder tissue. WAS is a model of physical and psychological stress and accepted as moderate stress [25-27].

WAS is a life stress causing psychological effects. It was reported that WAS applied for 10 days caused increased anxiety and pain in rats [28]. Studies have shown that repeated psychological stress in rodents induced bladder dysfunction [29]. In the studies using WAS model, bladder epithelium was observed to be injured and epithelial morphology was changed [26, 30]. It was also observed that WAS induced bladder overactivity in mice [31]. WAS mimics features of urinary frequency and bladder hyperalgesia seen in patients with IC [32]. In the present study, WAS was applied for 5 days in the rats. As a result, in line with the previous studies, bladder epithelium was injured. This injury demonstrated itself as opening of intercellular junctions, shedding of epithelial cells into the lumen, and decrease in the number of fusiform vesicles.

Injury in the GAG layer causes activation of the submucosal sensory neurons and pain [10]. Proinflammatory mediators are released due to triggering of substance P release. As a result, damage in the surface urothelial cells occur and epithelial cells are shed into the lumen. In the present study, GAG layer damage was demonstrated by PAS reaction, and in light and electron microscopic examinations, opening of intercellular junctions and epithelial cells in the lumen was observed in WAS group.

Clinical and laboratory studies proved that mast cells play a central role in the pathophysiology of IC [33]. Mast cell increase and activation, and prevention of this increase by medical treatment and deactivation of proinflammatory mediators was observed in patients with IC. Mast cells in smooth muscle and mucosal layers of urinary bladder in these patients were increased [34]. Mast cells are activated by a series of mechanisms in the bladder wall. Epithelial permeability increase due to potassium ion flow may cause mast cell activation. It was reported that there was an anatomical and functional relation between mast cells and neurons. Scanning electron microscopic studies showed that mast cells were located in the neighborhood of endothelial cells and neuronal projections [35, 36]. In human studies and animal models, a series of free neuropeptides and neurotransmitters were shown activating submucosal nerves and mast cells in the epithelium. Neuroinflammatory and neuroendocrine alterations in the bladder of patients with IC contribute to pain symptoms and frequent urination in these patients [33]. The inflammatory progression of IC was found to be related to the expression levels of inflammatory cytokines such as IL-6, IL-17, and IL-1β, and also related to mast cell activation [6, 37, 38]. In the present study, only mucosal mast cells were counted and mast cells in the smooth muscle layer were not included. Degranulated mast cells were increased in number in WAS group. Degranulated cells were observed in smooth muscle layer, although quantitative analysis was not performed. Antioxidant ALA treatment was observed to repress degranulation.

Alpha-lipoic acid might be useful clinically in the treatment of IC [39]. Urothelial expression of fractalkine and its receptor was reported to be increased in chronic cystitis model in mouse [40]. Fractalkine is a protein belonging to cytokine family [41]. Fractalkine receptors were increased in cytitis caused by cyclophosphamide in rat [40]. Because the cystitis caused by cyclophosphamide exposure corresponds to IC in humans, reducing fractalkine in the treatment of this clinical situation by ALA treatment was accepted as a potential target [40, 42]. Two hypotheses were suggested in the mechanism of effect of ALA. The first one is that ALA is a potential agent reducing fractalkine mRNA and its protein expression [43]. ALA also decreases fractalkine mediated inflammatory processes. The second hypothesis is that ALA has the capacity to inhibit TNF-α induced fractalkine expression.

In chronic cystitis models in rat, a prominent decrease in the antioxidant defense parameters was observed. Harmful effects of oxidative stress were reversed by powerful antioxidant effects of ALA in inflamed bladder [42]. It was also reported that ALA might prevent contractile function disorder in bladder tissue *in vitro* [44]. It was suggested that ALA might be used as a new strategy in treatment of IC [42]. In the present study, it was observed that bladder injury findings due to oxidative stress decreased by ALA treatment; therefore, similar to the above studies, we suggest it should be supported by clinical studies so that ALA can be used as a therapeutic agent.

Previous studies have shown that increased free oxygen radicals and lipid peroxidation played a role in the pathogenesis of many diseases [45]. In chronic WAS studies, increased MDA levels and decreased GSH levels were observed [46]. Similarly, we demonstrated significantly increased MDA levels in the bladder tissue of stress model in rat, which is a sign of lipid peroxidation; and in parallel with this finding, we observed significantly decreased GSH levels. ALA treatment decreased MDA levels and increased GSH levels. These findings indicate that ALA decreases oxidative stress in the bladder in IC model [47]. ALA was shown previously to be an antioxidant in the bladder tissue [48].

5. CONCLUSION

In conclusion, in the present study, it was shown that bladder mucosa injury and mast cell degranulation, related to oxidative stress, in WAS model in rat were reversed by ALA tratment.

http://doi.org/10.5472/marumj.1191211
Marmara Med J 2022;35(3): 263-269

We suggest that these findings should be supported by clinical studies and ALA should be tried as a therapeutic agent in the treatment of IC.

Compliance with Ethical Standards

Ethical Approval: All experiments were done according to the National Guidelines on Animal Experimentation and were approved by the Marmara University Local Ethical Committee for Experimental Animals (approval number 03.2009.mar).

Financial Support: This study was supported by Marmara University Research Fund (SAG-C-YLP-090909-0280).

Conflict of Interest: The authors declare that there are no conflicts of interest.

Author Contributions: NY and EC: did the experiments, HZT: did the biochemical analysis and critical revision of the article, SS: analyzed the data and wrote the article. All authors approved the final version of the article.

REFERENCES

[1] Akiyama Y, Hanno P. Phenotyping of interstitial cystitis/bladder pain syndrome. Int J Urol 2019; 26(Suppl 1):17-19. doi: 10.1111/iju.13969.

[2] Akiyama Y, Luo Y, Hanno PM, Maeda D, Homma Y. Interstitial cystitis/bladder pain syndrome: The evolving landscape, animal models and future perspectives. Int J Urol 2020; 27(6):491-503. doi: 10.1111/iju.14229.

[3] Koziol JA, Clark DC, Gittes RF, Tan EM. The natural history of interstitial cystitis: a survey of 374 patients. J Urol 1993; 149:465-9. doi: 10.1016/s0022-5347(17)36120-7.

[4] Sant GR. Interstitial cystitis. Curr Opin Obst Gynecol 1997; 9:332-6.

[5] Atuğ F, Canoruç N. İnterstisyel sistit: Kronik pelvik ağrı sendromu. Dicle Tıp Dergisi 2005; 32:204-10.

[6] Logadottir Y, Delbro D, Fall M, Gjertsson I, Jirholt P, Lindholm C, Peeker R. Cytokine expression in patients with bladder pain syndrome/interstitial cystitis ESSIC type 3C. J Urol 2014; 192:1564-8. doi: 10.1016/j.juro.2014.04.099.

[7] Kochiashvili G, Kochiashvili D. Urinary IL-33 and galectin-3 increase in patients with interstitial cystitis/bladder pain syndrome. Georgian Med News 2014; 232-233:12-5.

[8] Fall M, Johansson SL, Aldenborg F. Chronic interstitial cystitis: A heterogeneous syndrome. J Urol 1987; 137:35-8. doi: 10.1016/s0022-5347(17)43863-8.

[9] Kandere-Grzybowska K, Gheorghe D, Priller J, Esposito P, Huang M, Gerard N, Theoharides TC. Stress-induced dura vascular permeability does not develop in mast cell-deficient and neurokinin-1 receptor knockout mice. Brain Res 2003; 980:213-20. doi: 10.1016/s0006-8993(03)02975-5.

[10] Jasmin L, Janni G, Ohara PT, Rabkin SD. CNS induced neurogenic cystitis is associated with bladder mast cell degranulation in the rat. J Urol 2000; 164:852-5. doi: 10.1097/00005.392.200009010-00061.

[11] Letourneau R, Pang X, Sant GR, Theoharides TC. Intragranular activation of bladder mast cells and their association with nerve processes in interstitial cystitis. Br J Urol 1996; 77:41-54. doi: 10.1046/j.1464-410x.1996.08178.x.

[12] Ercan F, Çetinel Ş. Mast hücrelerinin enflamasyondaki rolü: insan ve deneysel hayvan modelleri üzerinde yapılan çalışmaların değerlendirilmesi. Marmara Medical Journal 2008; 21:179-86.

[13] Chatterjea D, Martinov T. Mast cells: versatile gatekeepers of pain. Mol Immunol 2015; 63:38-44. doi: 10.1016/j.molimm.2014.03.001.

[14] Parsons CL, Lilly JD, Stein P. Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). J Urol 1991; 145:732-5. doi: 10.1016/s0022-5347(17)38437-9.

[15] Ercan F, Akıcı A, Ersoy Y, Hürdag C, Erin N. Inhibition of substance P activity prevents stress-induced bladder damage. Regul Pept 2006; 133:82-9. doi: 10.1016/j.regpep.2005.09.024.

[16] Salehi B, Berkay Yılmaz Y, Antika G, Boyunegmez Tumer T, Fawzi Mahomoodally M, Lobine D, Akram M, Riaz M, Capanoglu E, Sharopov F, Martins N, Cho WC, Sharifi-Rad J. Insights on the use of α – lipoic acid for therapeutic purposes. Biomolecules 2019; 9:356. doi: 10.3390/biom9080356.

[17] Senoglu M, Nacitarhan V, Kurutas EB, Senoglu N, Altun İ, Atli Y, Ozbag D. Intraperitoneal alpha-lipoic acid to prevent neural damage after crush injury to the rat sciatic nerve. J Brachial Plex Peripher Nerve Inj 2009; 4:22. doi: 10.1186/1749-7221-4-22.

[18] Ghibu S, Lauzier B, Delemasure S, Amoureux S, Sicard P, Vergely C, Muresan A, Mogosan C, Rochette L. Antioxidant properties of alpha-lipoic acid: effects on red blood membrane permeability and adaptation of isolated rat heart to reversible ischemia. Mol Cell Biochem 2009; 320:141-8. doi: 10.1007/s11010.008.9916-0.

[19] Zhang J, Wang M, Zhao Y, Zhang Y, Gao Y, Zhang X, Yang G. Alpha-lipoic acid improved motor function in MPTP-induced Parkinsonian mice by reducing neuroinflammation in the nigral and spinal cord. Neurosci Lett 2022; 781:136669. doi: 10.1016/j.neulet.2022.136669.

[20] Jiang Y, Gong D, Liu H, Yang C, Sun Z, Kong C. Ability of alpha-lipoic acid to reverse the diabetic cystopathy in a rat model. Acta Pharmacol Sin 2008; 29(6):713-9. doi: 10.1111/j.1745-7254.2008.00790.x.

[21] Biewenga GP, Haene GR, Bast A. The pharmacology of the antioxidant lipoic acid. Gen Pharmacol 1997; 29:315-31. doi: 10.1016/s0306-3623(96)00474-0.

[22] Packer L. α-Lipoic acid: metabolic antioxidant which regulates NF-κB signal transduction and protects against oxidative injury. Drug Metab 1998; 30:245-75. doi: 10.3109/036.025.39808996311.

[23] Beuge JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52:302-11. doi: 10.1016/s0076-6879(78)52032-6.

[24] Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882-8.

[25] Cikler E, Ercan F, Çetinel S, Contuk G, Sener G. The protective effects of melatonin against water avoidance stress-induced mast cell degranulation in dermis. Acta Histochem 2005; 106:467-75. doi: 10.1016/j.acthis.2004.10.001.

[26] Cikler E, Ersoy Y, Çetinel Ş, Ercan F. The leukotriene D4 receptor antagonist, montelukast, inhibits mast cell degranulation in the dermis induced by water avoidance stress. Acta Histochem 2009; 111:112-8. doi: 10.1016/j.acthis.2008.04.006.

[27] Çetinel Ş, Ercan F, Conturk G, Sener G. Protective effect of melatonin on water avoidance stress induced degeneration of the bladder. J Urol 2005; 173:267-70. doi: 10.1097/01.ju.000.014.5891.35810.56.

[28] Robbins MT, DeBerry J, Ness TJ. Chronic psychological stress enhances nociceptive processing in the urinary bladder in high-anxiety rats. Physiol Behav 2008; 91:544-50. doi: 10.1016/j.physbeh.2007.04.009.

[29] West EG, Sellers DJ, Chess-Williams R, McDermott C. Bladder overactivity induced by psychological stress in female mice is associated with enhanced bladder contractility. Life Sci 2021; 265:118735. doi: 10.1016/j.lfs.2020.118735.

[30] Zeybek A, Ercan F, Cetinel S, Cikler E, Sağlam B, Sener G. Taurine ameliorates stress-induced degenerations of gastrointestinal tract and liver. Dig Dis Sci 2007; 51:1853-61. doi: 10.1007/s10620.006.9425-5.

[31] Mills KA, West EG, Sellers DJ, Chess-Williams R, McDermott C. Psychological stress induced bladder overactivity in female mice is associated with enhanced afferent nerve activity. Sci Rep 2021; 11:17508. doi: 10.1038/s41598.021.97053-5.

[32] Sanford MT, Yeh JC, Mao JJ, Guo Y, Wang Z, Zhang R, Holschneider DP, Rodriguez LV. Voluntary exercise improves voiding function and bladder hyperalgesia in an animal model of stress-induced visceral hypersensitivity: a multidisciplinary approach to the study of urologic chronic pelvic pain syndrome research network study. Neurourol Urodyn 2020; 39:603-12. doi:10.1002/nau.24270.

[33] Grannum RS, Duraisamy K, James ME, Theoharides TC. The mast cell in interstitial cystitis: Role in pathophysiology and pathogenesis. Urology 2007; 69(4 Suppl.):34-40. doi: 10.1016/j.urology.2006.08.1109.

[34] Kurtoğlu B, Yörükoğlu K, Sevinç M, Kırkalı Z. İnterstisyel sistitin patogenezi üzerine histokimyasal ve immunohistokimyasal bir çalışma. Turgut Özal Tıp Merkezi Dergisi 1998; 5:126-32.

[35] Theoharides TC, Kempuraj D, Sant GR. Mast cell involvement in interstitial cystitis: A review of human and experimental evidence. Urology 2001; 57(6 Suppl. 1): 47-55. doi: 10.1016/s0090-4295(01)01129-3.

[36] Theoharides TC. Mast cells and stress – a psychoneuroimmunological perspective. J Clin Psychopharmacol 2002; 22:103-6. doi: 10.1097/00004.714.200204000-00001.

[37] Grover S, Srivastava A, Lee R, Tewari AK, Te AE. Role of inflammation in bladder function and interstitial cystitis. Ther Adv Urol 2011; 3:19-33. doi: 10.1177/175.628.7211398255.

[38] Offiah I, Didangelos A, Dawes J, Cartwright R, Khullar V, Bradbury EJ, O'Sullivan S, Williams D, Chessell IP, Pallas K, Graham G, O'Reilly BA, McMahon SB. The expression of inflammatory mediators in bladder pain syndrome. Eur Urol 2016; 70:283-90. doi: 10.1016/j.eururo.2016.02.058.

[39] Pastuszka A, Slusarczyk K, Koszutski T, Kawalski H, Kudela G. Alpha-lipoic acid may be a clinically useful therapy in interstitial cystitis. Med Hypotheses 2007; 69:957-65. doi: 10.1016/j.mehy.2007.01.058.

[40] Yuridullah R, Corrow KA, Malley SE, Vizzard MA. Expression of fractalkine and fractalkine receptor in urinary bladder after cyclophosphamide (CYP)-induced cystitis. Auton Neurosci 2006; 126-127:380-9. doi: 10.1016/j.autneu.2006.02.030.

[41] El-Mansoury M, Boucher W, Sant GR, Theoharides TC. Increased urine histamine and methylhistamine in interstitial cystitis. J Urol 1994; 152:350-53. doi: 10.1016/s0022-5347(17)32737-4.

[42] Bilska A, Wlodek L. Lipoic acid – the drug of the future? Pharmacol Rep 2005; 57:570-7.

[43] Sung MJ, Kim W, Ahn SY, Cho CH, Koh GY, Moon SO. Protective effect of alpha lipoic acid in lipopolysaccharide induced endothelial fractalkine expression. Circ Res 2005; 97:880-90. doi: 10.1161/01.RES.000.018.6522.89544.4D.

[44] Levin RM, Danek M, Whitbeck C, Haugaard N. Effect of ethanol on the response of the rat urinary bladder to in vitro ischemia: protective effect of alpha-lipoic acid. Mol Cell Biochem 2005; 271:133-41. doi: 10.1007/s11010.005.5870-2.

[45] Altan N, Dinçel AS, Koca C. Diabetes mellitus ve oksidatif stress. Turk J Biochem 2006; 31:51-6.

[46] Paris M, Kidd PhD. Glutathione: systemic protectant against oxidative and free radical damage. Alt Med Rev 1997; 2:155-76.

[47] Busse E, Zimmer G, Schopohl B. Influence of alpha-lipoic acid on intracellular glutathione in vitro and in vivo. Arzneimittelforschung 1992; 42:829-31.

[48] Ekiz A, Özdemir-Kumral ZN, Erşahin M, Tuğtepe H, Velioğlu Öğünç A, Akakın D, Kıran D, Özsavcı D, Biber N, Hakan T, Çağlayan Yeğen B, Şener G, Toklu HZ. Functional and structural changes of the urinary bladder following spinal cord injury; treatment with alpha lipoic acid. Neurourol Urodyn 2017; 36:1061-8. doi: 10.1002/nau.23083.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Vitamin D receptor gene polymorphisms in pediatric patients with leukemia-lymphoma: Does it have an impact on malignancy?

Sinem GULCAN KERSIN[1], Ayse Gulnur TOKUC[2], Ahmet ARMAN[3], Baris YILMAZ[2]

[1] Department of Child Health and Pediatrics, School of Medicine, Marmara University, Istanbul, Turkey.
[2] Division of Pediatric Hematology and Oncology, Department of Child Health and Pediatrics, School of Medicine, Marmara University, Istanbul, Turkey.
[3] Department of Medical Genetics, School of Medicine, Marmara University, Istanbul, Turkey.

Corresponding Author: Sinem GULCAN KERSIN
E-mail: sinemgulcan@hotmail.com

Submitted: 24.06.2022 **Accepted:** 27.08.2022

ABSTRACT

Objective: Genetic variations have been identified in specific regions of the Vitamin D receptor (VDR) gene and many studies were investigating whether these variations are associated with malignancy. Studies in the VDR on children are scarce. In this study, we aimed to investigate the VDR gene polymorphisms in pediatric patients with the diagnosis of leukemia and lymphoma.

Patients and Methods: Of the 99 participants included in this cross-sectional study, 59 were control, 40 were patients. Fok-I, Bsm-I and Taq-I polymorphism of the VDR gene were investigated in both groups.

Results: While no significant difference was found in the genotype distribution of the three polymorphisms between the patient and control groups, significant results were obtained in Bsm-I and Taq-I allele frequencies (Odds ratio=0.489; CI95%=0.275-0.871 and 0.519; CI95%=0.280-0.964) ($p<0.05$).

Conclusions: In this study, we found that the frequency of allele "A" for Bsm-I and allele "C" for Taq-I was lower in the patient group. Contrary to most publications in the literature, polymorphisms were not found to be risk factors in our study.

Keywords: Childhood cancers, Leukemia, Lymphoma, VDR, Polymorphism

1. INTRODUCTION

Approximately two million new cases of malignancies are reported each year in the United States, of which sixteeen thousand are reported to be in children [1]. There are many studies about the causative factors of cancer. One of the suspected causative factors is vitamin D. Vitamin D is a hormone that acts by binding to the intracellular specific receptor and plays a role in cell proliferation, inflammation, hormone receptor. In some studies, it is suggested that low vitamin D levels may be associated with some autoimmune and allergic diseases, metabolic syndrome, infectious diseases and cancer [2]. Rather than the plasma level, it has been seen that genetic variations detected in the vitamin D receptor (VDR) gene cause cancer and have effects on mortality [3].

Vitamin D receptor is a nuclear receptor and is associated with intracellular signaling pathways. The VDR protein is encoded by the VDR gene located on chromosome 12q12–q14. Polymorphic numerous variations are detected on the VDR gene and these variations are thought to increase the cancer risk [3]. To date, more than sixty polymorphisms have been found in the promoter region, around exon 2-9, and at the 3' end [4]. For example; Fok-I with start codon polymorphism at the 5' end of exon 2 has been shown to encode a shorter VDR protein and show less function [5]. Bsm-I polymorphism at the 3' end of exon 8 does not make any change on either the translated protein or the transcribed mRNA; Taq-I in exon 9 causes silent codon change (ATT→ATC) to add isoleucine to the 352nd position; both of them are involved in VDR gene regulation and mRNA stability [6].

In some studies, it has been stated that VDR Fok-I, Bsm-I, Taq-I polymorphisms may be associated with prostate, breast, kidney and colon cancers (4). In some studies, however, no association was found. There are not many studies on VDR gene polymorphism in children and our study aimed to investigate the relationship between VDR gene polymorphisms and lymphoproliferative malignancies in children.

How to cite this article: Gulcan Kersin S, Tokuc A G, Arman A, Yilmaz B. Vitamin D receptor gene polymorphisms in pediatric patients with leukemia-lymphoma: Does it have an impact on malignancy?. Marmara Med J 2022; 35(3): 270-274, doi: 10.5472/marumj.1191178

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1191178
Marmara Med J 2022;35(3): 270-274

2. PATIENTS and METHODS

Design

This is a cross-sectional study, which was approved by Marmara University School of Medicine Clinical Researches Ethics Committee (Protocol number: 09.01.2015-09.2014.0336/70737436-050.06.04). This study was supported by Marmara University Scientific Research Fund (SAG-C-TUP-080.415.0097).

Participants

In this study, the patient group consisted of 30 acute lymphoblastic leukemia (9 low-risk, 16 standard risk, 3 high-risk) three acute myeloid leukemia, four Hodgkin's lymphoma and three non-Hodgkin's lymphoma patients who were at the course of treatment and followed up in the pediatric hematology and oncology outpatient clinic. Only one of the patients was cured during this time period when the study was conducted. The control group consisted of 59 patients who applied to pediatric outpatient clinics with complaints such as upper respiratory tract infection and had no family history of cancer or any chronic disease. The study was conducted following the principles of the Declaration of Helsinki. Written informed consent was obtained from all the participants.

Procedures and Instruments

Fok-I, Bsm-I and Taq-I polymorphism of VDR gene were investigated in both patient and control groups. 4 cc peripheral blood samples were obtained from the participants and taken into a 0.5 M Ethylenediaminetetraacetic acid (EDTA) tube (BD Medical, NJ, USA). Deoxyribonucleic acid (DNA) was obtained by several serial procedures. The isolated DNAs were stored at – 20°C. Polymerase Chain Reaction (PCR) was used to amplify polymorphisms sites of the VDR gene. PCR was carried by total volume of 25 µl reaction containing 12.8 µl distilled water, 2.5 µl 10x buffer solution, 0.7 µl FP, 0.7 µl RP, 0.1 µl Taq polymerase, 2 µl template, 3.7 µl deoxynucleotide triphosphates [dATG, dGTP, dCTP, dTTP] and 2.5 µl of MgCl2.

Primers for Fok-I (rs2228570) polymorphism region;

VDRFF(23b) 5' – AGGATGCCAGCTGGCCCTGGCAC – 3'

VDRFR(26b) 5'-TGGCTGTGAGCGCCGCATGTTCCATG – 3'

- Primers for Bsm-I (rs1544410) polymorphism region;

VDRBF 5' – GCAACCTGAAGGGAGACGTAGC – 3'

VDRBR 5' – TCCTTGAGCCTCCAGTCCAGG – 3'

- Primers for Taq-I (rs731236) polymorphism region;

VDRTF 5' – AGAGCATGGACAGGGAGCAAGGC – 3'

VDRTR 5' – TAGCTTCATGCTGCACTCAGGCTGG – 3'

The gene region was amplified by PCR using these primers. Fok-I polymorphism was found 265 base pairs (bp), Bsm-I polymorphism was found 825 bp, and Taq-I polymorphism was found 740 bp. Using restriction endonuclease enzymes, the digested PCR products were run on 2% agarose gel. It was carried out for 30-50 minutes at 90-100V current and the results were examined in the gel imaging system. Digestion of Fok-I gives T/T (263 bp, 80 bp for homozygote wild type), T/C (343 bp, 263 bp, 80 bp for heterozygote) and C/C (343 bp for homozygote mutant). The digestion of Bsm-I gives G/G (331 bp, 200 bp for homozygote wild type), G/A (531 bp, 331 bp, 200 bp for heterozygote) and A/A (531 bp for homozygote mutant). The digestion of Taq-I gives T/T (479 bp for homozygote wild type), C/T (479 bp, 290 bp, 189 bp for heterozygote) and C/C (290 bp, 189 bp for homozygote mutant).

Statistical Analysis

All data were recorded electronically and SPSS 20.0 statistics program (SPSS Inc, Chicago, USA) was used. Chi-square ($\chi 2$) and Fisher's exact tests were used to compare categorical descriptive data in the study. Whether the measurement data showed normal distribution or not was determined by the Kolmogorov-Smirnov test. A value of $p < 0.05$ was considered statistically significant. The risk probability of alleles was evaluated using odds ratio (OR) and 95% confidence interval (CI).

3. RESULTS

This study consisted of 99 participants. DNA could not be obtained from six patients because four were lymphopenic and two patients died during outpatient follow-up. The patient group consisted of 40 people. 59 participants were included in the control group. 47 (47.5%) participants were male. The mean age of patients was 8.27±4.8 and the mean age of controls was 9.66±4.1. There was no statistical significance in age and gender distribution between groups (p=0.057, p=0.107, respectively).

Genotypic and phenotypic characteristics of participants

Vitamin D receptor gene genotypes and allele frequencies were examined in this study. The distributions of the genotype of Fok-I, Bsm-I, Taq-I polymorphisms in both control and patients are shown in Table I. There was no significant difference in genotype distribution of the three polymorphisms between the patient and control groups (p>0.05).

The allele frequencies of Fok-I, Bsm-I and Taq-I polymorphisms are shown in Table II. There was a significant difference between groups in Bsm-I and Taq-I allele frequencies, but not in Fok-I. Frequency of Bsm-I G allele was 57.5% (n=46) within patient group and 40% (n=47) within control group and frequency of Bsm-I A allele was 42.5% (n=34) within patient group and 60% (n=71) within control group (Odds ratio=0.489; CI 95%=0.275-0.871) (p<0.05). Frequency of Taq-I T allele was 73.8% (n=59) within patient group and 59.3% (n=70) within control group. Frequency of Taq-I C allele was 26.2% (n=21) within patient group and 40.7% (n=48) within control group (Odds ratio=0.519; CI 95%=0.280-0.964) (p<0.05).

http://doi.org/10.5472/marumj.1191178
Marmara Med J 2022;35(3): 270-274

Table I. *Genotype distribution of VDR gene polymorphisms in patient and controls*

Polymorphism	Patient	Control	P value	Odd's ratio (95% CI)
Fok-I genotype				
T/T	2.5% (n=1)	8.4% (n=5)	0.396*	3.61 (0.405-32.160)
T/C	45% (n=18)	44% (n=26)		
C/C	52.5% (n=21)	47.6% (n=28)		
Bsm-I genotype				
G/G	35% (n=14)	18.7% (n=11)	0.109**	0.425 (0.169 – 1.07)
G/A	45% (n=18)	42.3% (n=25)		
A/A	20% (n=8)	39% (n=23)		
Taq-I genotype				
T/T	55% (n=22)	37.3%(n=22)	0.125**	0.486 (0.215-1.101)
T/C	37.5% (n=15)	44% (n=26)		
C/C	7.5% (n=3)	18.7% (n=11)		

**Fisher's Exact Test p value, ** The two-sided p values*

Table II. *VDR allele frequencies in patients and controls*

Polymorphism region	Patient	Control	P value	Odd's ratio (95% CI)
Fok-I				
T allele	25% (n=20)	30.5% (n=36)	0.398*	1.31 (0.694-2.498)
C allele	75% (n=60)	69.5% (n=82)		
Bsm-I				
G allele	57.5% (n=46)	40% (n=47)	0.015*	0.489 (0.275-0.871)
A allele	42.5% (n=34)	60% (n=71)		
Taq-I				
T allele	73.8% (n=59)	59.3% (n=70)	0.037*	0.519 (0.280-0.964)
C allele	26.2% (n=21)	40.7% (n=48)		

** The two-sided p values*

4. DISCUSSION

Mortality due to malignancy has an important place in childhood. In the literature, it has been reported that Fok-I, Bsm-I, Taq-I polymorphisms are frequently associated with cancer [3,4]. Many studies have shown that Fok-I C/C, Bsm-I A/A, Taq-I C/C genotypes are associated with higher cancer risk [5]. In a meta-analysis involving the prostate, breast, skin, ovarian, colorectal cancers and non-Hodgkin lymphoma, it was stated that a significantly increased cancer risk was observed in C/C genotypes compared to T/T genotypes, while the risk was slightly increased in T/C genotypes in Fok-I; whereas in patients with Bsm-I G/G or G/A genotypes, the risk has been reported to be low [5]. On the contrary, Beysel et al., found that the Fok-I T/T genotype was associated with advanced stage in thyroid papillary carcinoma [7]. Yu et al., reported that Bsm-I A allele has a negative association with cancer risk compared to the G allele in lung cancer [8]. In our study, the majority of both groups (93%) were genotypically T/C or C/C in Fok-I and the distribution of patients and controls with the T/T genotype was 2.5% and 8.4%, respectively, which was not statistically significant. Also, Taq-I C allele was seen in lower frequency in the patient group. Similar to the study of Yu et al., in our study, the Bsm-I A allele was significantly more frequent in the control group and we thought that it might be negatively associated with cancer [8].

In a study by Oh and Barrett-Connor, it was reported that 35% of the population had the Bsm-I homozygous mutant genotype and the risk of colon cancer was two times higher than the homozygous wild genotype [9]. Rasool et al., also reported that cancer risk was 2.7 times more in homozygous mutant genotype than wild genotype [10]. Some studies have revealed an increased risk of breast cancer in the mutant Bsm-I genotype [11,12]. In the meta-analysis by Zhang and Song they found an association between breast cancer and the Fok-I polymorphism, they reported that poly-A, Bsm-I, Taq-I and

http://doi.org/10.5472/marumj.1191178
Marmara Med J 2022;35(3): 270-274

Apa-I polymorphisms had no effect [13]. Similarly, in the meta-analysis by Luo et al., it was shown that Apa-I polymorphism did not have a determining role in breast cancer [14]. In the meta-analysis by Tang et al. many polymorphisms were investigated and breast cancer risk was found to be increased in the Fok-I homozygous mutant genotype (OR: 1.16, 95% CI: 1.04–1.30) [15]. Contrary to these studies, Yang et al., reported in a meta-analysis that Fok-I, Bsm-I, Taq-I and Apa-I polymorphisms had no association with breast cancer in Caucasian women [16].

Although, there are many studies in adults, there are limited data on VDR gene polymorphisms in childhood [17,18]. Purdue et al., did not find any association between Fok-I, Bsm-I and Taq-I polymorphisms and non-Hodgkin's lymphoma, and reported that Bsm-I gene polymorphism may lead to an increased risk of diffuse large B cell lymphoma [19]. Tekgündüz et al., did not find any association between Cdx2, Fok-I, Bsm-I, Apa-I, Taq-I polymorphisms and malignancy in patients diagnosed with childhood Hodgkin's lymphoma [20]. Also, Yılmaz et al., found no association between the Taq-I, Fok-I and Bsm-I polymorphisms and pediatric brain cancers [18]. In another study involving pediatric patients with solid cancer, it was stated that in Fok-I CT and CC genotypes were weakly associated with a reduced risk of malignancy formation [21]. In our study, no statistically significant association was observed between VDR Fok-I, Bsm-I, Taq-I genotypes and malignancy. However, there was a significant difference between Bsm-I and Taq-I allele frequencies. We found out that carrying the mutant Bsm-I A allele and the mutant Taq-I C allele has a negative association with cancer risk compared to carrying the Bsm-I G allele and Taq-I T allele.

The small number of participants in the research is the biggest limitation. Our other limitation is; in this cross-sectional study, the control group was defined as those who had no complaints or symptoms suggestive of malignancy at that time and those with no family history of cancer. This should not mean that malignancy will not be seen in this group at all. Perhaps this was the reason why there was no significant difference between the groups. Long-term follow-up of the patients in the control group, re-evaluation of the results of this study, and comparison of the groups will be meaningful.

Conclusion

In conclusion, the association between VDR polymorphisms and malignancy is still controversial, and no association was found between Fok-I and malignancy in our study. Contrary to most publications in the literature, the incidence of mutant Bsm-I A and Taq-I C allele polymorphisms was lower in our study in the patient group, and polymorphisms were not found to be risk factors. It is thought that more meaningful results can be obtained in a study with long-term follow-up with a larger number of participants, perhaps including all childhood malignancies.

Compliance with Ethical Standards

Ethical Approval: The study protocol was approved by the Marmara University School of Medicine, Clinical Researches Ethics Committee (Protocol number: 09.01.2015-09.2014.0336/70737436-050.06.04).

Financial Support: This study was supported by Marmara University Scientific Research Fund (SAG-C-TUP-080.415.0097).

Conflict of Interest Statement: None of the authors has any conflict of interest regarding the data and conclusions reported in this study.

Authors' Contributions: SGK: Writing – reviewing and editing, data curation, AGT: Investigation, project administration, methodology, AA: Formal analysis,conceptualization, supervision, BY: Data curation. All authors read and approved the final version of the article.

REFERENCES

[1] Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin 2021;71:7-33. doi: 10.3322/caac.21654.

[2] Pearce SH, Cheetham TD. Diagnosis and management of vitamin D deficiency. BMJ 2010;340:b5664. doi:10.1136/bmj.b5664

[3] Deeb KK, Trump DL, Johnson CS. Vitamin D signaling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 2007; 7:684-700. doi:10.1038/nrc2196

[4] Köstner K, Denzer N, Muller CSL, Klein R, Tilgen W, Reichranth J. The Relevance of Vitamin D Receptor (VDR) gene polymorphisms for cancer: A review of the literature. Anticancer Res 2009; 29:3511-36. PMID: 19667145.

[5] Raimondi S, Harriet Johansson H, Patrick Maisonneuve P, Gandini S. Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. Carcinogenesis 2009; 30:1170-80. doi: 10.1093/carcin/bgp103.

[6] Thorne J, Campbell MJ. The vitamin D receptor in cancer. Proc Nutr Soc 2008; 67:15-22. doi: 10.1017/S002.966.5108006964.

[7] Beysel S, Eyerci N, Pinarli FA, et al. VDR gene FokI polymorphism as a poor prognostic factor for papillary thyroid cancer. Tumour Biol 2018; 40:101.042.8318811766. doi: 10.1177/101.042.8318811766.

[8] Yu ZH, Chen M, Zhang QQ, Hu X. The association of vitamin D receptor gene polymorphism with lung cancer risk: An update meta-analysis. Comb Chem High Throughput Screen 2018; 21:704-10. doi: 10.2174/138.620.7322666.190.125150557.

[9] Oh JY, Barrett-Connor E. Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults: The Rancho Bernardo Study. Metabolism 2002; 51:356–9. doi: 10.1053/meta.2002.29969.

[10] Rasool S, Kadla SA, Rasool V, et al. Role of the VDR Bsm1 and Apa1 polymorphisms in the risk of colorectal cancer in Kashmir. Oncol Res Treat 2014; 37:345-9. doi: 10.1159/000362859.

[11] Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW. Vitamin D receptor gene

polymorphisms are associated with breast cancer risk in a UK Caucasian population. Br J Cancer 2001; 85:171-5. doi: 10.1054/bjoc.2001.1864.

[12] Guy M, Lowe LC, Bretherton-Watt D, Mansi JL, Colston KW. Approaches to evaluating the association of vitamin D receptor gene polymorphisms with breast cancer risk. Recent Results Cancer Res 2003; 164:43-54. doi: 10.1007/978-3-642-55580-0_3.

[13] Zhang K, Song L. Association between vitamin D receptor gene polymorphisms and breast cancer risk: a meta-analysis of 39 studies. PLoS One 2014; 9:e96125. doi: 10.1371/journal.pone.0096125.

[14] Luo S, Guo L, Li Y, Wang S. Vitamin D receptor gene ApaI polymorphism and breast cancer susceptibility: a meta-analysis. Tumour Biol 2014; 35:785-90. doi: 10.1007/s13277.013.1107-2.

[15] Tang C, Chen N, Wu M, Yuan H, Du Y. Fok1 polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: a meta-analysis. Breast Cancer Res Treat 2009; 117:391-9. doi: 10.1007/s10549.008.0262-4.

[16] Yang B, Liu S, Yang X, et al. Current evidence on the four polymorphisms of VDR and breast cancer risk in Caucasian women. Meta Gene 2014; 2:41-9. doi: 10.1016/j.mgene.2013.09.008.

[17] Ruza E, Sotillo E, Sierrasesúmaga L, Azcona C, Patiño-García A. Analysis of polymorphisms of the vitamin D receptor, estrogen receptor, and collagen I genes and their relationship with height in children with bone cancer. J Pediatr Hematol Oncol 2003; 25:780-6. doi: 10.1097/00043.426.200310000-00007.

[18] Yilmaz B, Tokuc AG, Koc A, Yesil E. Investigation of vitamin D receptor gene polymorphism in pediatric patients with brain cancer. Indian J Med Paediatr Oncol 2017; 38:128-32. doi: 10.4103/ijmpo.ijmpo_22_16.

[19] Purdue MP, Lan Q, Kricker A, Vajdic CM, Rothman N, Armstrong BK. Vitamin D receptor gene polymorphisms and risk of non-Hodgkin's lymphoma. Haematologica 2007; 92:1145-6. doi: 10.3324/haematol.11053.

[20] Tekgündüz SA, Yeşil Ş, Ören AC, et al. Vitamin D receptor (VDR) polymorphisms in pediatric patients presenting with Hodgkin's lymphoma. J Pediatr Hematol Oncol 2017; 39:e59-e61. doi: 10.1097/MPH.000.000.0000000664.

[21] Bienertová-Vašků J, Drábová K, Zlámal F, et al. Pre-treatment VD levels and VDR receptors as potential predictors of occurrence and overall survival in paediatric patients with solid tumours-a single institution pilot study. Tumour Biol 2016; 37: 9209-19. doi: 10.1007/s13277.016.4820-9.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Demographic and microbiological characteristics of tuberculous lymphadenitis and other extrapulmonary tuberculosis cases

Lutfiye Nilsun ALTUNAL[1], Buket ERTURK SENGEL[2], Ayse Serra OZEL[1], Mehtap AYDIN[1], Ayten KADANALI[3]

[1] Department of Infectious Diseases, Faculty of Medicine, Health Sciences University, Istanbul, Turkey.
[2] Department of Infectious Diseases, Marmara University Pendik Training and Research Hospital, Istanbul, Turkey.
[3] Department of Infectious Diseases, School of Medicine, Biruni University, Istanbul, Turkey.

Corresponding Author: Lutfiye Nilsun ALTUNAL
E-mail: l.nilsun@hotmail.com

Submitted: 23.03.2022 **Accepted:** 02.06.2022

ABSTRACT

Objective: Tuberculosis (TB) is a significant public health problem that remains important worldwide. This study aimed to examine the demographic characteristics and clinical features of patients with extrapulmonary tuberculosis (EPTB) and also identify the comorbidities of EPTB. Our secondary aim was to compare tuberculous lymphadenitis (TBL) with other forms of EPTB.

Patients and Methods: This single-center, retrospective, observational study was conducted on patients diagnosed with EPTB in a training hospital in Turkey between January 1, 2011 and December 31, 2020. The demographic characteristics and clinical features of the patients were examined. TBL cases were compared with other forms of EPTB cases.

Results: The most common clinical form was TBL. Fever, night sweats, and accelerated erythrocyte sedimentation rate were significantly more common in the other forms of the EPTB group compared to TBL. The rate of tuberculin skin test positivity was significantly higher in patients with TBL. The complication rate was 3%, and mortality rate was 4% among all patients.

Conclusions: Extrapulmonary tuberculosis should be considered in the differential diagnosis by evaluating the medical history of patients who present with organ-specific symptoms, especially in TB endemic countries. The clinic may differ according to the organ involvement. TBL may be seen with milder symptoms compared to other clinical forms.

Keywords: Extra pulmonary tuberculosis, Tuberculous lymphadenitis, Mycobacterium tuberculosis

1. INTRODUCTION

Tuberculosis (TB) is a crucial infectious disease that causes mortality and morbidity all around the world, especially in developing countries. According to the World Health Organization (WHO) global report, TB is the 13th cause of death worldwide [1]. In 2020, approximately 10.4 million new TB cases and 1.4 million deaths were reported globally, especially in low and middle-income countries [2]. This rate is equivalent to127 cases per 100.000 people all around the world. TB is an endemic disease in our country. According to the Turkish Ministry of Health, the total number of cases in our country is 14.4 per 100,000 people [3].

Tuberculosis may appear as a multisystemic disease and pulmonary tuberculosis (PTB) is the most common clinical form. Moreover, TB affects other systems with the hematogenous and lymphomatous spread [4,5]. Although the exact cause is unknown, the number of extrapulmonary tuberculosis (EPTB) cases are increasing. It might be due to increased risk factors such as human immunodeficiency virus (HIV) positivity, anti-tumor necrosis factor alfa therapies, foreign-born status [6,7]. Earlier studies have shown a high proportion (20%-53%) of cases presenting with EPTB in all TB cases [8-11]. In our country, the rate of EPTB has been found as 34.3% in all TB patients [3]. The most common form of EPTB is tuberculous lymphadenitis (TBL) in countries with a low TB prevalence, while it is tuberculous pleurisy in countries with a high prevalence [12].

Nonspecific symptoms such as fever, night sweats, and a wide spectra of clinical manifestations are seen in EPTB. These patients usually receive treatments for alternative diagnoses

How to cite this article: Altunal LN, Erturk Sengel B, Ozel AS, Aydin M, Kadanali A. Demographic and microbiological characteristics of tuberculous lymphadenitis and other extrapulmonary tuberculosis cases. Marmara Med J 2022; 3; 35(3):275-280. doi: 10.5472/marumj.1191093

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1191093
Marmara Med J 2022;35(3): 275-280

on their first visit to the primary care setting. The difficulty of sampling specimens and the low rate of microbiological positivity cause delayed diagnosis or misdiagnosis of EPTB patients.

Gold standard diagnostic laboratory tests are microscopic identification of acid-fast mycobacteria from any body fluid by acid-resistant bacilli (ARB) staining or TB polymerase chain reaction (PCR) or mycobacterial culture. Also, tuberculin skin test (TST) demonstrates cellular immunity against mycobacteria and delayed-type hypersensitivity reactions against mycobacterial antigen [12,13].

This study aimed to examine the demographic characteristics and clinical features of patients with EPTB. We also examined the differences between TBL and the other forms of EPTB such as bone/joint, gastrointestinal, genitourinary, meningeal, skin, breast, and eye.

2. PATIENTS and METHODS

Study design

The study was conducted in an 805-bed training and research hospital in Turkey, between January 1, 2011and December 31, 2020. Older than 18 years old patients were included in the study. TBL cases were compared with other forms of EPTB. Demographic and clinical characteristics of the patients (age, gender, and nationality) were collected from the hospital database. The systemic symptoms such as fever, night sweats, loss of weight, and previous TB histories of the patients and their families were recorded.

To confirm the diagnosis of EPTB, appropriate clinical samples (cerebrospinal fluid, urine, peritoneal fluid, etc.) and biopsy materials were examined histopathologically and microbiologically (ARB staining or TB PCR or mycobacterial culture).

The obtained specimens were cultured in solid (Lowenstein-Jensen, Salubris inc, Istanbul Turkey) and liquid (Becton Dickinson / BD BACTEC MGIT 960, Sparks MD, USA) media. Real-time PCR (GenXpert Ultra, Cepheid) was used for quantitative PCR. In histological examination, necrotizing granulomatous lesions were interpreted as tuberculosis specific. Tuberculin skin test ≥10mm was considered as skin test positive.

Patients with a diagnosis of PTB or coexistence of pulmonary with EPTB were excluded.

The ethics committee of the Health Sciences University of Turkey, Research and Training Hospital approved the study on June 17, 2021, with decision number 191.

Statistical Analysis

Analyzes were performed with Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) 21. software statistical programs. Normality control of the tests was done with Kolmogorov Smirnov. Since the distribution of the data was not normal, the values were given as median and 25%-75%. Non-parametric measurement data were compared with the Mann-Whitney U test in independent groups. Demographic characteristics of the patients were evaluated with the chi-square test. $P < 0.05$ was considered significant.

3. RESULTS

Characteristics of the study patients

A total of 110 patients, (76 (69 %) female), were included in this study and the mean age was 42.8±6.3. Seven of the patients were non-Turkish citizens. Sixteen patients (15 %) had a history of previously treated tuberculosis. Fifteen patients (14 %) had a family history of tuberculosis. The most common comorbidity was malignancy (n:6, 6 %), followed with hypertension (n:3, 3 %), diabetes mellitus (n:3, %3), anti-HIV positivity (n:3, 3 %), and chronic renal failure (n:1, 1 %) (Table I).

Of the 110 EPTB, the most commonly seen form was TBL (n:63; 57 %), followed by bone/joint tuberculosis (n: 13; 12 %), gastrointestinal tuberculosis (n:12; 11 %), genitourinary tuberculosis (n:12; 11 %), meningeal tuberculosis (n:7; 6 %), skin tuberculosis (n:1; 1 %), breast tuberculosis (n:1; 1 %), and eye tuberculosis (n:1; 1 %).

While the diagnosis was confirmed histologically in 85 (77%) cases, the number of microbiologically confirmed cases was 63 (57%). Twenty (18%) of microbiologically confirmed cases were culture positive, nine of them (8%) were ARB positive, and 37 (34%) were tuberculosis PCR positive in tissue. TST was positive in 42 cases (38%).

We did not detect any multidrug-resistant isolates. While in the TBL group, only one patient had streptomycin resistance, one patient had streptomycin, and one had pyrazinamide resistance in the other EPTB group.

Among all patients, three patients with tuberculous meningitis and one patient with bone/joint tuberculosis died in the follow-up. Chronic renal failure has been developed in one patient, and infertility has been developed in two patients as a complication of genitourinary tuberculosis. Relapse occurred in a patient with bone/joint tuberculosis. The mortality rate of our study was 4%, and the complication rate was 3% in all patients.

Patients diagnosed with tuberculous lymphadenitis and other extrapulmonary tuberculosis

The TBL and the other EPTB cases were compared. There was no difference in the gender, incidence of malignancy (6 % versus 4 %, p<0.39), diabetes mellitus (0 % versus 3 %, p<0.07), hypertension (3 % versus 0 %, p<0.25), chronic renal failure (0 % versus 1%, p<0.427), previously treated for tuberculosis (16 % versus 13 %, p<0.85), and family history of tuberculosis (14 % versus 13 %; p=1) between both of two groups.

In other forms of EPTB patients, fever (36% versus 8 %, p <0.001), night sweats (36 % versus 13 %, p <0.007), accelerated ESR (49 versus 29, p<0.00) were significantly more common compared to TBL. TST positivity rate was significantly higher in patients with TBL (46 % versus 28 %; p<0.002) (Table I).

http://doi.org/10.5472/marumj.1191093
Marmara Med J 2022;35(3): 275-280

Table I. *Demographic characteristics of all patients*

	Total	Tuberculous Lymphadenitis (n %)	Other extrapulmonary tuberculosis (n %)	p
Gender				
Female	76 (69)	46 (73)	30 (64)	0.302
Male	34 (31)	17 (27)	17 (36)	
Mean age	42.8±6.3	45±17.1	39.9±17.5	
Nationality				
Turkish citizen	103 (94)	61 (97)	42 (89)	0.135
Comorbidities				
Malignancy	6 (6)	4 (6)	2 (4)	0.391
Diabetes mellitus	3 (3)	0 (0)	3 (6)	0.075
Hypertension	3 (3)	3 (5)	0 (0)	0.259
Chronic renal failure	1 (1)	0 (0)	1 (2)	0.427
Anti-HIV				
Positive	3 (3)	1 (2)	2 (4)	0
Complaint				
Fever	22 (20)	5 (8)	17 (36)	0.001
Weight loss	8 (7)	3 (5)	5 (11)	0.283
Night sweats	25 (23)	8 (13)	17 (36)	0.007
Previously treated for tuberculosis	16 (15)	10 (16)	6 (13)	0.854
Family history of tuberculosis	15 (14)	9 (14)	6 (13)	1
Tuberculin skin test positive	42 (38)	29 (46)	13 (28)	0.002
Accelerated ESR	57 (52)	29(15-40)	49(27-63)	0.003
Mycobacterial PCR				
Positive	37 (33.6)	18 (29)	19 (40)	0.664
Pathology				
Tuberculosis specific histology	85 (77)	55 (87)	30 (64)	1
Mycobacterial culture				
Positive	20 (18)	6 (10)	14 (30)	0.228

ESR: Erythrocyte sedimentation rate, PCR: Polymerase Chain Reaction

4. DISCUSSION

Most of the patients in our study were diagnosed with TBL and were female. Fever, night sweats, and accelerated ESR were significantly common in the other forms of the EPTB group compared to TBL. TST positivity rate was significantly higher in patients with TBL.

In the developing countries, TB is an important disease that may affect all tissues and organs. Although, it varies according to the development levels of the countries, the incidence of EPTB is increasing worldwide [2]. The diagnosis of EPTB is more difficult than PTB because of the nonspecific symptoms and clinical findings.

Previous studies have demonstrated the risk factors for TB. These rısk factors included; born in high prevalence countries, poverty, crowded living conditions, undernourishment, low levels of education, use of glucocorticoids, malignancy, smoking, and genetic predisposition [14-18].

Sreeramareddy et al., have found that female patients were more likely to have EPTB in Nepal, a developing country [19]. Also, Djannah et al., have shown that the incidence of EPTB in women is higher than in men in Indonesia [20]. In our study, the female gender is dominant at the rate of 69%. Our study is in line with other studies [21-24]. On the contrary, some articles have shown that being male is a risk factor for EPTB [25-27]. The reason for this gender difference is unclear in different cities and countries. These may be associated with the variability among male-dominant communities where women experience worse living conditions.

Although, there is no consensus on the possible effect of aging, it has been found that younger age may be an independent risk factor for EPTB [19,28]. Other studies have also demonstrated that EPTB is more common in the younger age groups of patients [19,29]. The mean age was 42.8±6.3 in our study and it was similar to other studies from Turkey [28,29]. Older people are more prone to EPTB than the youngers. This result may be associated with the altered immune function of the body of older people, or it could be due to differences in the prevalence of host-related factors.

In previous studies, extrapulmonary involvement was most common in the lymph nodes 12%, 49%, 26,5%, and 40%, respectively [7,21,25,30]. In our study, the most frequent form was TBL (57%), followed by bone/joint (12%), gastrointestinal (11%), and genitourinary TB (11%). Also, some studies have shown that a common form of EPTB is bone/joint TB, genitourinary TB, or meningeal TB [28,31,32]. The difference may be associated with dietary habits, developing levels, and social restrictions between populations [25].

In a large-scale study, diabetes mellitus, hypertension, anti-HIV positivity, and using immunosuppresive drugs have been found associated with different sites of EPTB [21]. Also, Oztop et al., found that the most common accompanying diseases were diabetes mellitus, and hypertension [24]. The immunosuppressive conditions such as anti-HIV positivity, and using immunosuppresive drugs were known to be associated with EPTB, however little is known about the epidemiological or clinical relationship between malignancy and EPTB. In our study, malignancy was the most common comorbid disease (6%); followed by diabetes mellitus, hypertension, anti-HIV positivity (3%), and chronic renal failure (1%) with EPTB.

Since, the organ-specific symptoms are predominant and variable in each case, the constitutive symptoms are less common in EPTB. In a study from Iran, fever, fatigue, and night sweats were the most common complaints of the patients [33]. Bal et al., found that common symptoms in our country were fever, night sweats, and weight loss [30]. With this finding, most TBL (up to 57%) present with no systemic symptoms and manifest with local symptoms like mass lesions [34]. A small number of

http://doi.org/10.5472/marumj.1191093
Marmara Med J 2022;35(3): 275-280

the TBL patients presented with low-grade fever, weight loss, fatigue, and less frequent night sweats [35, 36]. TB bacilli spread via the lymphatics to the lymph nodes initially. Because of the limited disease, patients may not become aware of the illness, and immunity may restrict it. After this period, some bacilli may spread to organs and may be clinically symptomatic with uncontrolled multiplication of TB bacilli [37]. In our study, the major clinical features of all EPTB were night sweats, fever, and the loss of weight. Similar to the other studies, we found that systemic symptoms such as fever and night sweats were less common in the TBL group with statistical significance, respectively (p=0,001; 0,007).

Erythrocyte sedimentation rate is an acute phase reactant widely used in the initial diagnosis of TB. In the study of Mandal et al., in patients with PTB and EPTB, the mean ESR was 67.6 mm/hr. Also, there was no statistically significant difference between the two groups in terms of ESR value [38]. We found that ESR was elevated in 52% of patients, and the median ESR level was significantly higher in the other forms of EPTB than in TBL (49% versus 29%; p=0,003). Moreover, we did not evaluate nutritional status, hemoglobin, albumin levels, or other infectious diseases, which may also raise the ESR.

When TB is suspected, it is important to ask about previous contact, treatment, or family history of patients. In studies conducted in Turkey, TB contact history has been reported between 5.1% and 65.8% [39]. Previous articles showed that 25–65.8% of patients had a history of contact with a case of TB [29,40]. In this study, 15% of all patients have been treated for previous TB and 14% had a family history, and there was no difference between TBL and other forms.

Although, the site of the TST in infection is debated, it is used for diagnostic purposes [34]. Test values may be affected by BCG vaccination, other infections, malnutrition, malignity, immunosuppression, older age, or improper test application [34,38]. Previous studies have shown that the TST positivity rate in TBL is higher than in other forms of EPTB [41,42]. Similarly, in our study, the TST positivity rate was significantly higher in the TBL group (46% versus 28%; p <0.02). However, we did not evaluate the BCG vaccination status, which could affect the test positivity of the patients.

In TB cases, providing appropriate and sufficient tissue materials is essential for diagnosis. Histopathology is an essential part of the diagnosis for all forms of TB. Sinna et al., found that the rate of classified epithelioid granuloma with fine-needle aspiration was 71.3%, and the rate of ARB positivity with smear was 39.8% [43]. Also, Güler et al., established the diagnosis as pathological in 73.8% of the cases, and found the ARB positivity rate was 30.3% [25]. In previous studies, ARB positivity was shown to range from 23% to 45%, and culture positivity from 20.8% to 83% [44]. In the present study, the diagnosis was confirmed in 77% of cases by histopathological examination, 57% with culture, and 34% with ARB positivity, similar to previous studies. Also, we found that histopathological confirmation of tissue material was higher in TBL than in other EPTB (87 % versus 64 %; p=1)

This is the first study that investigated the differences between TBL and other forms of EPTB. In addition, it reflects ten years of data about patients with EPTB. In our study, the most common clinical form was TBL. No significant difference was found between TBL and other EPTB groups in gender, age, comorbid factors, and laboratory tests except for TST and ESR. Symptoms such as fever, and night sweats were more common in the other forms of EPTB. This can be explained by the fact that the other forms of TB are more likely to present with active symptoms with hematogenous spreads to organs. Also, median ESR levels were higher in the other forms of the EPTB, and the TST positivity rate was higher in the TBL group, statistically.

Our research was a single-center observational study in a TB endemic country with a limited number of patients. We did not evaluate nutritional status, hemoglobin, albumin levels, or other infectious diseases which may also raise the ESR, and the BCG vaccination status, which could affect the TST positivity of the patients. We observed that multicenter, randomized controlled studies with large populations are needed to investigate the differences between TBL and other forms of EPT.

In conclusion, EPTB should be considered in the differential diagnosis by evaluating the medical history of patients, especially in TB endemic countries. It is essential to obtain sufficient tissue material for both pathological and microbiological analysis to confirm the diagnosis and early treatment of the patients. The organ involvement may lead to different clinical findings. Nonspecific symptoms in TBL may be seen as milder than in other clinical forms.

Compliance with Ethical Standards

Ethical Approval: The ethics committee of Umraniye Health Sciences University of Turkey, Research and Training Hospital approved the study on June 17, 2021, with decision number 191.

Financial Support: No special funding was obtained.

Conflict of Interest Statement: There is no conflict of interest.

Authors' Contributions: LNA: Concept and design, LNA and ASO: Collected the data, wrote the mnauscript and designed the tables, BES: Analyzed and interpreted the data, BES, MA and AK: Revized the manusctipt critically for intellectual content, All authors approved the final version of the article.

REFERENCES

[1] World Health Organization [WHO]. Cause specific mortality. https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-leading-causes-of-death. Accessed: 10 March 2022.

[2] World Health Organization [WHO]. Global tuberculosis report 2021. https://www.who.int/publications/digital/global-tuberculosis-report-2021. Accessed: 10 March 2022

[3] T.C Ministry of Health. Istatistics of tuberculosis. https://hsgm.saglik.gov.tr/tr/tuberkuloz-istatistikler. Accessed: 10 March 2022

[4] Holden IK, Lillebaek T, Andersen PH, Bjerrum S, Wejse C, Johansen IS. Extrapulmonary tuberculosis in Denmark from 2009 to 2014; Characteristics and predictors for treatment outcome. Open Forum Infect Dis 2019;6:ofz388. doi: 10.1093/ofid/ofz388.

[5] Cukic V, Ustamujic A. Extrapulmonary tuberculosis in Federation of Bosnia and Herzegovina. Mater Sociomed 2018;30:153-6. doi: 10.5455/msm.2018.30.153-156.

[6] Natali D, Cloatre G, Brosset C, et al. What pulmonologists need to know about extrapulmonary tuberculosis. Breathe (Sheff) 2020;16:200216. doi: 10.1183/20734.735.0216-2020.

[7] Peto HM, Pratt RH, Harrington TA, LoBue PA, Armstrong LR. Epidemiology of extrapulmonary tuberculosis in the United States, 1993-2006. Clin Infect Dis 2009;49:1350-7. doi: 10.1086/605559.

[8] Sama JN, Chida N, Polan RM, Nuzzo J, Page K, Shah M. High proportion of extrapulmonary tuberculosis in a low prevalence setting: a retrospective cohort study. Public Health 2016;138:101-7. doi: 10.1016/j.puhe.2016.03.033.

[9] Sandgren A, Hollo V, van der Werf MJ. Extrapulmonary tuberculosis in the European Union and European Economic Area, 2002 to 2011. Euro Surveill 2013;18:20431.

[10] Norbis L, Alagna R, Tortoli E, Codecasa LR, Migliori GB, Cirillo DM. Challenges and perspectives in the diagnosis of extrapulmonary tuberculosis. Expert Rev Anti Infect Ther 2014;12:633-47. doi: 10.1586/14787.210.2014.899900.

[11] World Health Organization (2013). Using the Xpert MTB/RIF assay to detect pulmonary and extrapulmonary tuberculosis and rifampicin resistance in adults and children: expert group meeting report: 2013 (No. WHO/HTM/TB/2013.14).

[12] Deveci HS, Kule M, Kule ZA, Habesoglu TE. Diagnostic challenges in cervical tuberculous lymphadenitis: A review. North Clin Istanb 2016;3:150-5. doi: 10.14744/nci.2016.20982.

[13] Bauer CM, Schmähl A, Kreuter M. Bildgebung und Labordiagnostik bei Tuberkulose [Imaging and Laboratory Diagnostics for Tuberculosis]. Klin Monbl Augenheilkd. 2016;233:587-93. doi: 10.1055/s-0042-104062.

[14] Nhlema S, Kemp J, Steenbergen S, Theobald S, Tang S, Squire S. Asystematic analysis of TB and poverty. Technical Paper Commissioned by the STOP TB Partnership. Geneva: World Health Organization; 2003.

[15] Antunes JL, Waldman EA. The impact of AIDS, immigration and housing overcrowding on tuberculosis deaths in São Paulo, Brazil, 1994-1998. Soc Sci Med 2001;52:1071-80. doi: 10.1016/s0277-9536(00)00214-8.

[16] Wandwalo ER, Mørkve O. Delay in tuberculosis case-finding and treatment in Mwanza, Tanzania. Int J Tuberc Lung Dis 2000;4:133-8.

[17] Targeted tuberculin testing and treatment of latent tuberculosis infection. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. This is a Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). This statement was endorsed by the Council of the Infectious Diseases Society of America. (IDSA), September 1999, and the sections of this statement. Am J Respir Crit Care Med 2000;161(4 Pt 2):S221-47. doi: 10.1164/ajrccm.161.supplement_3.ats600.

[18] Kamboj M, Sepkowitz KA. The risk of tuberculosis in patients with cancer. Clin Infect Dis 2006;42:1592-5. doi: 10.1086/503917.

[19] Sreeramareddy CT, Panduru KV, Verma SC, Joshi HS, Bates MN. Comparison of pulmonary and extrapulmonary tuberculosis in Nepal – a hospital-based retrospective study. BMC Infect Dis 2008;8:8. doi: 10.1186/1471-2334-8-8.

[20] Djannah F, Massi MN, Hatta M, Bukhari A, Hasanah I. Profile and histopathology features of top three cases of Extra Pulmonary Tuberculosis (EPTB) in West Nusa Tenggara: A retrospective cross-sectional study. Ann Med Surg (Lond) 2022;75:103318. doi: 10.1016/j.amsu.2022.103318.

[21] Khan AH, Sulaiman SAS, Laghari M, et al. Treatment outcomes and risk factors of extra-pulmonary tuberculosis in patients with co-morbidities. BMC Infect Dis. 2019;19:691. doi: 10.1186/s12879.019.4312-9.

[22] Forssbohm M, Zwahlen M, Loddenkemper R, Rieder HL. Demographic characteristics of patients with extrapulmonary tuberculosis in Germany. Eur Respir J 2008;31:99-105. doi: 10.1183/09031.936.00020607.

[23] Rieder HL, Snider DE Jr, Cauthen GM. Extrapulmonary tuberculosis in the United States. Am Rev Respir Dis 1990;141:347-51. doi: 10.1164/ajrccm/141.2.347.

[24] Oztop A, Unsal I, Gunay T, et al. The study of evaluation of tuberculous patients who registered in Kahramanlar tuberculous struggle dispensary. Tuberk Toraks 2000;48:243-7. doi: 10.1159/000365511.

[25] Guler SA, Bozkus F, Inci MF, et al. Evaluation of pulmonary and extrapulmonary tuberculosis in immunocompetent adults: a retrospective case series analysis. Med Princ Pract 2015;24:75-9. doi: 10.1159/000365511.

[26] Cailhol J, Decludt B, Che D. Sociodemographic factors that contribute to the development of extrapulmonary tuberculosis were identified. J Clin Epidemiol 2005;58:1066-71. doi: 10.1016/j.jclinepi.2005.02.023.

[27] Kang W, Yu J, Du J, et al. The epidemiology of extrapulmonary tuberculosis in China: A large-scale multi-center observational study. PLoS One 2020;15:e0237753. doi: 10.1371/journal.pone.0237753.

[28] Gunal S, Yang Z, Agarwal M, Koroglu M, Arıcı ZK, Durmaz R. Demographic and microbial characteristics of extrapulmonary tuberculosis cases diagnosed in Malatya, Turkey, 2001-2007. BMC Public Health 2011;11:154. doi: 10.1186/1471-2458-11-154.

[29] Sevgi DY, Derin O, Alpay AS, et al. Extrapulmonary tuberculosis: 7 year-experience of a tertiary center in Istanbul. Eur J Intern Med 2013;24:864-7. doi: 10.1016/j.ejim.2013.08.704.

[30] Bal İA, Tartar AS, Akbulut, A, Demirdağ K. Mediterr J Infect Microb Antimicrob 2021;10: 58.

[31] Yang Z, Kong Y, Wilson F, et al. Identification of risk factors for extrapulmonary tuberculosis. Clin Infect Dis 2004;38:199-205. doi: 10.1086/380644.

[32] Lin JN, Lai CH, Chen YH, et al. Risk factors for extra-pulmonary tuberculosis compared to pulmonary tuberculosis. Int J Tuberc Lung Dis 2009;13:620-5.

[33] Shirzad-Aski H, Hamidi N, Sohrabi A, Abbasi A, Golsha R, Movahedi J. Incidence, risk factors and clinical characteristics of extra-pulmonary tuberculosis patients: a ten-year study in the North of Iran. Trop Med Int Health. 2020;25:1131-9. doi: 10.1111/tmi.13452.

[34] Mohapatra PR, Janmeja AK. Tuberculous lymphadenitis. J Assoc Physicians India 2009;57:585-90.

[35] Lee KC, Tami TA, Lalwani AK, Schecter G. Contemporary management of cervical tuberculosis. Laryngoscope. 1992;102:60-4. doi: 10.1288/00005.537.199201000-00012.

[36] Levin-Epstein AA, Lucente FE. Scrofula—the dangerous masquerader. Laryngoscope 1982;92(8 Pt 1):938-43.

[37] Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am J Respir Crit Care Med 2000;161(4 Pt 1):1376-95. doi: 10.1164/ajrccm.161.4.16141.

[38] Mandal SK, Laxmikant C. Erythrocyte sedimentation rate values in cases of active tuberculosis without HIV co-infection. J Med Sci Clin Res 2016;4:13156-9. doi: 10.18535/jmscr/v4i10.58.

[39] T.C. Ministry of Health, General Directorate of Public Health, Department of Tuberculosis. Distribution of Total Tuberculosis Cases by Country of Birth, 2005-2018. https://hsgm.saglik.gov.tr/depo/birimler/tuberkuloz_db/dosya/Istatistikler/Yeni/Tuberkuloz_Olgularinin_Dogdugu_Ulkeye_Gore_Dagilimi.pdf. Accessed: 19 April 2019

[40] Ilgazli A, Boyaci H, Basyigit I, Yildiz F. Extrapulmonary tuberculosis: clinical and epidemiologic spectrum of 636 cases. Arch Med Res 2004;35:435-41. doi: 10.1016/j.arcmed.2004.05.008.

[41] Sirin Y, Coskunol I. Yuzyetmisuc ekstrapulmoner tuberkuloz olgusu. Tuberkuloz ve Toraks 2002;50:272-7.

[42] Olson NR. Nontuberculous mycobacterial infections of the face and neck—practical considerations. Laryngoscope 1981;91:1714-26. doi: 10.1288/00005.537.198110000-00018.

[43] Sinha SK, Chatterjee M, Bhattacharya S, et al. Diagnostic evaluation of extrapulmonary tuberculosis by fine needle aspiraton (FNA) supplemented with AFB smear and culture. J Indian Med Assoc 2003;101:588, 90-1.

[44] Das DK. Fine-needle aspiration cytology in the diagnosis of tuberculous lesions. Lab Med 2000;31:625-32.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Partial healing effects of St. John's wort oil on the rat excisional wound model

Ayse Nilhan ATSU[2], Tayfun BILGIC[3], Cavit Kerem KAYHAN[4], Zumrut Mine ISIK SAGLAM[5], Nazli CAF[1]

[1] Vocational School of Health Services, Istanbul Kent University, Istanbul, Turkey.
[2] Vocational School, Nisantasi University, Istanbul Turkey.
[3] Department of Pathology, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey.
[4] Medical Biochemistry, Istanbul Education and Research Hospital, Istanbul, Turkey.
[5] Dermatology Clinic, Basaksehir Cam and Sakura City Hospital, Istanbul, Turkey.

Corresponding Author: Nazli CAF
E-mail: naslicaf@hotmail.com

Submitted: 23.02.2022 **Accepted:** 15.07.2022

ABSTRACT

Objective: St. John's wort (SJW) oil (Hypericum perforatum) has been used for its immunomodulatory and anti-inflammatory effects. Several studies have shown the efficacy of SJW on wound healing. The aim of this study is to assess the effectiveness of SJW using a combination of biochemical, histopathological and laser Doppler evaluations.

Materials and Methods: Sixteen young Wistar albino rats were used as case and control groups (having 8 in each group). After anesthesia protocol, 6 mm punch biopsy was taken from six separate sites on the rats' dorsal skin. Three wounds were stitched (closed wounds); three wounds were left as they were (open wounds). SJW oil was administered topically to case group once a day for 14 days. Controls did not receive any treatment.

Results: There was no statistical difference in blood perfusion between the groups. No statistical difference was present between the groups in GPx (glutathione peroxidase)values. Rat MDA (malonyldialdehyde) values were higher in the case group compared to the control group. SJW oil was found to be beneficial and effective within some histological parameters.

Conclusion: SJW may be an effective salve within some parameters. Nevertheless, this judgment is uncertain due to the low sample size. We encourage further studies on this promising natural medicine.

Keywords: Antiinflammatory, fibroblast, glutathione peroxidase, St. John's wort, wound

1. INTRODUCTION

St. John's wort (SJW), also known as *Hypericum perforatum (H. perforatum),* has been used for immunomodulatory, anti-inflammatory and antiseptic purposes for centuries. Its sedative and antidepressive effects are also known in psychiatric science [1-3].

Wound healing begins from the moment the wound is formed and consists of three main phases: inflammation, proliferation, and maturation [4]. After a wound is formed, the first reaction of the body is vasoconstriction and activation of platelets. Then various inflammatory cells such as neutrophils, mast cells and T cells flow to the injured area. Angiogenesis occurs when the inflammatory phase ends. Endothelial proliferation, migration, and branching occur for the formation of new vessels in angiogenesis. As new blood vessels form, fibroblasts proliferate and attack the clot to form granulation tissue that can contract. Some fibroblasts turn into myofibroblasts, allowing the wound edges to converge. Dividing fibroblasts store an extracellular matrix and transform the microenvironment of the wound from an inflamed state to a growth phase[5].

There are several studies showing the activity of SJW on wound healing. The active ingredient of SJW, *H. perforatum*, contains many active ingredients such as biapigenin, hypericin, flavonoids, and hyperforin. *H. perforatum* exerts its anti-inflammatory effect mainly with hyperforins and pseudohypericin compounds. This compound's anti-inflammatory effect triggers the migration of fibroblasts and collagen storage and shortens healing time[6].

Oxidant substances act by destroying the DNA, lipids and membranes of cells. When looking specifically at wound healing, DNA damage affects a wound's proliferation phase by causing collagen degradation. Antioxidants protect cells and tissues against the harmful effects of reactive oxygen species (ROS). Superoxide dismutase and glutathione peroxidase are

How to cite this article: Atsu AN, Bilgic T, Kayhan CK, Isık Saglam A, Caf N. Partial healing effects of St. John's wort oil on the rat excisional wound model. Marmara Med J 2022; 35(3): 281-287, doi: 10.5472/marumj.1186858

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1186858
Marmara Med J 2022;35(3): 281-287

aid wound healing because these antioxidants play an important role in enzymatically inactivating ROS[7].

St. John's wort is widely used, but its clinical benefit has not been demonstrated in appropriate conditions and in such a way as to evaluate several parameters simultaneously. This study was conducted to study the curative effectiveness of SJW on experimental animals by examining macro and micro factors within specific healing parameters.

2. MATERIALS and METHODS

2.1. Study Design and Ethical Approval

This animal experiment protocol was approved by the Local Ethics Committee for Animal Experimentation, University of Health Sciences, Istanbul Mehmet Akif Ersoy Animal Experiments Production and Experimental Research Center (Approval number: 2019/11 on 17.05.2019)

Sixteen female young Wistar albino rats, weights ranging from 220-250 grams, were used in this study. The rats were housed in separate sterile cages under standard conditions with a temperature of 26 ± 2 °C and a 12 hour day-night cycle. Standard rodent food and ad libitum water were provided.

The rats were randomly divided equally into two groups: Group A (St. John's wort treatment group) and Group B (control group). The dorsal skin of the rats in both Group A and B were shaved and cleaned with 70% isopropyl alcohol before being wounded with a 6 mm punch biopsy instrument at six separate sites 1 cm apart from the midline and from each other. Intraperitoneal anesthesia was administered with 7 mg/kg xylazine and 35 mg/kg ketamine before surgical wound formation. The wounds of each rat were numbered from 1 to 6. Wounds numbered 1, 3 and 5 were left as they were (non-stitched=open wounds); and wounds number 2, 4 and 6 were stitched with 4/0 vicryl sutures and defined as closed wounds (sutured wounds).

St. John's wort oil was administered topically to Group A, once a day for 14 days of treatment. The administration of the oil started right after surgical wound formation. *St. John's extract oil was provided by Zadevital ®, Konya, Turkey*. The animals in Group B did not receive any treatment.

Rats in both groups were inspected during the whole process for any possible infections, toxicity or side effects.

Punch biopsy was obtained from both open and closed wounds for histopathologic examination. These samples were evaluated to determine polymorphonuclear nuclei leukocytes (PMN) infiltration, angiogenesis, collagen and reepithelization on the 7th, 14th, and 21st days of the experiment. Another punch biopsy was taken from a separate wound for the evaluation of superoxide dismutase, glutathione peroxidase, malonyldialdehyde (SOD, GPx, and MDA) by using ELISA on the 14th day. In addition, both the open and sutured wounds of Group A and Group B were evaluated by PeriScan PIM 3 System Laser Doppler Blood Perfusion Imager (Perimed. Järfälla, Sweden) in order to calculate perfusion on the 0th, 7th, 14th and 21st days. The rats were photographed on the 0th, 7th, 14th and 21st days.

2.2. Evaluation of the Wounds

The PeriScan PIM 3 system was used to evaluate the wounds with laser Doppler. This system measures blood flow at the microcirculation level without touching the tissue, then transfers the data to computer software. Two-dimensional mapping can thus be done easily. In this study, blood flow was evaluated in healing tissue using the PeriScan PIM 3 system.

Antioxidant capacity was evaluated with superoxide dismutase (SOD) and glutathione peroxidase (GPX), while malondialchehyche (MDA) was used as an oxidative marker. Tissue samples obtained by punch biopsy were used for ELISA method evaluation of these items on day 14. Standard OD (optical density) and concentration values (ng / ml) were evaluated by an optic reader at 450 nm. Sunred Biotechnology (Shanghai, China) provided Ready-ELISA kits. (Catalogue numbers of MDA, GPX and SOD respectively: 201-11-0157/ 201-11 – 1705/ SOD: 201-11-0169).

Neovascularization, inflammation, fibroblast activation levels and granulation of both groups were evaluated histopathologically by a pathologist blinded for this study. The pathologist applied a specific scale for histopathological grading in this experiment. After fixing the tissues in paraffin, 4 µm tissue pieces with a thickness of 4 meters were cut. The histological evaluation was performed under a light microscope. All prepared slides were scanned using a digital pathology system (3D Histech company, P250 – Flash III Digital Scanner, 20x), and microscopic photos were taken using special software (3D Histech company, CaseViewer program, tiff format and 300 dpi). Microscopic areas involving neovascularization, inflammation, granulation and fibroblast regeneration were calculated with this software. Ratios of the above-mentioned parameters were proportioned over the entire tissues. According to this software, the proportioned structures were divided into 5 subgroups. Level 1: 0-20%, Level 2: 21-40%, Level 3: 41-60%, Level 4: 61-80%, Level 5: 81-100% of the total tissue.

Statistical Analysis

SPSS 22.0 Statistical package program was used for statistical analysis. Certain comparisons were made to examine the effects of St. John's wort oil of control and treatment groups on subjects. The conformity of the values of the subjects to the normal distribution was tested by the Shapiro Wilk method. Parametric tests (independent t-test) were applied on values that are suitable for normal distribution. Nonparametric tests (Mann-Whitney U test) were used to assess values that were not suitable for normal distribution. In addition, Chi-Square analysis was made for comparison of categorical data. Comparison results in 95%; it was evaluated at a significance level of $p < 0.05$.

3. RESULTS

PeriScan PIM 3 System Laser Doppler Blood Perfusion Imager was used to calculate perfusion of the wounded rats.

Open Wounds

There was no statistically significant difference between Group A (St. John's wort, 66.71 ± 10.80) and Group B (control, 61.83 ± 4.60) on day 0 in subjects with open wounds (p> 0.05). On the 7th day, there was no statistically significant difference between the A and B groups (p> 0.05, 79.17 ± 11.18 and 74.68 + 10.98, respectively). There was no statistically significant difference in groups A and B on day 14 (p> 0.05, 77.76 ± 18.07 and 80.95 + 20.11, respectively). On day 21, no statistically significant difference was found between the control (78.91 ± 7.63) and St. John's Wort (69.00 ± 17.29) group types (p> 0.05).

Closed Wounds

On day 0, there was no statistically significant difference between A (76.88 ± 14.80) and B (75.99 ± 14.80) groups (p> 0.05). There was no statistically significant difference between the A (78.43 ± 8.63) and B (81.35 ± 9.97) groups in the measurement values on the 7th day (p> 0.05). There was no statistically significant difference between A (78.03 ± 16.22) and B (75.17 ± 13.61) groups on day 14 (p> 0.05). On the 21st day, there was no statistically significant difference between the groups (A: 63.52 ± 9.74, B: 69.64 ± 8.57) (p> 0.05).

RAT GPX

Open wounds

There was no statistically significant difference in the standard OD values between Group A (0.38 ± 0.16) and Group B (0.52 ± 0.16) (p> 0.05). In addition, the concentration (ng/ml) values did not differ statistically between Group A (5.27 ± 2.48) and Group B (21.78 ± 8.54) (p> .05).

Closed wounds

For the standard OD values, there was no statistically significant difference between Group A (0.40 ± 4.69) and Group B (0.40 ± 0.097) (p> 0.05). In addition, the concentration (ng/ml) values were not statistically different between Group B (15.66 ± 4.69) and Group A (5.27 ± 2.48) (p> 0.05).

RAT MDA

Open wounds

There was no statistically significant difference between Group A (0.27 ± 0.11) and Group B (0.24 ± 0.11) in terms of standard OD values (p> 0.05). Concentration (ng/ml) values were also not statistically different between Group A (5.27 ± 2.48) and Group B (4.82 ± 2.53) (p> 0.05).

Closed wounds

A statistically significant difference was found between Group A (0.36 ± 0.053) and Group B (0.15 ± 0.029) in the standard OD values (p = 0.002; p <0.05). In this case, it was found that the MDA values were higher in subjects belonging to Group A. There was also a statistically significant difference in the concentration (ng / ml) values between Group A (7.35 ± 1.66) and B (2.84 ± 0.35) (p = 0.002; p <0.05). MDA values were higher in the Group A.

Table 1. *PeriScan PIM 3 System Laser Doppler Blood Perfusion Imager-Evaluation of Blood Perfusion*

Open	**Group type**			
	B (n=3 wounds)	**A (n=3)**	**t**	**p**
Day 0	61.83±4.60	66.71±10.80	-0.721	0.511
Day 7	74.68±10.98	79.17±11.18	-0.496	0.646
Day 14	80.95±20.11	77.76±18.07	0.204	0.848
Open	**Group type**			
	B (n=3)	**A (n=3)**	**U**	**p**
Day 21	78.91±7.63	69.00±17.29	2.000	0.400
Closed	**Group type**			
	B(n=3	**A (n=3)**	**t**	**p**
Day 0	75.99±14.80	76.88±14.80	-0.076	0.943
Day 7	81.35±9.97	78.43±8.63	0.383	0.721
Day 14	75.17±13.61	78.03±16.22	-0.234	0.826
Closed	**Group type**			
	B (n=3)	**A (n=3)**	**U**	**p**
Day 21	69.64±8.57	63.52±9.74	2.000	0.400

p<0.05

Table 2. *Determination of SOD, GPX, and MDA by ELISA method*

Rat_GPX	**Group Type**			
Open	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.52±0.16	0.38±0.16	2.013	0.090
Concentration(ng/ml)	21.78±8.54	14.29±2,56	2.057	0.086
Closed	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.40±0.097	0.40±4.69	0.156	0.879
Concentration (ng/ml)	15.66±4.69	15.19±2.76	0.210	0.838
Rat_MDA	**Group Type**			
Open	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.24±0.11	0.27±0.11	-0.360	0.726
Concentration (ng/ml)	4.82±2.53	5.27±2.48	-0.311	0.763
Closed	**B (n=6)**	**A (n=6)**	**U**	**p**
Standard OD	0.15±0.029	0.36±0.053	0.000	0.002*
Concentration (ng/ml)	2.84±0.35	7.35±1.66	0.000	0.002*
Rat_SOD	**Group Type**			
Open	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.49±0.051	0.31±0.068	5.083	0.000*
Concentration (ng/ml)	8.54±0.879	5.69±1.04	5.109	0.000*
Closed	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.64±0.21	0.26±0.037	4.133	0.008*
Concentration (ng/ml)	11.41±4.10	4.97±0.54	3.811	0.012*

**p<0.05*

http://doi.org/10.5472/marumj.1186858
Marmara Med J 2022;35(3): 281-287

RAT SOD

Open wounds

A statistically significant difference was found between Group A (0.31 ± 0.068) and Group B (0.49 ± 0.051) in the standard OD values (p = 0.000; p <0.05). There was also a statistically significant difference in the concentration (ng / ml) values between Group A (5.69± 1.04) and Group B (8.54 ± 0.879) (p = 0.000; p <0.05). SJW has not been found to be effective within this parameter.

Closed wounds

There was a statistically significant difference between Group A (0.26 ± 0.037) and Group B (0.64 ± 0.21) in terms of the standard OD values (p = 0.008; p <0.05). There was also a statistically significant difference between Group A (4.97 ± 0.54) and Group B (11.41 ± 4.10) groups for concentration (ng/ml) values (p = 0.012; p <0.05). SJW has not been found to be effective within this parameter.

Histopathogical results

Open wounds

There was no statistically significant difference between the inflammation and granulation levels on the 7th day in open wounds depending on group type (p> 0.05).

According to day 14 values, a statistically significant difference was found in inflammation levels depending on group type (p = 0.000; p <0.05). When this difference was examined, the first level inflammation rate was higher in the control group than the first level inflammation in Group A. In Group A, 2nd and 3rd level inflammation rates were higher than in Group B. There was also a relationship between neovascularization levels and group type. According to these values, there was a statistically significant difference in neovascularization levels depending on group type (p = 0.001; p <0.05). While 1st level neovascularization was high in Group B, 2nd level neovascularization was dominant in Group A. In addition, there was a statistically significant difference in fibroblast levels depending on group type (p = 0.000; p <0.05). While 1st and 3rd levels fibroblasts were higher in Group B, 2nd level fibroblasts were higher in Group A.

According to day 21 values, it was observed that there was a statistically significant relationship between inflammation levels and group type (p = 0.000; p <0.05). The first-level inflammation rate in the control group was higher than in Group A, whereas 2nd level inflammation was higher in Group A. There was no statistically significant relationship between neovascularization and granulation levels and group type (p> 0.05). There was a statistically significant difference between fibroblast levels depending on group type (p = 0.000; p <0.05). The ratio of first – and second-level fibroblasts in Group Bwas higher than in Group A. In Group A, the rate of third-level fibroblasts was higher than Group B.

Table 3. Histopathological Evaluation of the Open Wounds

Day	Open	Group type				
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 7	Inflammation	2	0(0%)	1(12.5%)	1.067	0.302
		3	8(100%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Granulation	2	0(0%)	1(12.5%)	1.067	0.302
		3	8(100%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 14	Inflammation	1	8(100%)	0(0(0%)	16.000	0.000*
		2	0(0%)	7(87.5%)		
		3	0(0%)	1(12.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Neovascularization	1	7	0(0%)	12.444	0.001*
		2	1(12.5%)	8(100%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Fibroblast	1	1(12.5%)	0(0%)	16.000	0.000*
		2	7(87.5%)	0(0%)		
		3	0(0%)	8(100%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 21	Inflammation	1	8(100%)	1(12.5%)	12.444	0.000*
		2	0(0%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Neovascularization	1	1(12.5%)	0(0%)	1.067	0.302
		2	7(87.5%)	8(100%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Granulation	1	1(12.5%)	0(0%)	1.874	1.000
		2	7(87.5%)	7(87.5%)		
		3	0(0%)	1(12.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Fibroblast	1	1(12.5%)	0(0%)	16.000	0.000*
		2	7(87.5%)	0(0%)		
		3	0(0%)	8(100%)		

*p<0.05

Closed wounds

There was no statistically significant relationship between neovascularization and granulation levels and group type according to 7th-day values (p> 0.05).

When values were examined again on the 14th day, a statistically significant difference was found between neovascularization levels depending on group type (p = 0.000; p <0.05). The 1st level neovascularization rate in Group A was higher than in the control group, whereas 2nd level neovascularization was higher in the control group. There is no statistically significant

http://doi.org/10.5472/marumj.1186858
Marmara Med J 2022;35(3): 281-287

difference between the granulation and fibroblast levels on day 14 depending on the group type (p> 0.05).

According to day 21 values, there is a statistically significant difference between inflammation levels depending on group type (p = 0.000; p <0.05). The 1st level inflammation rate was higher in Group A compared to the control group. However, the 2nd level inflammation rate was higher in the control group. There is a statistically significant difference between neovascularization levels depending on group type (p = 0.001; p <0.05). The rate of first-level neovascularization in Group A was higher compared to the control group. In the control group, the rate of second-level neovascularization was higher compared to Group A. There was also a significant relationship between granulation levels depending on group type on day 21 (= 0.000; p <0.05). While the 1st level granulation rate was higher in the control group, 2nd level granulation was found to be higher in Group A. There was no statistically significant difference between fibroblast levels depending on group type (p> 0.05).

Table 4. *Histopathological Evaluation of the Closed Wounds*

Day	Closed	Group type				
		Level	**B(n=8)**	**A (n=8)**	Chi square	p
Day 7	Neovascularization	2	0(0%)	1(12.5%)	1.067	0.302
		3	8(100%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Granulation	2	0(0%)	1(12.5%)	1.067	0.302
		3	8(100%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 14	Neovascularization	1	0(0%)	8(100%)	16.000	0.000*
		2	8(100%)	0(0%)		
		Level	**B (n=8)**	**A(n=8)**	Chi square	p
	Granulation	1	0(0%)	2(25%)	2.286	0.467
		2	8(100%)	6(75%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Fibroblast	2	1(12.5%)	1(12.5%)	0.000	1.000
		3	7(87.5%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 21	Inflammation	1	0(0%)	8(100%)	16.000	0.000*
		2	8(100%)	0(0%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Neovascularization	1	1(12.5%)	8(100%)	12.444	0.001*
		2	7(87.5%)	0(0%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Granulation	1	8(100%)	0(0%)	16.769	0.000*
		2	0(0%)	7(87.5%)		
		3	0(0%)	1(12.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Fibroblast	2	1(12.5%)	1(12.5%)	0.000	1.000
		3	7(87.5%)	7(87.5%)		

**p<0.05*

No adverse effects, toxic effects, or infections were observed in rats in either the control or treatment groups.

4. DISCUSSION

A wound is formed by a physical or infective process in the anatomical and physiological structure of the skin, which causes a set of inflammatory events. Wound healing and the effect of St John's wort oil as a salve have been much discussed in the literature. However, no study was found in which several parameters were co-examined. Our experiment differs from previous studies by showing that SJW may not be effective in every phase and parameter of wound healing. Unlike findings presented in the literature, we did not detect any improvement

http://doi.org/10.5472/marumj.1186858
Marmara Med J 2022;35(3): 281-287

in GPx, SOD, and MDA values in our study. However, we did find that St. John's wort oil may have a positive effect on the neovascularization healing phase. This substance may be effective in the epithelialization and contraction phases as well, but larger series may be required to verify our hypothesis.

Inflammation is the result of complex events. Even a simple inflammatory change is the result of various, interrelated biochemical events and cellular activities. Each successive stage of inflammation depends on the functioning of regulatory and inhibitory systems that prevent excessive response to the stimulus that initiated the event. During development of the event, active cells, pharmacological substances, cell surface adhesion molecules, and receptors all change. It should be considered that the cells or mediators involved in inflammation are the results of dynamic, interlinked changes[8,9].

During wound healing, many growth factors, cytokines, enzymes, and other various molecules are secreted from local tissues to promote recuperation. Fibroblast growth factor 2 (FGF-2) and vascular endothelial growth factor (VEGF) are necessary for stimulating angiogenesis and forming granulation tissue[3,9]. Disruption of the balance between free oxygen radicals and antioxidants fighting against them has been associated with tissue damage, oxidative stress, and retardation of wound healing[10].

The effective use of St. John's wort oil may be due to its stimulating effect on keratinocyte differentiation, collagen production, and fibroblast motility, as well as its anti-inflammatory and antimicrobial properties. Other compounds including hyperforin, hypericin, and their derivatives like SJW have also been found to be useful in the treatment of skin abrasions, ulcers and burns[11]. Therefore, our research used a rat model to test this hypothesis for the first time by evaluating the effect of St John's wort extract on wound healing, inflammation, free oxygen radicals, and perfusion.

Antioxidant enzymes effective in preventing or repairing the damage of free oxygen radicals include catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) [12]. In many studies examining the effect of St. John's wort extract on the release of pro-inflammatory cytokines, it was observed that IL-6 synthesis decreased with inhibition of the release of substance P[13,14]. The antioxidant effect of St John's wort extract has also been demonstrated in many in vivo and in vitro experiments. This extract protects many cell types against extensive free oxygen radical injury[15-17]. It is reported that the antioxidant effect acts by directly reducing ROS due to the flavonoids contained in St John's wort extract[16], an outcome made possible by increasing the gene expression of the main antioxidant enzymes[18]. Thus, it is reasonable to evaluate antioxidant enzymes such as CAT, SOD, and GSH in granulation tissue[10].

In our analysis, GPx, SOD, and MDA levels were measured on the 14th day. These levels were not found to be in keeping with expected values. One of the reasons may be that St. John's wort oil was used directly without a carrier such as a liposome. A second reason may be related to whether SJW is effective. Although studies prove the positive curative effect of SJW, our findings in antioxidant parameters are not correlated in this study design. However, in an excisional mouse wound model made by Han et al., GPx and SOD levels measured on the 7th and 14th days were found to be higher in the hypericum perforatum group compared to the control group. Also, MDA levels were found to be higher in the control group[19]. Large-scale studies are required to elucidate conflicting data.

In both acute and chronic wounds, the activity of enzymatic antioxidants such as superoxide dismutase, calatase and glutathione peroxidase decreases, since excessive amounts of antioxidants are released when high oxidative stress occurs. In addition, this high oxidative stress load causes a decrease in non-enzymatic antioxidants such as glutathione and vitamins C and E[20]. It has been reported that the phytochemicals contained in SJW extract include substances such as vitamin C, flavonoids (such as quercetin and kaempferol), and carotene [2]. Furthermore, it can be said that these substances may have been effective in increased epithelization and wound contraction on the 14th day in the open wound group. However, this must be confirmed by further research.

This study could not demonstrate the effects of St John's wort total extract formula on the healing of epithelial wounds and wound closure. For example, wound perfusion was not statistically different between the control and SJW group. However, histopathologically, 14th day neovascularization levels were higher in the treatment group (open wounds). The incompatibility between the two parameters may be due to the periscan laser system being user-dependent. In addition, although the fact that it causes improvement within some of the parameters mentioned above cannot be denied, St. John's wort oil still needs further investigation. We are optimistic this herbal extract, which is an ethnomedical drug, is likely to reveal new wound treatment options in studies designed to test its healing properties on humans.

Acknowledgments:

None

Funding:

This work was funded by Nişantaşı University Scientific Research Projects Coordination Unit (Support ID: NIISHBAP 00012).

Author Contributions:

TB: Study construction, data collection and interpretation, manuscript writing; NC: Data interpretation, literature research, manuscript writing; ZMIS: Data collection and interpretation, laboratory analysis; CKK: Laboratory analysis and data collection; ANA: Manuscript writing, statistical analysis, critical analysis and supervision.

REFERENCES

[1] Belwal T, Devkota HP, Singh MK, et al. St. John's Wort (Hypericum Perforatum) in Nonvitamin and Nonmineral Nutrirional Supplements. Nabavi SM, Silva AS. Elsevier Inc.; 2019. Chapter 3.40. https://doi.org/10.1016/C2016-0-03546-5

[2] Klemow KM, Bartlow A, Crawford J, Kocher N, Shah J, Ritsick M. Medical Attributes of St. John's Wort (Hypericum perforatum). In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 11. PMID: 22593920

[3] Barrientos S, Brem H, Stojadinovic O, et al. Clinical application of growth factors and cytokines in wound healing. Wound Repair Regen 2014;22:5:569-78. doi:10.1111/wrr.12205

[4] Topal Hançer A, Yılmaz Pınar. Editöre mektup: Balın Yara İyileşmesi Üzerine Etkisinin Değerlendirilmesi. Bozok Tıp Derg 2019;9:1:152-9. doi:10.16919/bozoktip.399768

[5] Rodrigues M, Kosaric N, Bonham CA, et al. Wound healing: A cellular perspective. Physiol Rev 2019;99:1:665-706. doi:10.1152/physrev.00067.2017

[6] Gunpinar S, Kilic OA, Duran I, et al. Tosun M, Firat T, Soyler G. Evaluation of the Effect of Topical Hypericum perforatum Oil on Excisional Palatal Wound Healing in Rabbits. J Investig Surg 2020;33:1:49-58. doi:10.1080/08941.939.2018.1474980

[7] Abd Jalil MA, Kasmuri AR, Hadi H. Stingless bee honey, the natural wound healer: A review. Skin Pharmacol Physiol 2017;30:2:66-75. doi:10.1159/000458416

[8] Trautinger F, Schwarz T, Luger T. Cytokines in dermatotherapy. J Dermatolog Treat 1992;3:3:143-152. doi:10.3109/095.466.39209088710

[9] Morelli T, Neiva R, Nevins ML, et al. Angiogenic biomarkers and healing of living cellular constructs J Dent Res. 2011;90:4:456-62. doi:10.1177/002.203.4510389334

[10] Murthy S, Gautam MK, Goel S, et al. Evaluation of in vivo wound healing activity of Bacopa monniera on different wound model in rats. Biomed Res Int 2013;2013. doi:10.1155/2013/972028

[11] Yücel A, Kan Y, Yesilada E, Akın O. Effect of St.John's wort (Hypericum perforatum) oily extract for the care and treatment of pressure sores; a case report. J Ethnopharmacol 2017;196:236-41. doi:10.1016/j.jep.2016.12.030

[12] Grundmann O, Lv Y, Kelber O, et al. Mechanism of St. John's wort extract (STW3-VI) during chronic restraint stress is mediated by the interrelationship of the immune, oxidative defense, and neuroendocrine system. Neuropharmacology 2010;58:4-5:767-73. doi:10.1016/j.neuropharm.2009.12.014

[13] Fiebich BL, Höllig A, Lieb K. Inhibition of substance P-induced cytokine synthesis by St. John's wort extracts. Pharmacopsychiatry 2001;34: 1:26-8. doi:10.1055/s-2001-15462

[14] DeRose V, Robbins R, Snider R, et al. Substance P increases neutrophil adhesion to bronchial epithelial cells. J Immunol 1994;152:3:1339–46

[15] Lu YH, Du C Bin, Liu JW, et al. Neuroprotective effects of Hypericum perforatum on trauma induced by hydrogen peroxide in PC12 cells. Am J Chin Med 2004;32:3:397-405. doi:10.1142/S0192415X04002053

[16] Silva B, Oliveira PJ, Dias A, et al. Quercetin, kaempferol and biapigenin from hypericum perforatum are neuroprotective against excitotoxic insults. Neurotox Res 2008;13:3-4:265-79. doi:10.1007/BF03033510

[17] Zou Y, Lu Y, Wei D. Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. in vitro. J Agric Food Chem 2004;52:16:5032-39. doi:10.1021/jf049571r

[18] Sánchez-Reus MI, Gómez del Rio MA, Iglesias I, et al. Standardized Hypericum perforatum reduces oxidative stress and increases gene expression of antioxidant enzymes on rotenone-exposed rats. Neuropharmacology 2007;52:2:606-16.doi:10.1016/j.neuropharm.2006.09.003

[19] Han MC, Durmuş AS, Sağliyan A, et al. Effects of Nigella sativa and Hypericum perforatum on wound healing. Turkish J Vet Anim Sci 2017;41:1:99-105. doi:10.3906/vet-1603-26

[20] Bilgen F, Ural A, Kurutas EB, Bekerecioglu M. The effect of oxidative stress and Raftlin levels on wound healing. Int Wound J 2019;16:5:1178-84. doi:10.1111/iwj.13177

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Prevalence of cervical high-risk human papillomavirus and cytological abnormalities in elderly Turkish women

Cem YALCINKAYA

Gynecologic Oncology Clinic, Umraniye Training and Research Hospital, Istanbul, Turkey.

Corresponding Author: Cem YALCINKAYA
E-mail: yalcinkayacem@gmail.com

Submitted: 23.04.2022 **Accepted:** 17.08.2022

ABSTRACT

Objective: Cervical cancer (CC) screening ends at the age of 65 in Turkey, as in many countries. However, about a quarter of CCs occurs in women over the age of 65. Persistent high-risk human papillomavirus(HR-HPV) infection is the most important factor leading to CC. In this study, we aimed to investigate the prevalence of cervical HR-HPV and cytological abnormalities in Turkish women aged 65 and over.

Patients and Methods: A total of 467 women between the ages of 66-84 who were referred to the gynecology outpatient clinics for any reason between January 2010 and December 2021 were included in the study. Pap smears (liquid-based) of patients were screened for HPV DNA typing using the COBAS test system and evaluated cytopathologicaly.

Results: The mean age of women was 72.4 years. The prevalence of HR – HPV was 5.1% (n = 24), HPV-16 and HPV-18 subtypes constituted 58.4% of all positive cases. The rate of cervical HR-HPV and cytological abnormalities were not statistically different between age groups. Of the women with a positive HR-HPV test in Pap smear, cytological abnormalities were found to be more frequent when compared to HR-HPV negatives (95.8%, n=23 vs 4.3%, n=19, p<0.00001).

Conclusion: We demonstrated a strong correlation between HR-HPV positivity and cytological abnormality in the elderly women population. Age groups were not significantly different regarding the prevalence of HR-HPV and abnormal cytology. We also revealed that the prevalence of HR-HPV and cytological abnormalities in the elderly population were not high enough to explain the second peak of cervical cancer seen in older ages. More studies are needed to explain which testing methods should be used in the elderly patient population.

Keywords: Cervical high-risk human papillomavirus, Cytological abnormality, Elderly Turkish women

1. INTRODUCTION

Human papillomavirus (HPV) is a small, non-enveloped double-stranded DNA virus that infects cervicovaginal epithelial cells in women, leading to cervical cancer [1]. There are more than 200 genotypes of HPV and HPV infection is common in the world [2]. Cervical cancer, which occurs because of high-risk Human Papillomavirus (HR-HPV) infection, is the fourth most common cancer after breast cancer, colorectal cancer, and lung cancer in women all over the world, according to GLOBOCAN data [3]. It is estimated that approximately 1.4 million women worldwide are living with cervical cancer. While it is leading cancer among gynecological tract cancers all over the world, it is the third most common in Turkey after endometrial and ovarian cancer [4].

Human papillomavirus infection, which is more common, especially at young ages, is often spontaneously eradicated in middle and advanced ages and does not cause any histological abnormalities. Although, its prevalence varies from society to society, socioeconomic status, age, other concomitant diseases, and sexual life characteristics also affect the frequency of HPV. Knowing the prevalence of HPV by age group may be very useful data that can guide public health policies, epidemiological studies, and screening programs.

Istanbul, with its population of over 20 million, is a small-scale replica of Turkey and most likely represents the country as a whole. The center where the study was conducted, is the largest

How to cite this article: Yalcinkaya C. Prevalence of cervical high-risk human papillomavirus and cytological abnormalities in elderly Turkish women. Marmara Med J 2022 (3):288-292. doi: 10.5472/marumj.1186851

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1186851
Marmara Med J 2022;35(3): 288-292

hospital in the Umraniye region, is a large district located on the Anatolian side of Istanbul, and may represent Istanbul with its population density and socioeconomic characteristics.

Turkey has started to use primary HPV screening for population screening in recent years and has been taken as an example by some other countries. The Ministry of Health of Turkey successfully carries out the national cervical cancer screening program based on the HPV test [5]. According to this program, population screening ends at age 65. Opportunistic screening is performed for women over 65 years of age. Cervical cancer shows a bimodal distribution according to age and approximately one-fourth of cervical cancers are seen in women over 65 years of age. In addition, cervical cancer cases over the age of 65 usually present at an advanced stage and the prognosis is poor in this age group [6].

There is a lack of information about the prevalence of HPV and cervical cytological abnormalities in women aged 65 and over.. There is a false prejudice in the general population, and even among health professionals, that the elderly population has very little sex life. However, many international studies show that women over the age of 65 lead an active sexual life [7]. In addition, in many developed countries and developing countries including Turkey, the average life expectancy is getting longer.

Due to the hypoestrogenic state caused by the effect of advancing age and menopause, the cervical transformation zone is pulled into the cervical canal and it becomes difficult to obtain a sufficient smear for cytological evaluation [8]. Therefore, HPV screening may be a more appropriate method for the elderly population [9]. The aim of our study is to reveal the prevalence of HPV and the prevalence of HPV-associated cervical dysplasia in the elderly population, which is outside the social screening age range according to the national screening program, and to discuss the national screening program from this perspective.

2. PATIENTS and METHODS

This retrospective cross-sectional descriptive study is based on 467 women aged between 65–84 years (mean age 72.4 years), attending gynecology or gynecologic oncology outpatient clinic and having an HPV test as part of a routine gynecological examination. Women with a surgically absent cervix (who had a total hysterectomy) or women with a known gynecological malignancy were excluded from the study. Cervicovaginal smears were taken from the patients who applied to the gynecology, gynecological oncology and urogynecology outpatient clinics of our hospital with any complaint and all smears were taken by a gynecologist, gynecological oncology specialist or obstetrics and gynecology assistant. The smear samples were transferred to the pathology department in ThinPrep® PreservCyt® (Hologic Inc., Marlborough, MA) solution. Specimens were evaluated by pathologists for cytological abnormalities and tested for HPV. Samples in which cytological evaluation could not be done, or HPV test could not be performed due to insufficient sample quality were excluded from the study.

As it is known, the national screening program of the Turkish Ministry of Health has two steps. In Pap smears, the HPV test is done first. Cytology is also studied in HPV-positive samples. However, in our study, the screening strategy of our hospital was applied and simultaneous cytological evaluation was performed with the HPV test.

Cytological abnormalities were graded according to the 2014 Bethesda classification [10]. In our study, smear results with atypical squamous cells of uncertain significance (ASCUS) and low-grade disease (LSIL) were categorized as LSIL, while high-grade squamous intraepithelial lesion (HSIL), atypical squamous cells from which high-grade lesion cannot be excluded (ASC-H), and atypical glandular cells (AGC) were categorized as a high-grade disease (HSIL). Women with cytological abnormalities associated with HPV positivity, women with HPV 16 or HPV 18 positivity regardless of cytology, or women with ASC-H, HSIL, or AGC cytological abnormalities even if HPV negative were referred to colposcopy for further evaluation. Patients with normal cytology results and negative HPV, ASCUS, or LSIL cytology results but negative HPV results, and patients with normal cytology but other high-risk HPV positivity other than 16/18 HPV results were referred to have a cotest after 12 to 24 months.

The Cobas4800® HPV testing system (Roche Molecular Systems, Branchburg, NJ, USA) is a fully automated PCR-based HPV test that detects HPV DNA and detects 14 high-risk HPV types from a liquid-based cytology medium. This system is one of five HPV testing systems approved by the US Food and Drug Administration for HPV DNA typing. After detecting 14 high-risk HPVs, the Cobas HPV test reports HPV 16 and HPV 18 separately, while the other 12 HR-HPV types (31,33,35,39,45,51,52,56,58,59, 66,68) reports together as a pool.

The study was approved by the Umraniye Training and Research Hospital Ethical Committee (approval number B.10.1.TKH.4.34.H.GP.0.017281).

Statistical Analysis

All analyzes were performed using SPSS 16.0 software (SPSS INC., Chicago, Il, USA). Quantitative data are expressed as mean ± standard deviation. Categorical data were defined as percentages and numbers. A chi-squared test was used to compare the prevalence of cytological abnormalities and HPV subtypes in different age groups and the prevalence of abnormal cytology in groups according to HR-HPV positivity. p value less than 0.05 was considered significant.

3. RESULTS

The mean age of 467 women was 72.4 years. All participants were grouped according to ages of 65-69 (n=239), 70-74 (n=177), 75-79 (n=45) and 80-84 (n=6) (Table I, Figure 1).

http://doi.org/10.5472/marumj.1186851
Marmara Med J 2022;35(3): 288-292

Table I. *Prevalence of cytological abnormalities between age groups in elderly women*

	Age 66-69 (n=239)	*Age 70-74* (n=177)	*Age 75-79* (n=45)	*Age 80-84* (n=6)	Total (n=467)	p
Unsatisfactory smear, n(%)	28 (11.7)	13 (7.3)	5 (11.1)	1 (16.6)	47 (10.1)	
LSIL (ASCUS or LSIL), n(%)	25 (10.4)	9 (5.1)	2 (4.4)	0 (0)	36 (7.7)	
HSIL (ASCH or HSIL), n(%)	4 (1.7)	1 (0.6)	1 (2.2)	0 (0)	6 (1.3)	**P=0.103**
abnormal Cytology (total),n(%)	29 (12.1)	10 (5.7)	3 (6.6)	0 (0)	42 (9)	

LSIL: low grade squamous intraepithelial lesion, HSIL: high grade squamous intraepithelial lesion, ASCUS: atypical squamous cells of undetermined significance, ASC-H:atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion

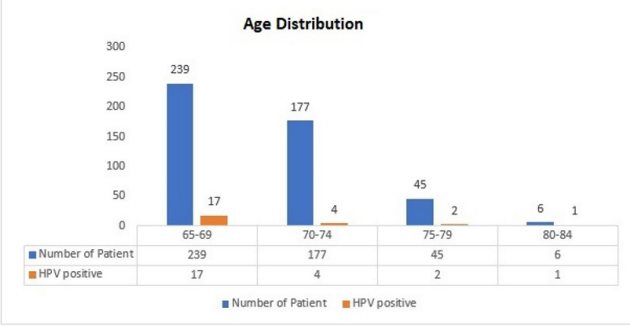

Figure1*. HPV positivity between age groups in elderly women*

Overall, abnormal cytology was found in 9% (n=42) of the population. The smear was reported as insufficient in 10.1%, LSIL were found in 7.7% and high-grade cytological abnormalities were seen in 1.3%. When evaluated according to age groups, the rate of abnormal cytology was 12.1% in '66-69 age', 5.7% in '70-74 age', 6.6% in '75-79 age' and 0% in '80-84 age' groups (Table I). The frequencies were not significantly different between groups (p=0.103).

High-risk human papillomavirus positivity was found in 5.1% (n=24) of the population; 1.7% was associated with HPV-16 /18 and 3.4% with other HR-HPV. When evaluated according to age groups, the rate of HR-HPV was 7.1% in '66-69 age', 2.8% in '70-74 age', 4.4% in '75-79 age' and 16.7% in '80-84 age' groups (Table II, Figure-1). The frequencies were not significantly different between groups (p=0.085).

Table II. *Prevalence of HPV subtypes between age groups in elderly women*

	Age 66-69 (n=239)	*Age 70-74* (n=177)	*Age 75-79* (n=45)	*Age 80-84* (n=6)	Total (n=467)	p
HPV 16/18, n(%)	5 (2.1)	2 (1.1)	1 (2.2)	0 (0)	8 (1.7)	
other HR-HPV, n(%)	12 (5)	2 (1.1)	1 (2.2)	1 (16.7)	16 (3.4)	
HPV positivity (total), n(%)	17 (7.1)	4 (2.8)	2 (4.4)	1 (16.7)	24 (5.1)	**P=0.085**

HPV: human papillomavirus, HR-HPV: high-risk HPV

Of the women with a positive HR-HPV test in Pap smear, cytological abnormalities were found to be more frequent when compared to HR-HPV negatives. (95.8%, n=23 vs 4.3%, n=19) (p=<0.00001) (Table III).

Women who participated in the study and required colposcopic evaluation according to cytology or HPV test results were referred to colposcopy. However, colposcopic evaluation results were excluded from the scope of this study due to insufficient numbers.

Table III. *Prevalence of abnormal cytology according to high-risk HPV positivity in elderly women*

	Abnormal cytology	Normal cytology	Total	P
HR-HPV (+)	**23** (95.8%) *within HR-HPV(+)*	1 (4.2%) *within HR-HPV(+)*	24 (100%)	
HR-HPV (-)	19 (4.3%) *within HR-HPV(-)*	424 (95.7%) *within HR-HPV(-)*	443 (100%)	**<0.00001**
total	42	425	467	

HR-HPV: high-risk human papillomavirus

4. DISCUSSION

To the best of our knowledge, our study is the first study on the prevalence of cervical HR-HPV and the cytological abnormalities in elderly Turkish women. This study reveals a strong association between abnormal cytology and HR-HPV positivity in elderly women despite lower prevalence compared to the younger population.

In our study, almost all of the HR-HPV-positive cases (23/24) had cytological abnormalities, while only 19 of 443 HPV-negative patients had abnormal cytology. The high rate of

http://doi.org/10.5472/marumj.1186851
Marmara Med J 2022;35(3): 288-292

cytological abnormalities in this patient group might be related to inadequate eradication of HPV despite advancing age.

In the total study group, we found the prevalence of HR-HPV was 5.1%. Petignat et al., in their study with a cohort over 60 years of age, found the prevalence of HPV to be 6.7% [11]. In another study, Ferenczy, and friends found that only 1% were HPV positive, out of 306 postmenopausal women aged 50-70 years [12]. Ferenczy et al., claimed that the reason for the low rate in their study was that it was carried out in a Jewish hospital and a private hospital.

Human papillomavirus-based screening programs emerge as a more economical and easily applicable alternative to cytological screening for community screening. This screening program has been used successfully in Turkey for many years. Several studies have shown that HPV DNA detection tests are more sensitive than cytological evaluation for primary screening of premalignant lesions of the cervix. We demonstrated that, in elderly women, cytological abnormalities were strongly associated with cervical HR-HPV, nonetheless we could not support colposcopic histopathologic verification to display any relation with cervical neoplasms. Therefore, HR-HPV testing as a primary cervical cancer screening tool for the elderly women population is arguable and uncertain to carry out which age period. As well known, the age-specific incidence of cervical cancer is bimodal and peaks at 35-40 and 70-80 years of age.

Our study had some limitations. First, the number of patients in some age groups, especially those over the age of 70, was low. Second, this study was single-centered and not carried out on a national basis, contrary to some studies of China and Scandinavian countries, on a larger scale with the participation of higher numbers [13-15]. Nonetheless, the total number of our study was sufficient, and the evaluated women population was representative of Turkey's population. Third, the sexual life and smoking history of the patients could not be questioned. Fourth, we did not include the colposcopic evaluations of the patients with HPV positivity and cytological abnormalities in our study, so we were unable to compare the specificity and sensitivity of HPV testing and liquid-based cytology in the elderly patient population.

In conclusion; we observed a strong correlation between HR-HPV positivity and cytological abnormality in the elderly patient population. Age groups were not significantly different in terms of HR-HPV and abnormal cytology prevalence. We also revealed that the prevalence of HR-HPV and cytological abnormalities in elderly ages are not high enough to explain the second peak of cervical cancer seen in older ages. Because the frequency of unsatisfactory cytology might be highly seen, cytological evaluation alone does not seem to be sufficient for screening cervical neoplasms in elder women. We believe that larger studies, including histological evaluation, are needed to decide whether cytology or HPV testing should be used as a screening test and which age periods are more appropriate to screen in the elderly patient population.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Umraniye Training and Research Hospital Ethical Committee (approval number B.10.1.TKH.4.34.H.GP.0.017281).

Financial Support: The author received no financial support for the research and/or authorship of this article.

Conflict of Interest: The author declare no conflicts of interest with respect to the authorship and/or publication of this article.

Author Contribution: CY: Designed, gathered and analyzed the data , did literature review and wrote the manuscript.

REFERENCES

[1] de Villiers EM. Cross-roads in the classification of papillomaviruses. Virology 2013;445:2-10. doi: 10.1016/j.virol.2013.04.023.

[2] Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-9. doi: 10.1002/(SICI)1096-9896(199909)189:1<12 :AID-PATH431>3.0.CO;2-F.

[3] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of ıncidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49. doi: 10.3322/caac.21660.

[4] Açikgöz A, Ergör G. Cervical cancer risk levels in Turkey and compliance to the national cervical cancer screening standard. Asian Pac J Cancer Prev 2011;12:923-7.

[5] Gultekin M, Karaca MZ, Kucukyildiz I, Dundar S, Keskinkilic B, Turkyilmaz M. Mega Hpv laboratories for cervical cancer control: Challenges and recommendations from a case study of Turkey. Papillomavirus Res 2019;7:118-122. doi: 10.1016/j.pvr.2019.03.002.

[6] Darlin L, Borgfeldt C, Widen E, Kannisto P. Elderly women above screening age diagnosed with cervical cancer have a worse prognosis. Anticancer Res 2014; 34: 5147–51.

[7] Beckman N, Waern M, Gustafson D, Skoog I. Secular trends in self reported sexual activity and satisfaction in Swedish 70 year olds: cross sectional survey of four populations, 1971-2001. BMJ 2008;337(7662):a279. doi: 10.1136/bmj.a279.

[8] Hermansson RS, Olovsson M, Hoxell E, Lindström AK. HPV prevalence and HPV-related dysplasia in elderly women. PLoS One 2018;13:e0189300. doi: 10.1371/journal.pone.0189300.

[9] Meijer CJ, Berkhof J, Castle PE, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer 2009;124:516-20. doi: 10.1002/ijc.24010.

[10] Nayar R, Wilbur DC. The Pap Test and Bethesda 2014. "The reports of my demise have been greatly exaggerated." (after a quotation from Mark Twain). Acta Cytol 2015;59(2):121-32. doi: 10.1159/000381842.

[11] Petignat P, Faltin D, Goffin F, Billieux MH, Stucki D, Sporri S, et al. Age-related performance of human papillomavirus testing used as an adjunct to cytology for cervical carcinoma

screening in a population with a low incidence of cervical carcinoma. Cancer 2005;105:126-32. doi: 10.1002/cncr.21060.

[12] Ferenczy A, Gelfand MM, Franco E, Mansour N. Human papillomavirus infection in postmenopausal women with and without hormone therapy. Obstet Gynecol 1997;90:7-11. doi: 10.1016/S0029-7844(97)00217-2.

[13] You W, Li S, Du R, Zheng J, Shen A. Epidemiological study of high-risk human papillomavirus infection in subjects with abnormal cytological findings in cervical cancer screening. Exp Ther Med 2018;15:412-8. doi: 10.3892/etm.2017.5357.

[14] Andersen B, Christensen BS, Christensen J, Ejersbo D, Heje HN, Jochumsen KM, et al. HPV-prevalence in elderly women in Denmark. Gynecol Oncol 2019;154:118-23. doi: 10.1016/j.ygyno.2019.04.680.

[15] St-Martin G, Viborg PH, Andersen ABT, et al. Histological outcomes in HPV-screened elderly women in Denmark. PLoS One 2021 11;16:e0246902. doi: 10.1371/journal.pone.0246902.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Investigation of the lawsuits regarding Down syndrome

Hande KAYMAKCALAN

Department of Medical Genetics, School of Medicine, Demiroğlu Bilim University, Istanbul, Turkey.

Corresponding Author: Hande KAYMAKCALAN
E-mail: doctorhande@yahoo.com

Submitted: 21.02.2022 **Accepted:** 30.05.2022

ABSTRACT

Objective: To examine the number of cases filed about Down syndrome in terms of its numbers, causes and consequences, to provide an overview of what doctors should pay attention to when informing and consulting patients and during follow-up and recommend solutions for decreasing the number of malpractice cases. (Discussing the legal aspect of the decisions is beyond the scope of this research.)

Materials and Methods: 'Down', 'Down sendromu' 'Down's, 'trizomi 21 ', 'trisomi 21' and 'trisomy 21' was written to ' https://karararama.yargitay.gov.tr/ ' and 'https://karararama.danistay.gov.tr/' web addresses search engines and the data was examined with Microsoft Excel or with R version 4.0.5 (2021-03-31) for bias and frequency table was used and the results were examined.

Results: A total of 53 cases were found. 49 supreme court and 4 Council of State court decisions are found. The cases are from 27.10.2009 to 13.10.2021.

Conclusion: A total of 39 different Down syndrome cases were examined, as 6 of the 53 cases were related to the same cases and 8 of them were cases not related to Down syndrome. 28 cases are "doctor negligence", 5 are "reckless killing", 1 "material mixing in the genetic center", 1 "unauthorized use of the child's photo", 1 "stealing money from the child", 1 "intentionally injuring the child", 1 'inheritance request for the child' and 1 on 'guardianship'.

Keywords: Down syndrome, Prenatal screening, Prenatal diagnosis, Malpractice, Lawsuit

1. INTRODUCTION

Down syndrome or trisomy 21 (having 3 of the 21st chromosomes) is the most common chromosomal disorder and the most common non-hereditary cause of intellectual disability [1-3]. It is seen in 1/600-800 people. According to the data analysis of the Turkish Statistical Institute (TUIK), the incidence of Down syndrome in the population is 1/530. In addition to intellectual disability, congenital heart anomalies, hypothyroidism, cataracts at an early age, hearing loss, intestinal anomalies are also common [4]. Early dementia and Alzheimer's are important causes that shorten life expectancy. People with Down syndrome have a characteristic facial appearance such as upslanting palpepral fissures, epicanthal folds, small ears and flat nasal bridge (OMIM#190685). Failure of chromosomes to separate during meiosis is the underlying cause of 95% of the cases [4]. The remaining causes include translocations and mosaicism. While the risk is 1/1400 for each live birth at the age of 24, this rate increases to 1/350 at the age of 35 and 1/45 at the age of 43 [5].

Although, advanced maternal age (over 35 years of age) is an important risk factor, giving birth to a baby with Down syndrome is more common in mothers under the age of 35 since most of the pregnancies occur in women under the age of 35. According to Turkish Statistical Institution (TUIK) data, there have been a total of 7,841.668 deliveries in Turkey in the last 5 years. 14% of them (n=1,097,834) are by women over 35 years old and 86% (n=6.743,834) are by women under 35 years old [6].

Double, triple and combined tests and detailed ultrasound examinations are recommended for all pregnant women, regardless of age, in the prenatal screening guidelines of the Ministry of Health in Turkey. These are suggestions only and not mandatory. The 'extracellular free DNA' test, which has a sensitivity of approximately 99% in the detection of Down

How to cite this article: Kaymakcalan H. Investigation of the lawsuits regarding Down syndrome. Marmara Med J 2022; 3; 35(3):293-296. doi: 10.5472/marumj.1195921

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1195921
Marmara Med J 2022;35(3): 293-296

syndrome and called non-invasive prenatal test (NIPT), is not among the recommendations made by the Ministry of Health and is not performed in public hospitals.

According to the Biomedical Contract and the Patient Rights Regulation, there is no need to obtain written consent when ordering these screening tests and since, there is no document of proof, the majority of the cases brought to the court due to the birth with Down syndrome doctors are accused of negligence for not offering these tests to the pregnant women [6]. These accusations may be the result of not informing the patient enough or the patients not understanding the information.

Even in high-risk pregnancies (in our country, if the risk is less than 1/270 for having a baby with Down syndrome, it is considered as high risk), definitive diagnostic tests such as amniocentesis and chorionic villus sampling (CVS), are offered to the patient as a recommendation only and again are not mandatory. CVS is usually done between 10-14 weeks of pregnancy and amniocentesis is done after the 16th week of pregnancy until the 20th week. Since, it is an invasive test, complications such as infection and bleeding may be seen. Death of the fetus and the mother are seen very rarely. Although, consent is obtained from the pregnant woman for these tests (some experts also obtain consent from the father), some of the lawsuits are filed for the crime of "reckless killing" due to the death of the fetus or the pregnant woman [7].

The purpose of this research is to examine the number, reasons and results of the lawsuits filed regarding Down syndrome and to provide a general perspective on what doctors should pay attention to during informing, consulting and follow-up of patients and to recommend solutions for reducing the number of malpractice cases. To the best of my knowledge, this is the first study examining the lawsuits for Down syndrome in Turkey.

2. MATERIALS and METHOD

In this observational study, we examined each of the results for searches on the terms "Down", ""Down sendromu", "Down's", "trisomi 21", "trizomi 21", and "trisomy 21" in the two search engines https://karararama.yargitay.gov.tr/ (supreme court cases) and "https://karararama.danistay.gov.tr/' (state of council cases) .Search on "Down" produced 49 supreme court decisions, and search on "Down sendromu" produced 21 supreme court and 4 state of counsil decisions. Search on "Down's", "trisomi 21", "trizomi 21", and "trisomy 21" produced no results. The dates of the decisions ranged from 27.10.2009 to 13.10. 2021.The focus of this study is to examine the reasons of the court cases, and the court outcomes (Table I).

This study was approved by the Clinical Research Ethics Committee of Demiroglu Bilim University (approval number 44140529).

Statistical Analysis

All statistical evaluations as well as handling descriptive data (e.g., frequency table) throughout this study was carried with Microsoft Excel or with R version 4.0.5 (2021-03-31). To check whether there is any bias in selection of cases with respect to the decision, Chi-square test is used with the null hypothesis that there is no difference between expected number and observed number of cases, therefore there is no bias. P value less than 0.05 was considered as significant.

3. RESULTS

We took into account 39 of the 53 court case results. Of the 14 court case results we did not take into account, 6 were related to the same court cases and 8 were court cases unrelated to Down syndrome (they just happened to have the terms "Down" or "sendrom" in the decision texts; for example one court case was about a rap song that contained the word "Down" in its lyrics, and two cases related to hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome.

Of the 39 court cases, 71.7 % (28 cases) were for doctor's negligence, 12.8% (5 cases) for manslaughter through negligence, 1 for mixing-up of sample material at the genetic testing center, 1 for unauthorized use of the photograph of a child with Down syndrome in a test book, 1 for stealing money from a child with Down syndrome, 1 for injury with intent of a child with Down syndrome, 1 for demanding the inheritance rights for a child with Down syndrome, and 1 for guardianship (Table I). There were no bias in the selection of cases with respect to the decision (p=0.12).

Table I. *Cases related to Down syndrome and their results that are found in data on 'karararama.yargitay.gov.tr' and 'karararama.danistay.gov.tr' websites*

Cases	Outcome			
w	Acceptance/ Compensation	None/ Dismissal / Acquittal	Appeal	Total and Frequency
Doctor's negligence	4	9	15	28 (71.7%)
Unauthorized use of photos	1			1 (2.58%)
Material mixing		1		1 (2.58%)
Deliberate injury			1	1 (2.58%)
Inheritance	1			1 (2.58%)
Stealing money	1			1 (2.58%)
Reckless killing		2	3	5 (12.8%)
Guardianship		1		1 (2.58%)
Total	7	13	19	39 (100%)

In each of the court cases for; stealing from a child with Down syndrome, injury with intent of a child with Down syndrome, unauthorized use of the photograph of a child with Down syndrome, and demanding the inheritance rights for a child with Down syndrome, the court ruled in favor of the plaintiff.

Manslaughter through negligence

One court case was related to the death of a mother and baby with Down syndrome after birth. One court case was related to the death of the fetus and of the mother from septic shock, following a second amniocentesis after an unsuccessful first

http://doi.org/10.5472/marumj.1195921
Marmara Med J 2022;35(3): 293-296

amniocentesis, two court cases were related to the death of the fetus after an amniocentesis, and one court case was related to the death of a child with Down syndrome the following morning after being sent home with a diagnosis of acute tonsillitis and being prescribed antibiotics. Three of these cases were re-opened on appeal, and in two of them the doctors were acquitted. The outcome of the appeals is not known.

Mixing-up of sample material at the genetic testing center

The parents of a child born with Down syndrome opened this court case with the claim that they did not have an abortion because their amniocentesis test result was normal, and that another set of parents had an abortion because their amniocentesis test showed Down syndrome whereas their baby was normal. It is not known whether the other parents who had an abortion even though their fetus was normal, opened a court case or not. As it was not able to be proven that the samples were mixed up at the genetic testing center, the center was found not guilty.

Doctor's negligence (malpractice)

In these court cases the plaintiffs sued for medical malpractice accusing the doctors of having not sufficiently informed them regarding screening tests, did not get informed consent, did not act correctly regarding the diagnosis, did not recommend advanced testing, were negligent in performing ultrasound, did not analyze the ultrasound results, did not request consultation, and did not perform amniocentesis. Of the 39 court cases, 28 were in this category. In 9 of the cases the doctors were acquitted, and in 4 cases the doctor was ordered to pay compensation. The remaining 15 cases went to appeals, and the result of the appeals are not known.

4. DISCUSSION

Definitive diagnosis of Down syndrome during pregnancy can only be made by invasive diagnostic methods such as CVS or amniocentesis. If the fluorescent in situ hybridization (FISH) and chromosome analysis of the samples taken by these methods show the presence of three chromosomes in the 21st chromosome, Down syndrome diagnosis is made. A meta-analysis study in 2019 showed that amniocentesis caused approximately 0.91% and CVS caused 1.39% pregnancy loss [8]. In the same article, the miscarriage rate in the control group was 1.23% and the authors concluded that the procedure related risks of miscarriage following amniocentesis and CVS are lower than expected. In a study by O'Connor et al, 11.7% of mothers of a fetus with Down syndrome and who continued their pregnancy had miscarriage and 26.4% had stillbirth [9]. If these types of invasive methods are performed by expert teams, the miscarriage rate is very low and maternal death was not reported in the literature. However, complications such as fetal loss, amniotic fluid leakage, chorioamnionitis, needle injury and vaginal bleeding may occur in all of these procedures. During these procedures, evaluation by ultrasound, evaluation in terms of infection risk and Rh prophylaxis should be performed [10].

In this study 12.8% of cases were about complications of the procedures that resulted in either the death of the mother, the fetus or both. This is a high number compared to the expected deadly complication rates of these procedures [8]. In the literature review, we could not find any prevalence data in our country showing the complications of these invasive methods. Studies were published only as single center experiences. There is a need for a multicenter study on this subject in our country. Considering that maternal death occurred in two of the cases in our article, the importance of the subject becomes even more understandable.

Screening tests only show the magnitude of the risk and if this risk is greater than 1/270 in our country, invasive diagnostic methods are recommended for the mother [11]. The social security institution pays the fees for double, triple, quadruple screening tests and second-level ultrasonography performed by radiologists. It also covers the cost of CVS and amniocentesis. It does not pay for the NIPT tests which is being used widely in the developed world [12]. However, studies have shown that NIPT tests reduce the number of invasive tests [13]. At the same time, Huang et al., concluded in their study that the NIPT test screening is the most cost-effective screening test and can increase the effectiveness of prenatal aneuploidy screening by reducing the number of patient visits and providing earlier results [14]. There is a need for research on the effectiveness of NIPT tests for our country, which are currently more costly than double and triple screening tests.

The results of screening tests are reported as 'normal', 'negative' or 'positive' by some laboratories. This situation leads families to reach the wrong conclusion that these tests give definite results. In most of the 'doctor negligence' cases filed, there are accusations that the doctor did not sufficiently inform the patient and did not direct them to invasive diagnostic methods. As written in the guide of the Ministry of Health, pregnant women are informed about prenatal screening and diagnosis, and a screening test is done if requested and written consent is not obtained when ordering or not ordering these tests [11]. However, obtaining written consent does not always end the legal disputes because what is written in the consent may be found insufficient [12]. It is especially important that the obstetrician takes time to explain these tests to the family in detail and make sure that they understand. If possible, the obstetrician's directing the family to a medical geneticist before invasive diagnosis will help the family to make a more informed decision and reduce possible lawsuits. At the same time, the use of more reliable NIPT tests will reduce the use of invasive diagnostic tests and ultimately reduce the number of cases. These tests should be easily accessible to everyone, and these tests should be carried out in our country (most of the tests are sent abroad).

If the pregnant woman does not want a definitive diagnosis, a letter of rejection should be obtained. If she wants a definitive diagnosis, she is directed to the physician who will perform amniocentesis or other invasive diagnostic methods. The obstetrician should inform the pregnant woman in terms of the amniocentesis procedure and obtain written consent. To perform genetic analysis from the amniotic fluid sample, the

http://doi.org/10.5472/marumj.1195921
Marmara Med J 2022;35(3): 293-296

genetic diagnosis center must obtain written consent also. It is also helpful to get written consent from the baby's father.

On another note, while talking about prenatal screening and diagnostic tests; it should also be explained that these tests do not make a diagnosis for all diseases. Diagnostic tests only look for trisomy 21, trisomy 13, and trisomy 18 syndromes unless a different test is requested.

The limitations of this study are there are not many cases that are present in the supreme court and state of council web sites. Also, the results of most of the appeals are not known. These websites should be improved by adding more cases and the results of these cases.

As a result, false positive and false negative results can be reduced by using screening tests with high sensitivity and specificity. It should also be explained that prenatal screening and diagnostic tests are not informative for all diseases.

During patient consultation, care must be taken that the consult is not directive, psychological support should be provided to the parents after the diagnosis, the decision to continue pregnancy should be left to the parents and the decision should not be judged [15]. Efforts should be made to reduce the number of patients per doctor so that doctors can give patients the required time. Written consents should be taken, and doctors must make sure that the patients understand what is written on the consent form. All these measures can improve the doctor-patient relationship, enable healthier decisions, and reduce the number of lawsuits.

Compliance with the Ethical Standards

Ethical Approval: Ethical approval for this study was obtained from Clinical Research Ethical Committee of Demiroglu Bilim University (approval number 44140529)

Financial Support: No funding was used.

Conflict of Interest: The author has no potential conflicts of interest to disclose.

Author Contribution: HK: Designed, searched the cases in the websites , did literature review and wrote the manuscript.

Acknowledgment:

I would like to thank the lawyer Emine Gülbin Hapçıoğlu for her legal support while examining the lawsuits.

REFERENCES

[1] Canfield MA, Honein MA, Yuskiv N, et al. National estimates and race/ethnic-specific variation of selected birth defects in the United States, 1999-2001. Birth Defects Res A Clin Mol Teratol 2006;76:747-56. doi:10.1002/bdra.20294

[2] Sherman SL, Allen EG, Bean LH, Freeman SB. Epidemiology of Down syndrome. Ment Retard Dev Disabil Res Rev 2007;13:221-27. doi:10.1002/mrdd.20157

[3] Daunhauer LA, Fidler DJ, Hahn L, Will E, Lee NR, Hepburn S. Profiles of everyday executive functioning in young children with down syndrome. Am J Intellect Dev Disabil 2014;119:303-18. doi:10.1352/1944-7558-119.4.303

[4] Bull MJ. Down Syndrome. N Engl J Med 2020;382:2344-52. doi:10.1056/NEJMra1706537

[5] Morris JK, Mutton DE, Alberman E. Revised estimates of the maternal age specific live birth prevalence of Down's syndrome. J Med Screen 2002;9:2-6. doi:10.1136/jms.9.1.2

[6] https://data.tuik.gov.tr/ Kategori/GetKategori?p=Saglik-ve-Sosyal-Koruma-10. Accessed on 24.05.2022

[7] https://www.mevzuat.gov.tr/ #kanunlar Accessed on 24.05.2022

[8] Salomon LJ, Sotiriadis A, Wulff CB, Odibo A, Akolekar R. Risk of miscarriage following amniocentesis or chorionic villus sampling: systematic review of literature and updated meta-analysis. Ultrasound Obstet Gynecol 2019;54:442-51. doi:10.1002/uog.20353

[9] O'Connor C, Moore R, McParland P, et al. The natural history of trisomy 21: outcome data from a large tertiary referral centre. Fetal Diagn Ther 2021;48:575-81. doi:10.1159/000517729

[10] Çalış P, Bayram M. Techniques, complications and difficulties of prenatal ınvasive diagnosis. Turkiye Klinikleri J Med Genet-Special Topics 2018;3:38-43

[11] https://hsgm.saglik.gov.tr/depo/birimler/Kadin_ve_Ureme_Sagligi_Db/dokumanlar/rehbler/ dogum_oncesi_bakim_08-01-2019_1.pdf Accessed on 21.05.2022

[12] Dolen I. How should a fetus with down syndrome be diagnosed in prenatal care? Turk J Womens Health Neanotol 2021; 3: 39-49. doi: 10.46969/ezh.839710

[13] Alberry MS, Aziz E, Ahmed SR, Abdel-Fattah S. Non invasive prenatal testing (NIPT) for common aneuploidies and beyond. Eur J Obstet Gynecol Reprod Biol 2021; 258:424-29. doi: 10.1016/j.ejogrb.2021.01.008

[14] Huang T, Gibbons C, Rashid S, et al. Prenatal screening for trisomy 21: a comparative performance and cost analysis of different screening strategies. BMC Pregnancy Childbirth 2020;20:713. doi:10.1186/s12884.020.03394

[15] Dikmen U, Özbaş C, Özkan S. Turkiye Klinikleri J Med Genet-Special Topics. 2018;3:131-4.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Evaluation of the efficacy of several sclerosing agents for pleurodesis in rats

Cenk BALTA[1], Mustafa KUZUCUOGLU[2], Figen ASLAN[3], Ali Cem YEKDES[4]

[1] Department of Thoracic Surgery, Hatay Mustafa Kemal University Health Application and Research Hospital, Hatay, Turkey
[2] Department of Thoracic Surgery, İzmir Katip Çelebi University Health Application and Research Hospital, İzmir, Turkey
[3] Department of Pathology, Kutahya Health Sciences University Health Application and Research Hospital, Kutahya, Turkey.
[4] Department of Internal Medicine, Balıkesir University Health Application and Research Hospital, Balıkesir, Turkey

Corresponding Author: Cenk BALTA
E-mail: drcenkbalta@gmail.com

Submitted: 23.02.2022 **Accepted:** 30.05.2022

ABSTRACT

Objective: This study aims to evaluate the efficiencies and effects of different sclerosing agents.

Materials and Methods: Thirty-five female Wistar-albino rats were included in this study and divided into five groups randomly. Isotonic saline (group 1), iodopovidone (group 2), rifamycin (group 3), autologous blood (group 4), talc (group 5) were given into intrapleural space. On postoperative 28th day, ipsilateral and contralateral pleural and parenchymal fibrosis and inflammation were evaluated.

Results: There were statistically differences between control and other groups on macroscopically pleural fibrosis examination (p=0.004, p=0.001, p=0.001, p=0.000, respectively). Microscopically pleural fibrosis were determined in all groups when compared to control group (p=0.023, p=0.023, p=0.035, p=0.001, respectively). There were no significant differences between sclerosing agent groups (p>0.05). Significant differences were observed according to microscopic pleural inflammation evaluation between talc group and other groups. There was superiority in talc group according to intraparenchymal inflammation grade compared to other groups (p=0.010, p=0.042, p=0.030, respectively). Macroscopically, fibrosis and microscopically, inflammation were significantly observed between talc and other groups in the contralateral pleura (p = 0.037and p=0.009, respectively).

Conclusion: Iodopovidone, autologous blood, talc, and rifamycin can be used as effective pleurodesis agents. However, we found that rifamycin and autologous blood are safer as effective pleurodesis agents. We think that these two agents can be used in patients with secondary lung diseases due to their less intraparenchymal inflammation rates. It may be advantageous to prefer other sclerosing agents which has lower costs and less side effects comparing to talc.

Keywords: Pleurodesis, Pleural effusions, Pleural inflammation, Animal experiments

1. INTRODUCTION

Pleurodesis is a medical procedure to induce an inflammatory reaction between the visceral and parietal pleura to obtain adhesion of pleural layers. Chemical and mechanical pleurodesis are the two methods used for inducing pleurodesis. Chemical pleurodesis is mainly used for avoiding recurrences of effusions in patients with malignant pleural effusion. Sclerosing agents administered under videothoracoscopy (VATS) or through a thoracic catheter act on the mesothelial layer, leading to inflammation. The resulting fibrotic process induces adhesion between the visceral and parietal pleura [1].

Despite the use of several agents including tetracycline, bleomycin, talc powder, silver nitrate, iodopovidone, and autologous blood samples for this purpose; the debate for identifying the treatment of choice agent still continues [2]. An effective, available, low-cost, easy to administer, high molecular weight, and sterilizable agent with a low potential for side effects should be preferred in the treatment [3]. Tetracycline was the most commonly used agent for pleurodesis in the 1980s; however, its popularity has declined due to problems with its production and the risk of hemithorax. The efficacy of bleomycin is lower compared to other agents but it is an expensive agent. It is reported that the use of silver nitrate causes severe pain and hemothorax [3]. Today, the most commonly used agent for pleurodesis is talc powder. It has been demonstrated that talc powder administration is associated with the emergence of acute respiratory distress syndrome (ARDS) resulting from the penetration of small particles into alveoli [4].

How to cite this article: Balta C, Kuzucuoglu M, Aslan F, Yekdes AC. Evaluation of the efficacy of several sclerosing agents for pleurodesis in rats. Marmara Med J 2022;35(3): 297-302. doi: 10.5472/marumj.1191101

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1191101
Marmara Med J 2022;35(3): 297-302

In this study, we aimed to determine the efficacy of the sclerosing pleurodesis agents used in our clinical practice and to identify the most efficacious one by using comparative analyses.

2. MATERIALS and METHODS

The study was designed as a randomized and prospective trial. Because of rats' high tolerance to surgical procedures, low costs, and easy reproducibility, growth, and care characteristics; these animals were selected as the experimental animals to be used in our study. This study was conducted in the experimental animal laboratory of Balıkesir University following the approval obtained from the Animal Experiments Local Ethics Committee of Balıkesir University (Decision No: 2019/10-2).

Aim and the Study Design

In the study, 35 female albino Wistar rats of 250-300 grams body weight on the average were used. The rats were randomized into five groups.

- Group 1 was assigned as the control group and the rats in Group 1 were administered 1cc of 0.9% saline solution.
- The rats in Group 2 received 1 cc of 2% iodopovidone.
- The rats in Group 3 received 12.5 mg/kg rifamycin (RIF ampoules – Koçak Farma, Istanbul, Turkey) in a volume of 1cc.
- Autologous blood samples of 0.5 cc collected from the tail veins of the rats were administered to the rats in Group 4 at a dose of 2 ml/kg.
- Group 5 received 50 mg/kg sterile talc (NovatechSA – Steritalc®, Marseille, France) at a volume of 1 cc.

Surgical Technique

Ketamine hydrochloride (Alfamine vial, Ege-vet Pharmaceutical Co. Izmir, Turkey) and xylazine hydrochloride (Alfazyne vial, Ege-vet Pharmaceutical Co. Izmir, Turkey) were intramuscularly administered to the rats at doses of 100 mg/kg and 10 mg/kg, respectively, to obtain general anesthesia. After applying general anesthesia to the rats, they were brought to the right lateral decubitus position. The thoracic areas of the rats on the right side were shaved and cleaned. Asepsis was obtained by cleaning the site of surgery with iodopovidone. Access to the thoracic cavity was obtained through a 5 mm-incision made in the intercostal area in the midline of the left lateral hemithorax. The sclerosing agents were administered into the intrapleural space through a 24G venous catheter at the doses specified above. The rats were taken into the palm and they were turned around to make several circles in the air so that the active agent would be spread throughout the thoracic cavity. Then, the free air between the pleural layers was aspirated with an injector and catheter to avoid a potential pneumothorax. The incision was closed with 3/0 polypropylene sutures. Ventilator support was not applied during surgical intervention. Paracetamol was administered intraperitoneally at a dose of 10 mg/kg to all rats in the experiment before they woke up. The rats were followed up at separate cages in the postoperative period. A total of four rats died; 2 in the isotonic saline solution group and 2 in the blood group, during the surgical interventions and in the 24-hour period after surgery.

The rats were kept alive for 4 weeks under appropriate living conditions with optimum temperature and humidity and 12-hour light and 12-hour dark cycles. They were provided with access to water and they were fed with pellet feed. No movement disorders, feeding disorders, or any other pathologies were observed in the rats after the surgical procedures. All of the rats were sacrificed with cervical dislocation under general anesthesia obtained by the administration of 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride to each rat at the end of the fourth week. En-bloc resection of the whole thoracic wall was performed, dissecting the muscle and connective tissue (Figure 1). After the specimens were washed with saline solution, they were placed in 10% formaldehyde solution.

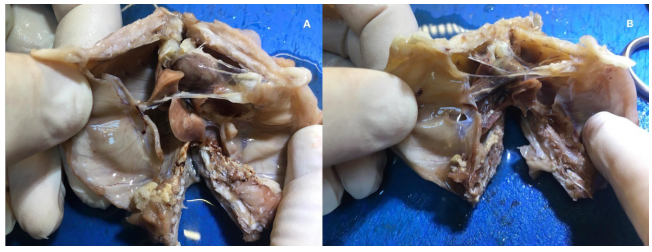

Figure 1. *En bloc resection of the whole thoracic wall A) adhesions in ipsilateral hemithorax (rifamycin received group) B) adhesions in both hemithoraces (talc received group)*

Pathological Examination

Both pleural cavities in each rat of all groups were exposed as described previously [3,5]. Firstly, both pleural cavities in each rat were exposed by incising all of the ribs bilaterally in both hemithoraces approximately along the midline and the diaphragm. Then, the medial parts of the anterior ribs and the sternum were excised so that the lungs and the pleural cavities could be observed appropriately.

The macroscopic degree of pleurodesis was determined in the groups in a blinded fashion as specified below [3,5] (Figure 2).

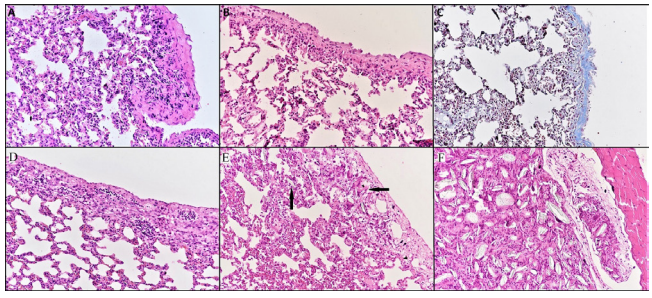

Figure 2. *(A) Microscopic section of Grade 3 visceral pleural inflammation and Grade 3 lung inflammation in the iodopovidone group (H-E x 200). (B) Microscopic section of Grade 2 visceral pleural inflammation and Grade 2 lung inflammation in the rifamycin group (H-E x 200). (C) Microscopic section of Grade 3 visceral pleural thickening in the rifamycin*

http://doi.org/10.5472/marumj.1191101
Marmara Med J 2022;35(3): 297-302

group (trichrome staining, x 200). (D) Microscopic section of Grade 3 visceral pleural inflammation and Grade 2 lung inflammation in the Blood group (H-E x 200). (E) Microscopic section of Grade 4 visceral pleural inflammation and Grade 3 lung inflammation in the Talc group. Granulomatous reaction rich in foreign body giant cells developing against talc crystals in the visceral pleura and lung parenchyma (arrow) (H-E x 200). (F) Microscopic section of Grade 4 parietal pleural inflammation in the Talc group. Granulomatous reaction rich in foreign body giant cells developing against talc crystals in the parietal pleura (H-E x 200).

- 0: The pleural cavities and the lungs looked normal.
- 1: No adhesions were observed; however, inflammation was observed in the pleural cavity, looking ragged with fibrin accumulation.
- 2: Presence of a few scattered adhesions
- 3: Presence of generally scattered adhesions
- 4: Complete adhesion resulting in the loss of the pleural space

Samples of parietal pleura, visceral pleura, and lung tissue were collected from both hemithoraces; from the areas, which represented macroscopic pleurodesis best. The samples were fixed in 10% formalin and underwent routine tissue procedures. Sections of 4 um thickness were obtained and stained with hematoxylin-eosin. Furthermore, Masson's trichrome staining was performed to evaluate fibrosis better. The prepared tissue sections were examined under a light microscope to evaluate inflammation and fibrosis. During these examinations, the investigator was blinded to the descriptive characteristics of the groups.

Degrees of microscopic inflammation and fibrosis [3,5]:

- 0: Not available
- 1: Insignificant/suspected
- 2: Mild
- 3: Moderate
- 4: Severe

Statistical Analysis

Sample size for the animal studies are suggested as minimum 5-7 animals per group [6]. All parameters were presented as mean ± standard deviation. The intergroup relationships of the parameters were evaluated with the one-way ANOVA test. Then, the Tukey test was performed for post hoc analysis to determine differences between the subgroups. In all statistical analyzes, p-values of less than 0.05 were considered significant.

3. RESULTS

The scores of macroscopic pleural fibrosis, microscopic pleural fibrosis, microscopic pleural inflammation, and intraparenchymal inflammation were presented in Table I as mean ± standard deviation.

Table I. *Mean values of sclerosing agents*

Groups	n	Macroscopically pleural fibrosis	Microscopically pleural fibrosis	Microscopically pleural inflammation	Intraparenchymal inflammation
		Mean±STD	Mean±STD	Mean±STD	Mean±STD
Saline	5	0.80±0.447	1.00±1.000	1.00±1.000	1.20±0.447
Iodopovidone	7	2.43±0.787	2.57±0.976	2.43±0.787	2.00±0.816
Rifamycin	7	2.71±0.488	2.57±0.535	2.43±0.535	1.57±0.535
Autologous Blood	5	2.80±1.095	2.60±0.894	2.20±0.837	1.40±0.894
Talc	7	3.00±0.577	3.14±0.690	3.71±0.756	2.71±0.756

n: number, STD: standard derivation

Table II. *Comparison of sclerosing agents in ipsilateral hemithorax*

Macroscopically pleural fibrosis					Microscopically pleural fibrosis				
	Talc	Autolooguous Blood	Rifamycin	Iodopovidone		Talc	Autologous Blood	Rifamycin	iodopovidone
Saline	0.000	0.001	0.001	0.004	Saline	0.001	0.035	0.023	0.023
iodopovidone	0.555	0.892	0.939		iodopovidone	0.691	1	1	
Rifamycin	0.939	1			Rifamycin	0.691	1		
Autologous Blood	0.988				Autologous Blood	0.789			
Microscopically pleural inflammation					**İntraparenchymal inflammation**				
	Talc	Autologous Blood	Rifamycin	iodopovidone		Talc	Autologous Blood	Rifamycin	iodopovidone
Saline	0.000	0.135	0.031	0.031	Saline	0.010	0.991	0.897	0.331
iodopovidone	0.034	0.986	1		iodopovidone	0.353	0.608	0.791	
Rifamycin	0.034	0.986			Rifamycin	0.042	0.994		
Autologous Blood	0.020				Autologous Blood	0.030			

http://doi.org/10.5472/marumj.1191101
Marmara Med J 2022;35(3): 297-302

Table III. *Mean values and comparison of sclerosing agents in contralateral hemithorax*

Groups	n	Macroscopically pleural fibrosis	Microscopically pleural fibrosis	Microscopically pleural inflammation	Intraparenchymal inflammation
		Mean±STD	Mean±STD	Mean±STD	Mean±STD
Saline	5	0.40±0.548	0.40±0.548	0.40±0.548	1.00±0.707
iodopovidone	7	1.00±0.577	1.29±1.113	1.29±0.756	1.86±0.900
Rifamycin	7	1.14±0.378	1.14±0.378	1.29±0.488	1.86±0.690
Autologous Blood	5	0.80±0.837	1.00±0.707	1.00±0.00	0.80±0.1.095
Talc	7	1.43±0.535	1.14±0.690	1.57±0.535	1.57±0.787
ANOVA test (p) between groups		0.059	0.016	0.339	0.129

n: number, STD: Standard derivation

Evaluation of the ipsilateral (left) hemithorax

It was observed that all sclerosing agents caused more pleural fibrosis macroscopically compared to the control group (iodopovidone: p=0.004, rifamycin: p=0.001, autologous blood: p=0.001, talc: p=0.000). However, no statistically significant differences were observed between the groups receiving sclerosing agents (Table II).

While all sclerosing agents caused statistically more pleural fibrosis microscopically compared to the control group (iodopovidone: p=0.023, rifamycin: p=0.023, autologous blood: p=0.035, talc: p=0.001), no superiority of a sclerosing agent over the other was observed (Table II).

In the group of rats; which received talc, microscopic pleural inflammation occurred to a statistically significantly higher degree compared to the other groups. Microscopic inflammation in the pleura was observed more in the rifamycin and iodopovidone groups when compared to the control group (p=0.031, p=0.031, respectively). It was observed that autologous blood administration caused less inflammation in the pleura microscopically compared to the other sclerosing agents used for pleurodesis in the other groups (Table II).

Intraparenchymal inflammation developed statistically significantly more in the talc pleurodesis group compared to the control group (p=0.010), rifamycin group (p=0.042), and the autologous blood group (p= 0.030). While the intraparenchymal inflammation was not statistically significantly different between the talc pleurodesis and iodopovidone groups (p=0.353), the intraparenchymal inflammation in the iodopovidone pleurodesis group was not statistically significant when compared to the control group (p=0.331) (Table III).

Evaluation of the contralateral (right) hemithorax

The examination of the macroscopic pleural fibrosis scores revealed that the macroscopic pleural fibrosis was statistically significant in the contralateral pleura of the group of rats; which were administered talc (p=0.037). However, no statistically significant contralateral pleural fibrosis was observed in the other groups (Table III).

Microscopic pleural inflammation was significant in the talc pleurodesis group compared to the control group (p=0.009); however, no significant pleural inflammation was observed in the other groups compared to the control group. There were no statistically significant differences in the microscopic pleural fibrosis and intraparenchymal inflammation scores among the groups in the experiment.

4. DISCUSSION

Pleurodesis can be used for the treatment of recurrent malignant effusions and prolonged air leakage. Effective induction of pleural inflammation and fibrosis is recommended for pleurodesis; however, the absolute superiority of sclerosing agents over each other has not been demonstrated yet [7]. This study compared the efficacy of three commonly used sclerosing agents in clinical practice; including talc, autologous blood, and iodopovidone, and a rarely used agent, rifamycin, for pleurodesis and compared the resulting degree of parenchymal inflammation induced by these agents.

Autologous blood was used by Robinson for the first time in 1987 with a success rate of 85% for the treatment of persistent air leakage [8]. Özpolat et al., conducted a study and reported that autologous blood pleurodesis was inexpensive, easy to administer, and an effective method [2]. They reported that the administration of 2-3 mL/kg, but not 1 mL/kg autologous blood, effectively resulted in macroscopic and microscopic pleurodesis. Lang-Lazdunski et al., did not advise the use of more than 50 mL of autologous blood for pleurodesis as that volume might cause empyema [9]. In our study, we administered autologous blood at a dose of 2 mL/kg. We determined that the administration of autologous blood resulted in statistically significant pleural fibrosis both macroscopically and microscopically when compared to the control group; however, the resulting microscopic pleural inflammation was not statistically significantly different. The failure to induce pleural inflammation with autologous blood in our experiment supported the Droghetti et al., study [10]; suggesting that autologous blood pleurodesis is a less painful procedure compared to other methods. Furthermore, we determined that the degree of intraparenchymal inflammation was not statistically significantly different compared to the control group; which was administered physiological saline solution only. These results showed that pleurodesis with the use of 2 mL/kg autologous blood induced effective pleural fibrosis and did not not cause inflammation in the pleura and the lung parenchyma.

Talc pleurodesis was first performed by Bethune in a patient; who developed pulmonary collapse associated with tuberculosis surgery [11]. In an experimental animal study; which evaluated

http://doi.org/10.5472/marumj.1191101
Marmara Med J 2022;35(3): 297-302

the efficacy of talc pleurodesis, Light et al., found that microscopic and macroscopic inflammation and fibrosis increased significantly with increasing doses [5]. Moreover; the authors reported that the risk of respiratory insufficiency, acute pneumonia, and adult respiratory distress syndrome (ARDS) increased with the increasing talc doses. Rehse et al., [12] reported that respiratory complications and ARDS developed in 33% and 9% patients, respectively, in association with talc pleurodesis in their study on 5 patients [12]. Yıldırım et al., reported that side effects such as fever, chest pain, vomiting, arrhythmia, hypotension, and convulsion were observed in their talc pleurodesis study with an 81.6% success rate [13]. Werebe et al., found talc particles in the coronary arteries, meninges, urinary tracts, pulmonary arteries, and myocardial tissue of rats 24 hours after the intrapleural talc administration regardless of the dose [14]. Yalçınkaya et al., found that non-steroidal anti-inflammatory drugs (NSAIDs) used in the symptomatic treatment of pleurodesis-associated pain reduced the efficacy of pleurodesis-inducing procedures [15]. We administered talc at a dose of 50 mg/kg to the rats in our study. We found out that the administration of talc was statistically superior in inducing macroscopic and microscopic pleural fibrosis compared to the control group; however, these results in the talc pleurodesis group were not statistically different compared to the iodopovidone, rifamycin, and autologous blood groups. The evaluation of the pleural inflammation under the microscope revealed the significant statistical superiority of talc over the saline solution and the other agents used in the study. Furthermore, we found out that talc induced more intraparenchymal inflammation compared to the control group and the rifamycin and autologous blood groups. Similarly, Yazıcıoğlu et al., found significantly more intraparenchymal inflammation in the talc group compared to the control group [16]. The evaluation of the contralateral hemithorax revealed that the rates of the macroscopic pleural fibrosis and microscopic pleural inflammation were significantly different in the talc group again compared to the other groups. In their study with talc pleurodesis; Gözübüyük et al., found statistically significantly higher incidences of acute and subacute oedema and haemorrhage in the contralateral lung, suggesting that these could be the causing factors resulting in ARDS, which was reported as a talc-associated complication [17].

Rifamycin was observed to cause fibrosis and inflammation during wound healing. Döngel et al., found out that 20 mg/kg rifamycin induced pleurodesis microscopically and macroscopically as much effective as talc and that the efficacy of rifamycin was statistically higher compared to autologous blood pleurodesis [18]. Furthermore, the authors stressed that rifamycin should be considered as an alternative to talc because it was less costly compared to talc and did not cause side effects statistically significantly. In our study, we administered 12.5 mg/kg rifamycin to the rats for pleurodesis. We found out that rifamycin caused macroscopic and microscopic pleural fibrosis significantly compared to the control group; however, these results were not statistically significantly different compared to the talc and autologous blood groups. We observed that talc induced more microscopic pleural inflammation compared to rifamycin. However, rifamycin caused less inflammation in the lung parenchyma compared to talc. Although, we did not observe any statistical differences in rifamycin-induced intraparenchymal inflammation compared to the two other sclerosing agents, iodopovidone and autologous blood; the mean scores of intraparenchymal inflammation were observationally less with rifamycin (mean=1.57±0.535) compared to iodopovidone (mean=2.00 ±0.816).

Iodopovidone is a topical antiseptic. It was used for pleurodesis in 1991 for the first time [19]. Agarwal et al., reported that iodopovidone was a safe and 90.6% effective agent for pleurodesis, causing fewer side effects compared to talc [20]. In a study comparing the efficacy of iodopovidone and bleomycin pleurodesis, no significant differences were found in the efficacy and associated side effects between those two sclerosing agents [21]. Although, the mechanism of iodopovidone pleurodesis is not fully known, it is suggested that it makes the environment acidic, resulting in an inflammatory response [20]. The study by Ibrahim et al., compared the efficacy and side effects of iodopovidone and talc but found no statistically significant differences between them [22]. Although, it has been suggested that the iodopovidone use may impair thyroid functions [23], Yeğinsu et al., did not detect any effects of 2% iodopovidone on thyroid functions in their study of pleurodesis [24]. In our study, we administered 2% iodopovidone to the rats in Group 2. It was observed that iodopovidone produced more pleural fibrosis macroscopically and microscopically compared to the control group. However, no significant differences of iodopovidone effects were observed compared to the other sclerosing agents used in the experiment. We found out that the administration of iodopovidone resulted in statistically significantly more microscopic pleural fibrosis compared to the control group but this effect was statistically insignificant compared to the rifamycin and autologous blood groups. We observed that talc induced more microscopic pleural inflammation compared to iodopovidone. The examination of intraparenchymal inflammation revealed no significant differences of iodopovidone compared to the other sclerosing agents used in the experiment.

5. CONCLUSION

In conclusion; iodopovidone, autologous blood, talc, and rifamycin can be used as effective pleurodesis agents. However, our findings demonstrate that rifamycin and autologous blood are safer agents for the treatment of patients with the diseases of pulmonary parenchyma compared to the other sclerosing agents used in this experiment because rifamycin and autologous blood induce intraparenchymal inflammation to a lesser degree. The sclerosing agents used in our study are less costly compared to talc. Therefore, the selection of these agents for pleurodesis may offer further advantages. Studies about the cost-effectiveness of these agents are warranted to obtain further information.

Compliance with Ethical Standards

Ethical Approval: The present study protocol was approved by the approval obtained from the Animal Experiments Local Ethics Committee of Balıkesir University (Decision No: 2019/10-2).

http://doi.org/10.5472/marumj.1191101
Marmara Med J 2022;35(3): 297-302

Financial Support: This manuscript did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Authors' Contribution: CB: Conception and design of the work, and the acquisition, analysis, and interpretation of the data for the work and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved; final approval of the version to be published; drafting the work; designing of the work; Interpretation of data for the work.

MK: Drafting the work or revising it critically for important intellectual content; Designing of the work; interpretation of data for the work.

FA: Substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data for the work, drafting the work or revising it critically for important intellectual content.

ACY: The acquisition, analysis, or interpretation of data for the work, drafting the work or revising it critically for important intellectual content. All authors approved the final version of the article.

REFERENCES

[1] Light RW, Vargas FS. Pleural sclerosis for the treatment of pneumothorax and pleural effusion. Lung 1997;175:213-23.

[2] Ozpolat B, Gazyagci S, Gözübüyük A, Ayva S, Atinkaya C. Autologous blood pleurodesis in rats to elucidate the amounts of blood required for reliable and reproducible results. J Surg Res 2010:15;161:228-32. doi: 10.1016/j.jss.2009.01.027.

[3] Teixeira LR, Vargas FS, Acencio MM, Bumlai RU, Antonangelo L, Marchi E. Experimental pleurodesis induced by antibiotics (macrolides or quinolones). Clinics 2006;61:559-64.

[4] Ahn HY, Cho JS, Kim YD, et al. Efficacy of mistletoe for chemical pleurodesis in rats without malignancy. Open Med (Wars) 2015:4;10:346-51. doi: 10.1515/med-2015-0051.

[5] Light RW, Wang NS, Sassoon CS, Gruer SE, Vargas FS. Talc slurry is an effective pleural sclerosant in rabbits. Chest 1995;107:1702-6.

[6] Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. Malays J Med Sci 2017; 24: 101-5. doi: 10.21315/mjms2017.24.5.11.

[7] Mitchem RE, Herndon BL, Fiorella RM, Molteni A, Battie CN, Reisz GR. Pleurodesis by autologous blood, doxycycline, and talc in a rabbit model. Ann Thorac Surg 1999;67:917-21.

[8] Robinson CLN. Autologous blood for pleurodesis in recurrent and chronic spontaneous pneumothorax. Can J Surg 1987;30:428-9.

[9] Lang-Lazdunski L, Coonar AS. A prospective study of autologous 'blood patch' pleurodesis for persistent air leak after pulmonary resection. Eur J Cardiothorac Surg 2004;26:897-900.

[10] Droghetti A, Schiavini A, Muriana P, et al. Autologous blood patch in persistent air leaks after pulmonary resection. J Thorac Cardiovasc Surg 2006;132:556-9.

[11] Bethune N. Pleural poudrage: new technique for deliberate production of pleural adhesions as preliminary to lobectomy. J Thorac Surg 1935; 4:251-61.

[12] Rehse DH, Aye RW, Florence MG. Respiratory failure following talc pleurodesis. Am J Surg 1999;177:4:437-40.

[13] Yildirim H, Metintas M, Ucgun I, Erginel S, Alatas F, Kurt E. Talc Plörodezise bağlı gelişen yan etkiler. Osman Gazi Tıp Dergisi 2005;27:51-6.

[14] Werebe EC, Pazetti R, Milanez de Campos JR, et al. Systemic distribution of talc after intrapleural administration in rats. Chest 1999;115:190-3.

[15] Yalçınkaya S, Yalçınkaya U. Non-steroid anti-inflammatory drugs reduce the efficacy of autologous blood pleurodesis. Turk Gogus Kalp Dama 2019;27:343-9. doi: 10.5606/tgkdc.dergisi.2019.17544

[16] Yazicioglu A, Uysal S, Sahinoglu T, Subasi M, Demirag F, Yekeler E. Does sericin, as a novel pleurodesis agent, have higher effectiveness compared to talcum powder, doxycycline, and silver nitrate pleurodesis? Arch Bronconeumol 2019;55:357-67. doi: 10.1016/j.arbres.2018.10.003.

[17] Gözübüyük A, Ozpolat B, Ciçek AF, et al. Comparison of side effects of oxytetracycline and talc pleurodesis: an experimental study. J Cardiothorac Surg 2010:13;5:128. doi: 10.1186/1749-8090-5-128.

[18] Dongel İ, Sunam GS, Külahçı Ö, Ceran S. A comparison of the efficacies of heterologous blood, rifamycin, and talc as pleural sclerosants in rabbits: An experimental study. Turk Gogus Kalp Dama 2012;20:590-95.

[19] Echavarría A, Pinzón V, Barés JP, Fernández E. Intracavitary treatment of malignant pleural effusion with iodine-povidone. Rev Med Panama 1991;16:69-74.

[20] Agarwal R, Aggarwal AN, Gupta D, Jindal SK. Efficacy and safety of iodopovidone in chemical pleurodesis: A meta-analysis of observational studies. Respir Med 2006;100:2043-7, doi:http://dx.doi.org/10.1016/j.rmed.2006.02.009.

[21] Bagheri R, Noori M, Rajayi M, et al. The effect of iodopovidone versus bleomycin in chemical pleurodesis. Asian Cardiovasc Thorac Ann 2018;26:382-386. doi: 10.1177/021.849.2318778485.

[22] Ibrahim IM, Dokhan AL, El-Sessy AA and Eltaweel MF. Povidone-iodine pleurodesis versus talc pleurodesis in preventing recurrence of malignant pleural effusion. J Cardiothorac Surg 2015;1;10:64. doi: 10.1186/s13019.015.0270-5.

[23] Bryant WP, Zimmerman D. Iodine-induced hyperthyroidism in a newborn. Pediatrics. 1995;95:434-6. Erratum in: Pediatrics 1995;96:779.

[24] Yeginsu A, Karamustafaoglu A, Ozugurlu F. Etikan I. Iodopovidone pleurodesis does not effect thyroid function in normal adults. Interact Cardiovasc Thorac Surg 2007;6: 563-4.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Assessment of the factors affecting the loss of workforce in patients with traumatic hand injury

Kardelen GENCER-ATALAY[1], Merve DEMIRCI[1], Ozgur BAYSAL[2], Canan SANAL TOPRAK[1]

[1] Department of Physical Medicine and Rehabilitation, School of Medicine, Marmara University, Istanbul, Turkey.
[2] Department of Orthopedics and Traumatology, School of Medicine, Marmara University, Istanbul, Turkey.

Corresponding Author: Kardelen GENCER-ATALAY
E-mail: kardelengencer@gmail.com

Submitted: 23.01.2022 **Accepted:** 29.04.2022

ABSTRACT

Objective: This study aimed to examine the factors affecting the loss of workforce, including the time to return to work and work-related situations in patients with traumatic hand injury who were taken to a hand rehabilitation program.

Materials and Methods: The patients aged between 18-65 years with a history of traumatic hand injury in the last five years were analyzed retrospectively. Demographic and clinic data were taken from the medical records, and work-related problems were obtained by telephone calls. The severity of hand injury was assessed with Modified Hand Injury Severity Score (MHISS).

Results: A total of 147 patients (129 males, 18 females; mean age 39.83±10.4 years) were included. The duration of return to work was correlated with total MHISS (rho=0.262 p=0.003) while not related to age, education level, gender, or injured hand's dominance (p>0.05). Duration of return to work after hand injury and total MHISS were lower in the patients who had job modifications (p<0.001, p=0.002). Job modification rate, salary reduction, and patient-reported hand dexterity loss were higher in patients with work-related injuries (p<0.05).

Conclusion: This study highlighted that the increasing severity of hand injury caused a prolonged time to return to work, or job scope changes.

Keywords: Hand injury, Modified Hand Injury Severity Score, Return to work, workplace engagement

1. INTRODUCTION

Traumatic hand injury is the leading cause of work-related disability in the productive aged population [1]. It ranges from 'simple' injuries such as an isolated fracture to complex crush injuries that places significant health and economic burdens on patients [2]. Most injuries, apart from their severity, limit daily and professional activities that result in personal, social, and work-related consequences. Work-related professional activities are known to be affected more than daily living activities [3,4]. Furthermore, the injuries that cause permanent hand dysfunctions are shown to affect returning to work directly [5]. The degree of the traumatic hand injury is found to be related to functional results that indicate the long-term disability [1,3]. It predicts the situation for returning to the same job, modifying the job scope, a complete change of position, or not working. Therefore, determining the degree of the injury is crucial for anticipating work-related problems and taking precautions. It is also important in terms of preparing patients psychologically and initiating early rehabilitation that minimizes the life-wide impacts of the injury [3].

Most countries support rehabilitation programs to facilitate patients with hand injuries for returning to work [2]. Consequently, the return to work rate for traumatic hand injury patients has been well investigated and found to be related to factors such as the degree of injury, injury site, and pre-injury salary [3,6]. However, knowledge about these factors after a comprehensive hand rehabilitation program is limited.

The aim of this study is to assess the factors affecting the time to return to work and work-related situations, including job modifications, salary changes, and indemnification in the

How to cite this article: Gencer-Atalay K, Demirci M, Baysal O, Toprak Sanal C. Assessment of the factors affecting the loss of workforce in patients with a traumatic hand injury. Marmara Med J 2022; 3; 35(3):303-307. doi: 10.5472/marumj.1186710

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1186710
Marmara Med J 2022;35(3): 303-307

patients with traumatic hand injuries who were taken to a comprehensive hand rehabilitation program.

2. MATERIALS and METHODS

This cohort study was conducted between September 2, 2020, and January 20, 2021. Participants were selected by a retrospective analysis of the patients taken into a hand rehabilitation program in the last five years at the Marmara University Physical Medicine and Rehabilitation Department, Istanbul, Turkey. The study was approved by Marmara University School of Medicine Ethics Committee (approval number 09.2020.721, date 24.07.2020), and verbal informed consent was obtained from all participants by telephone calls. The inclusion criteria were determined to have had a traumatic hand or hand and forearm injury and to be aged between 18 and 65 years by the time the patient was receiving the hand rehabilitation. Patients who had bilateral traumatic hand and forearm, who were unemployed before the traumatic hand injury, and who received a hand rehabilitation for less than fifteen sessions were excluded.

Age, gender, hand dominance, education, occupational status, the type of traumatic injury, localization of the injured area, size of the incision, the damaged structures, and surgically repaired structures, and the number of hand rehabilitation sessions were taken from the medical records. The Modified Hand Injury Severity Score (MHISS) was calculated by the same experienced orthopedist (OB). Then, time to return to work and work-related situations after the traumatic hand injury, including changes in the workplace, job scope, or salary amount, and indemnification status for the injuries due to a work-related accident were obtained from the telephone calls by two physiatrists (ZKA, MD). The time to return to work was defined as the duration between the date of the injury and the work start date. The current hand dexterity was also assessed by self-reports of patients with a 3-point Likert scale; 1: almost the same/minor as before the injury, 2: moderate decrease compared to pre-injury, and 3: severe decrease compared to pre-injury.

Modified Hand Injury Severity Score (MHISS)

Modified Hand Injury Severity Score was originally developed as a descriptive severity scoring system for hand injuries distal to the carpus (zone I, II, and III) [7]. The MHISS was designed to include injuries proximal to the carpus (zone VI, V) [3]. The MHISS has four subgroups: integument, skeletal, motor, and neurovascular components. These subgroups are calculated by considering all injured tendons and intrinsic muscles, injured zone, affected fingers, accompanying neurovascular injuries, lacerations or losses in the skin, fractures, dislocations, and ligament injuries. Each subgroup contains both absolute scores and weighted scores according to the functional importance of the affected finger. As an example, first finger injuries are weighted more significantly than other finger injuries. Additionally, the score of the subgroup is doubled by the factors such as wound contamination and crush injury. The total MHISS is finally calculated by the sum of the scores of four subgroups and expressed as Minor (MHISS < 20), Moderate (MHISS 21–50), Severe (MHISS: 51–100), or Major injury (MHISS >101) by Campbell and Kay [7].

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) 22.0 was used for the statistical analysis. The Shapiro-Wilk test was used to evaluate the normality of data distribution. The descriptive statistical methods (frequency, percentage, mean, standard deviation, minimum, maximum, median, and interquartile range) were performed. The Chi-Square test was used to examine the differences between categorical variables. The Kruskal–Wallis and Mann–Whitney U tests were used to compare the differences between continuous variables. Spearman rank correlation analysis was performed to analyze correlations. $P<0.05$ was set as statistical significance at a 95% confidence interval.

3. RESULTS

A total of 403 patients taken into a hand rehabilitation program in the last five years due to a traumatic hand or hand and forearm injury were retrospectively reviewed. Of these, 322 patients were found to be eligible for the study, and 218 were reached by telephone calls and informed about the study. One patient was excluded because of miscommunication, two patients were excluded because of receiving a hand rehabilitation program for less than fifteen sessions, six patients were excluded because of bilateral hand and forearm injuries, and six patients did not agree to participate and fifty-six patients were excluded because of being unemployed before the injury. Thus 147 patients were included in the study (Figure I). The demographic characteristics and clinical features of the participants are shown in Table I. Return to work periods in different MHISS subgroups and their comparisons are displayed in Table II. Since, the number of patients in severe and major injury subgroups were relatively small, it was decided to combine them to create a sufficient number of patients for further analysis. The work-related situations including returning to the same workplace or same job or not returning, a change in salary status, indemnification, and impairment of the injured hand skill according to the patient are expressed in Table III. The return to work period and the work-related situations according to a work-related accident are shown in Table IV.

The return to work period was found to be positively weak correlated with total MHISS ($r=0.262$ $p=0.003$). There was no relationship between return to work period and age, education level, gender, or injured hand's dominance ($p>0.05$). There was no difference in the time to return to work or total MHISS in the blue-collar workers compared to white-collar workers ($p=0.095$, $p=0.821$). The time to return to work and total MHISS were significantly lower in the patients who returned to the same job

http://doi.org/10.5472/marumj.1186710
Marmara Med J 2022;35(3): 303-307

at the same workplace when compared to the others who had job modifications (p<0.001, p=0.002).

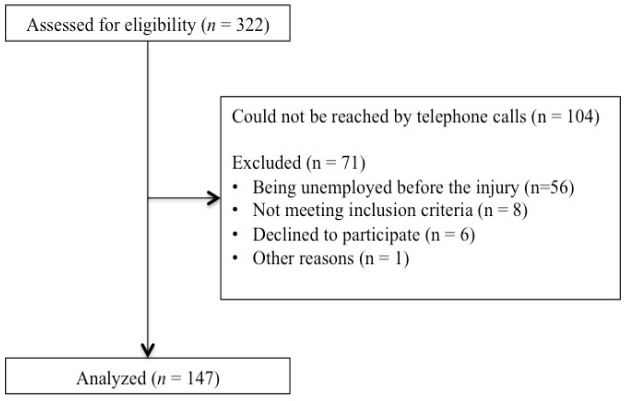

Figure I. *Flow diagram of participants*

Table I. *The demographic characteristics and clinical features of the participants*

Age (years)	*Min-Max*	18-65
	Mean±Sd	39.83±10.4
Gender	*Female*	18 (12.2%)
	Male	129 (87.6%)
Educational status	*Literate*	3 (2%)
	Primary school	44 (29.9%)
	Secondary school	19 (12.9%)
	High school	51 (34.7%)
	University	27 (18.4%)
	Postgraduate	3 (2%)
Pre-injury occupational category	*Blue collar worker*	129 (87.8%)
	White collar worker	18 (12.2%)
Reason of the traumatic hand and forearm injury	*Work-related accident*	77 (52.4%)
	Not	70 (47.6%)
Dominance of the traumatic hand and forearm injury	*Dominant side*	79 (53.7%)
	Non-dominant side	68 (46.3%)
Total MHISS	*Min-Max*	2-212
	Mean±Sd	27.27±28.5
MHISS classification	*Minor*	104 (70.7%)
	Moderate	23 (15.6%)
	Severe	14 (9.5%)
	Major	6 (4.1%)

Data= Mean±SD, n(%), MHISS= Modified Hand Injury Severity Score

Table II. *Comparison of the return to work period according to MHISS subgroups*

MHISS Subgroups	Return to work period (day) Median (IQR)	p value
Minor	60 (45-120)	[a]**<0.001**
Moderate	90 (40-180)	
Severe/Major	360 (108.75-905)	
Minor versus Moderate		[b]0.279
Minor versus Severe/Major		[b]**<0.001**
Moderate versus Severe/Major		[b]**0.003**

Data= Median (IQR), MHISS= Modified Hand Injury Severe Score, [a]Kruskal-Wallis Test, [b] Mann-Whitney U Test

Table III. *The work-related situations for the pre-injury working patients*

Return to work	*Same workplace-same job*	82 (55.7%)
	Same workplace – different job	11 (7.5%)
	Different workplace-same job	16 (10.9%)
	Different workplace – different job	21 (14.3%)
	Did not return	17 (11.6%)
Change in salary status	*Same*	111 (85.4%)
	Decreased	19 (14.6%)
Indemnification for the work-related accident	*Yes*	6 (7.8%)
	No	71 (92.2%)
The impairment of injured hand skill according to the patient	*None/minor*	61 (46.9%)
	Moderate	55 (42.3%)
	Severe	14 (10.8%)

Data= n(%)

Table IV. *The return to work period and the work-related situations for the pre-injury working patients according to a work-related accident*

		Work-related accident		*P value*
		Yes (n=71)	**No (n=59)**	
Return to work period (days)	*Median (IQR)*	70 (45-120)	60 (30-120)	[a]0.067
Total MHISS	*Median (IQR)*	20 (15-38)	20 (15-32)	[a]0.573
MHISS classification	*Minor*	42 (59.2%)	38 (64.4%)	[b]0.847
	Moderate	18 (25.4%)	13 (22%)	
	Severe/Major	11 (15.5%)	8 (13.6%)	
Job description	*Blue collar worker*	69 (95.8%)	43 (72.9%)	[c]**<0.001**
	White collar worker	2 (4.2%)	16 (27.1%)	
Job modification	*Yes*	34 (47.9%)	14 (23.7%)	[c]**0.006**
	No	37 (52.1%)	45 (76.3%)	
Change in salary status	*Same*	55 (77.5%)	56 (94.9%)	[c]**0.006**
	Decreased	16 (22.5%)	3 (5.1%)	
Dominance of the traumatic hand and forearm injury	*Dominant*	32 (45.1%)	44 (74.6%)	[c]**0.001**
	Non-dominant	39 (54.9%)	15 (25.4%)	
The impairment of injured hand skill according to the patient	*None/minor*	25 (35.2%)	36 (61%)	[c]**0.01**
	Moderate	38 (53.5%)	17 (28.8%)	
	Severe	8 (11.3%)	6 (10.2%)	

Data= Median (IQR), n (%), MHISS= Modified Hand Injury Severity Score, [a]Mann Whitney U Test, [b]Fisher Freeman Halton Test, [c]Pearson Chi-square Test

http://doi.org/10.5472/marumj.1186710
Marmara Med J 2022;35(3): 303-307

4. DISCUSSION

This study revealed that the degree of the traumatic injury had direct effects on the time to return to work and work-related situations even in the patients who were taken to a comprehensive hand rehabilitation program. The increasing severity of the injury was found to cause prolonged resting periods, changes in the workplace, and the job scope. Age, education level, gender, or injured hand dominance were not detected to be related to the return to work period. Similarly, in previous studies, the degree of the injury was defined to be an important determinant factor for return to work, while hand dominance did not have an effect [2,3]. The patients with severe or major MHISS were shown to have a longer return to work period than the patients with minor and moderate MHISS. Consistent with this finding, Urso-Baiarda et al., also demonstrated increasing resting durations at median values of 30, 30, 118, and 760 days with minor, moderate, severe, and major subgroups [3]. However, the correlation between total MHISS and return to work period was found to be weak (r=0.262). This was unconvincing as in the study conducted by Watts et al., despite several studies that reported stronger correlation coefficients, ranging from 0.40 to 0.98 [6-10]. The results of these studies, could indicate preferring to use subgroups rather than the total score of MHISS for predicting the return to work period after a traumatic hand and forearm injury. Because the severity of injury subgroups as a classification system could reflect the return to work prediction more accurately than the scoring system as a continuous variable. Another important finding of this study was that higher severity of injury scores was detected in the patients who had to modify their jobs after the injury. Although, traumatic hand injury was confirmed to have an enormous impact on work-related activities by many studies, the workplace and job scope changes have not been assessed extensively [2,5,9,11]. One study reported that motion area loss was more significant in the patients who returned to work with a job modification after a traumatic hand injury [5]. The current functional status of the patients was not evaluated in this study; however, both severity of injury scores and return to work period were shown to be higher in the patients who had job modifications. These results indicated that the patients with higher severity of injury required more time to recover, and they needed job modifications more frequently when they reached enough abilities to start work.

This study pointed out that work-related injury ended up with a longer duration of going back to work. Besides, job modification and salary reduction were found to be significantly higher in patients with work-related injuries. In the literature, traumatic hand injury was confirmed to cause many work-related limitations by a number of studies [2,5,9,11]. However, most of these studies have mainly included patients with work-related injuries, and barely of them assessed the comparison according to work-related and non-work-related injuries. One study highlighted that patients injured by work-related damages were more likely to take longer resting durations [12]. Other studies suggested that patients who held someone else responsible for their injury had a more extended return to work period [13-15]. An interesting finding of this study was that most of the patients with work-related injuries had mainly impaired their non-dominant hands while the other patients had their dominant hands. In addition, the patients with work-related injuries self-reported to have lost more hand dexterity, despite having similar injury severity scores to the others. This could be explained by the higher ratio of blue-collar workers in the patients with work-related injuries group. These patients might have perceived more decreased hand dexterity because of the blue-collar jobs that generally require hard manual labor and high physical demands [13].

We have some limitations. As the study was conducted during the pandemic period we could not be able to perform face-to-face examinations. Therefore, the main limitation of this study was that we could not be able to assess the current functional status of the patients. Another one was evaluating hand dexterity by a patient-reported Likert scale. However, it is still a sound study because all of the patients included had taken to a comprehensive hand rehabilitation program. Moreover, patients were also investigated according to work-related injuries.

In conclusion, the degree of injury came to the forefront as a determinant factor for returning to work and work-related changes in this study. In addition, the work-related injuries showed up to be an aggravator for longer return to work period, job modification, and salary reduction. This information should be taken into consideration in the prediction and administration of workforce loss.

Compliance with Ethical Standards

Ethical Approval: Ethical approval for this study was obtained from **the** Marmara University School of Medicine Ethics Committee (approval number 09.2020.721, date 24.07.2020) and verbal informed consent was obtained from all participants by telephone calls.

Financial Support: The authors received no financial support for the research and/or authorship of this article.

Conflict of Interest: The authors declare no conflicts of interest with respect to the authorship and/or publication of this article.

Author Contributions: KGA: Concept, design, data collection, analysis, literature research, writing, critical review, MD: Concept, design, materials, data collection, literature research, OB: Concept, design, supervision, materials, data collection, writing, critical review, CST: Concept, design, supervision, materials, data collection, writing, critical review. All authors read the article and approved the final version of the article.

REFERENCES

[1] Saxena P, Cutler L, Feldberg L. Assessment of the severity of hand injuries using 'hand injury severity score', and its correlation with the functional outcome. Injury 2004; 3:511-6. doi: 10.1016/S0020-1383(03)00211-0.

[2] Shi Q, Sinden K, MacDermid JC, Walton D, Grewal R. A systematic review of prognostic factors for return to work following work-related traumatic hand injury. J Hand Ther 2014; 27:55-62. doi: 10.1016/j.jht.2013.10.001.

[3] Urso-Baiarda F, Lyons RA, Laing J, Brophy S, Wareham K, Camp D. A prospective evaluation of the Modified Hand Injury Severity Score in predicting return to work. Int J Surg 2008; 6:45-50. doi: 10.1016/j.ijsu.2007.09.001.

[4] Cabral L, Sampaio RF, Figueiredo IM, Mancini MC. Factors associated with return to work following a hand injury: a qualitative/quantitative approach. Rev Bras Fisioter 2010; 14:149-57.

[5] Chang J-H, Wu M, Lee C-L, Guo Y-L, Chiu H-Y. Correlation of return to work outcomes and hand impairment measures among workers with traumatic hand injury. J Occup Rehab 2011; 21:9-16. doi: 10.1007/s10926.010.9246-4.

[6] Çapkın S, Cavit A, Yılmaz K, Erdoğan E, Kaleli T. Associations between initial injury severity in acute hand, wrist or forearm injuries and disability ratings and time to return to work. Turkish Journal of Trauma and Emergency Surgery 2020; 26:453-61. doi: 10.14744/tjtes.2019.40524.

[7] Campbell D, Kay S. The hand injury severity scoring system. J Hand Surg Am 1996; 21:295-8. doi: 10.1016/s0266-7681(05)80187-1.

[8] Watts A, Greenstock M, Cole R. Outcome following the rehabilitation of hand trauma patients: The importance of a subjective functional assessment. J Hand Surg Am 1998; 23:485-9. doi: 10.1016/s0266-7681(98)80128-9.

[9] Wong JY. Time off work in hand injury patients. J Hand Surg 2008; 33:718-25. doi: 10.1016/j.jhsa.2008.01.015.

[10] Mink Van Der Molen A, Ettema A, Hovius S. Outcome of hand trauma: the hand injury severity scoring system (HISS) and subsequent impairment and disability. J Hand Surg Am 2003; 28:295-9. doi: 10.1016/s0266-7681(03)00082-2.

[11] Feuerstein M, Huang GD, Ortiz JM, Shaw WS, Miller VI, Wood PM. Integrated case management for work-related upper-extremity disorders: impact of patient satisfaction on health and work status. J Occup Environ Med 2003; 45:803-12. doi: 10.1097/01.jom.000.007.9091.95532.92.

[12] Opsteegh L, Reinders-Messelink HA, Schollier D, et al. Determinants of return to work in patients with hand disorders and hand injuries. J Occup Rehabil 2009; 19:245-55. doi: 10.1007/s10926.009.9181-4.

[13] Neutel N, Houpt P, Schuurman AH. Prognostic factors for return to work and resumption of other daily activities after traumatic hand injury. J Hand Surg (European volume) 2019; 44:203-207. doi: 10.1177/175.319.3418812645.

[14] Grunert Bk, Dzwierzynski Ww. Prognostic factors for return to work following severe hand injuries. Tech Hand Up Extrem Surg 1997; 1:213-8. doi: 10.1097/00130.911.199709000-00008.

[15] Rusch MD, Dzwierzynski WW, Sanger JR, Pruit NT, Siewert AD. Return to work outcomes after work-related hand trauma: the role of causal attributions. J Hand Surg 2003; 28:673-7. doi: 10.1016/s0363-5023(03)00178-3.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Efficacy of tocilizumab therapy in severe COVID-19 pneumonia patients and determination of the prognostic factors affecting 30 days mortality

Leman DELEN[1], Umut Sabri KASAPOGLU[2], Abdullah GOK[1], Ozlem CAGASAR[3], Bora TETIK[4], Ersoy OKSUZ[5]

[1] Department of Anesthesiology and Reanimation, Malatya Training and Research Hospital, Malatya, Turkey.
[2] Department of Pulmonary and Critical Care Medicine, Malatya Training and Research Hospital, Malatya, Turkey.
[3] Department of Infectious Diseases and Clinical Microbiology, Training and Research Hospital, Malatya, Turkey.
[4] Department of Neurosurgery, Inonu University, School of Medicine, Malatya, Turkey.
[5] Department of Pharmacology, Malatya Turgut Ozal University, School of Medicine, Malatya, Turkey.

Corresponding Author: Umut Sabri KASAPOGLU
E-mail: umutkasapoglu@gmail.com

Submitted: 23.04.2022 **Accepted:** 15.06.2022

ABSTRACT

Objective: In coronavirus disease – 19 (COVID-19) patients, cytokine storm develops due to the increase of pro-inflammatory cytokines. Tocilizumab (TCZ), has been used in the treatment of COVID-19 patients and successful results have been obtained. The aim of this study was to determine the efficacy of TCZ and also investigate the prognostic factors affecting the success of treatment and mortality in COVID-19 patients treated with TCZ.

Patients and Methods: Between March 2020 and August 2021, a total of 326 confirmed severe COVID-19 pneumonia patients, treated in the intensive care unit, were included in the study.

Results: The mean age of the patients was 63.02±11.58 years, and 203 (62.3%) of the patients were male. Patients treated with TCZ had a longer survival time compared with the standard therapy (p=0.012). It was found that type of respiratory support (HR:2.19, CI:1.10-4.36, p=0.025) and hyperlactatemia on the day of TCZ therapy admission (HR:2.93 CI:1.53-5.64, p=0.001) were the significant and independent prognostic factors of survival in severe COVID-19 pneumonia patients treated with TCZ.

Conclusion: Tocilizumab therapy improved 30-days survival in critically ill COVID-19 pneumonia patients. Also, among the patients with TCZ, types of respiratory support and hyperlactatemia on the day of TCZ admission were the independent prognostic factors.

Keywords: SARS-CoV-2, COVID-19, Tocilizumab, Viral pneumonia, Cytokine storm, Prognostic factors

1. INTRODUCTION

The first cases of pneumonia caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) began to be seen at the end of 2019, in Wuhan, China, and subsequently, the novel coronavirus disease-2019 (COVID-19) spread the whole world and as a result of the rapid increase in the number of cases it was announced to be a pandemic [1-4].

Coronavirus disease-19 has a broad spectrum of clinical manifestations. In the majority of cases, the disease is mild to moderate, and the symptoms are similar to the typical symptoms of acute respiratory infection such as fever, cough, throat pain, and fatigue [5-7]. Although, it usually causes common cold symptoms, approximately 20% of the patients develop severe illness that requires supplemental oxygen therapy. Also, approximately 5% of patients develop critical illness with respiratory failure, which eventually progresses to multi-organ dysfunction and death [6-8].

In severe cases of COVID-19 pneumonia, the immune response to the viral induction plays a key role in the rapid progression of the disease to respiratory distress syndrome (ARDS) and multi-organ dysfunction [7, 9]. This uncontrolled response is defined as a cytokine storm and thought to result from the uncontrolled release of inflammatory cytokines. The clinical features of this hyper-inflammation process include, persistent fever, high levels of IL-6 and ferritin, gradually increasing C-reactive protein (CRP), D-dimer elevation, and increased liver enzymes [6, 9]. Since, COVID-19-related cytokine storm is associated with

How to cite this article: Delen L, Kasapoglu US, Gok A, Cagasar O, Tetik B, Oksuz E. Efficacy of tocilizumab therapy in severe COVID-19 pneumonia patients and determination of the prognostic factors affecting 30 days mortality. Marmara Med J 2022; 35(3): 308-315, doi: 10.5472/marumj.1191126

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1191126
Marmara Med J 2022;35(3): 308-315

increased mortality and morbidity, early diagnosis of it is of considerable importance [9, 10].

In studies conducted in the early period of the pandemic, the treatment with tocilizumab (TCZ), which is an IL-6 receptor antagonist, has been reported to be effective on mortality in cases of severe COVID-19 pneumonia and provided significant clinical recovery; although, it is not a specific treatment for the COVID-19-related cytokine storm [9-13]. However, in the studies conducted later in the pandemic process, it has been reported that TCZ treatment had no effect on survival and the need for mechanical ventilation in severe COVID-19 pneumonia cases [14, 15]. Therefore, clinicians need to know which patient group will benefit from TCZ treatment in cases of severe COVID-19 pneumonia.

The aim of the present study was to evaluate the efficacy of TCZ treatment in the critically ill COVID-19 pneumonia patients and also determine the prognostic factors for 30 days mortality to better select the most appropriate patients for the TCZ treatment.

2. PATIENTS AND METHODS

Patients and data

Between March 2020 and August 2021, a total of 326 confirmed severe COVID-19 pneumonia patients treated in intensive care units were included into the study.

The data were obtained from the hospital's medical record system. The demographic and clinical data, laboratory findings, the number of days between the onset of symptoms and the initiation of TCZ treatment, types of respiratory support, length of stay in the ICU, outcomes, scores on the Acute Physiology and Chronic Health Evaluation II (APACHE II) [16] and Sequential Organ Failure Assessment (SOFA) [17] were recorded. All data and scores reported in this study were collected within the first 24 hours following the admission of the intensive care unit (ICU).

The patients were divided into two groups as survivors and non-survivors. The clinical and laboratory data of the patients in both groups at the time of TCZ treatment administration were evaluated and compared.

Definitions

Confirmed COVID-19 case was defined as a clinical suspect case with positive SARS-CoV-2 real-time reverse transcription polymerase chain reaction (RT-PCR) from the upper respiratory sample (nasopharyngeal and/or oropharyngeal swab) or lower respiratory tract sample (tracheal aspirate). And, the COVID-19 pneumonia case was defined as a confirmed COVID-19 case that has pulmonary infiltrates in thorax computed tomography.

Tocilizumab treatment was administered intravenously at a dose of 8 mg / kg (to a maximum dose of 800 mg per infusion); it was administered 400 mg or 800 mg IV, depending on the severity of the patient's symptoms. When the first dose was administered as 400 mg, the dose was repeated as 200-400 mg for 24 hours, taking into account the changes in clinical and laboratory findings. Indications of the TCZ treatment in severe COVID-19 pneumonia patients have included clinical worsening due to COVID-19 pneumonia (persistent or increasing oxygen demand and fever), and high inflammatory biomarkers (elevated level of serum CRP, LDH, D-dimer and ferritin, decreased lymphocyte count and serum albumin level). Standard therapy defined as the patients received standard care according to national practice guidelines that include the concomitant use of antiviral treatment (hydroxychloroquine, azithromycin and favipiravir), antibiotics and supportive care.

APACHE-II and SOFA scores of the patients were used for the assessment of the severity of illness. The APACHE-II score is a number between 0 and 71 that is calculated based on current physiologic measures, age, and previous medical history; higher scores indicate more severe disease and a higher risk of mortality. The SOFA score is used to monitor a patient's organ dysfunction while in an ICU. This scoring system is composed of six independent scores for the respiratory, cardiovascular, hepatic, coagulation, renal, and neurological systems, with one score for each. The average and highest scores are the most accurate predictors of mortality. These scores were calculated based on the worst clinical and laboratory findings of the patients observed during the first 24 h following the admission to the ICU [16, 17].

All patients were followed during their ICU stay or until death and mortality was defined as death within 30 days after the ICU admission. Mortality data of the patients were collected from the hospital medical record system.

Statistical analysis

After the data obtained from the hospital database they were arranged and transferred to Microsoft Excel tables. The data compatible with normal and homogeneous distribution were expressed as mean ± standard deviation and data without normal or homogeneous distribution were expressed as min-max values by numbers and percentages. The distribution of the variables was controlled by the Skewness-Kurtosis. In the comparison of two independent groups, the independent samples t test was used for independent variables for the analysis of the parametric data, the Mann-Whitney-U test was used for the analysis of non-parametric data, and the chi-square test was used for the analysis of categorical data. The Kaplan-Meier method was used for the survival curve of the patients and the log-rank test was used to calculate the differences in survival between the groups. Independent variables associated with mortality in the univariate analysis were evaluated with multivariate Cox regression models. Results were evaluated at 95% CI and a value of $p < 0.05$ was accepted as statistically significant. SPSS (Statistical Package for Social Sciences) for Windows 25.0 program was used for statistical analysis.

3. RESULTS

Baseline characteristics of the study population

Between March 2020 and August 2021, a total of 326 severe COVID-19 pneumonia patients, treated at our intensive care unit, were included into the study. The mean age of the patients was 63.02±11.58 years, and 203 (62.3%) of the patients were male. We found that 264 (81.0%) patients had at least one comorbid disease. The most frequent comorbid diseases were hypertension (71.2%), diabetes mellitus (42.6%), and coronary artery disease (35.0%). The baseline characteristics of the study population are summarized in Table I.

Table I. *Baseline demographic characteristics of the patients*

		All patients (n=326)
Mean age, years (Mean ± SD)		63.02±11.58
Age	≥ 65 years	168 (51.5%)
	< 65 years	158 (485%)
Gender	Male, (%)	203 (62.3%)
	Female, (%)	123 (37.7%)
At least one comorbidity	Yes, (%)	264 (81.0%)
	No, (%)	62 (29%)
Comorbid diseases	Hypertension, (%)	232 (71.2%)
	Diabetes mellitus, (%)	139 (42.6%)
	Coronary artery disease, (%)	114 (35.0%)
	Chronic obstructive pulmonary disease, (%)	55 (16.9%)
	Chronic heart failure, (%)	38 (11.7%)
	Alzheimer disease, (%)	36 (11.0%)
	Cerebrovascular disease, (%)	19 (5.8%)
	Chronic kidney disease, (%)	14 (4.3%)
	Rheumatologic disease, (%)	8 (2.5%)
	Malignancy, (%)	8 (2.5%)
SOFA score, (Min-Max)		4 (2-9)
APACHE-II score, (Mean ± SD)		17.32±4.20

SOFA: sequential organ failure assessment, APACHE-II: acute physiology assessment and chronic health evaluation II, Min: minimum, Max: maximum, SD: standard deviation

Comparison of the baseline laboratory findings between patients treated with TCZ and patients treated with standard therapy

The patients were grouped as TCZ therapy group (n=110) and standard therapy group (n=216). We found that, mean age of the standard therapy group significantly higher compared with TCZ therapy group (59.86±10.91 vs 64.63±11.61, p<0.001), and also proportion of the patients with ≥65 years old higher in standard therapy group (44 (40.0%) vs 124 (57.4%), p=0.003).

Baseline laboratory parameters of the two groups on the day of ICU admission are summarized in Table II. We found that serum levels of LDH, CRP, PCT, ALT, ferritin and D-dimer on the day of ICU admission were respectively significantly higher in TCZ therapy group (p=0.004, p=0.004, p=0.001, p=0.001, p=0.002, p=0.045, respectively). And, counts of the lymphocytes were significantly lower in TCZ therapy group (p=0.015)

Table II. *Baseline laboratory findings of the patients*

	All patients (n=326)	TCZ therapy group (n=110)	Standard therapy group (n=216)	P value
White blood cells, mm³ (Mean±SD)	11791±5693	12444±5255	11459±5888	0.140**
Neutrophils, mm³ (Mean±SD)	10483±4876	10833±4468	10305±5072	0.356**
Lymphocytes, mm³ (Mean±SD)	852±669	726±333	916±780	**0.015****
Urea, mg/dL (min-max)	51 (14-263)	49 (15-213)	52 (14-263)	0.298*
Creatinine, mg/dL (min-max)	0.85 (0.36-9.29)	0.81 (0.36-3.64)	0.88 (0.41-9.29)	**0.016***
AST, U/L (min-max)	45 (10-1672)	48 (12-426)	43 (10-1672)	0.317*
ALT, U/L (min-max)	33 (8-814)	38 (7-265)	31 (6-814)	**0.001***
LDH, IU/L (min-max)	564 (14-1900)	618 (126-1604)	530 (14-1900)	**0.004***
Albumin, g/dL (Mean±SD)	2.74±0.45	2.74±0.44	2.74±0.47	0.975**
Ferritin, ng/dL (Mean±SD)	916±636	1067±581	839±651	**0.002****
D-Dimer, µg/mL (Mean±SD)	3.11±4.71	3.81±5.86	2.75±3.96	**0.045****
Fibrinogen, ng/dL (Mean±SD)	503±189	505±177	503±194	0.935**
CRP, mg/dL (Mean±SD)	13.18±8.12	14.98±7.16	12.26±8.44	**0.004****
PCT, ng/mL (Mean±SD)	0.60±1.18	0.76±1.42	0.30±0.27	**0.001****
pH, (Mean±SD)	7.42±0.07	7.44±0.05	7.42±0.07	**0.020****
pO_2, mmhg (min-max)	74.30 (47-205)	68.97 (47-195)	79.45 (52-205)	**<0.001***
pCO_2, mmhg (min-max)	35.20 (18-99)	40.92 (24-62)	34.30 (18-99)	0.006*
Lactate, mmol/L (Mean±SD)	2.11±1.26	2.10±0.95	2.12±1.40	0.923**

TCZ: tocilizumab, AST: aspartate aminotransferase, ALT: alanine transaminase, LDH: lactate dehydrogenase, CRP: C-reactive protein, PCT: procalcitonin, Min: minimum, Max: maximum, Me: mean, Std: standard deviation
** Mann–Whitney U test*
*** Independent samples t test*

http://doi.org/10.5472/marumj.1191126
Marmara Med J 2022;35(3): 308-315

There were statistically significant differences between the groups in terms of O_2 requirements and the Horowitz index (the ratio of partial pressure of oxygen in blood (PaO_2) and the fraction of oxygen in the inhaled air (FiO_2)), on the day of ICU admission. Baseline O2 requirements has been found to be higher in TCZ therapy group (91.74±13.46 vs 85.81±20.94, p=0.008), and Horowitz index has been found to be lower in TCZ therapy group compared with standard therapy (0.82±0.27 vs 1.13±0.58, p<0.001).

Efficacy of tocilizumab therapy

When we evaluated the impact of the TCZ therapy on 30-days survival, we found that patients with treated TCZ therapy had a longer survival time compared with the standard therapy (p=0.012) (Figure 1). Also, 30-days mortality rate of the overall study population was %49.1, and 30-days mortality rate has been found to be significantly lower in the TCZ treatment group compared with standard therapy group (40.9% vs 53.2%, p=0.035).

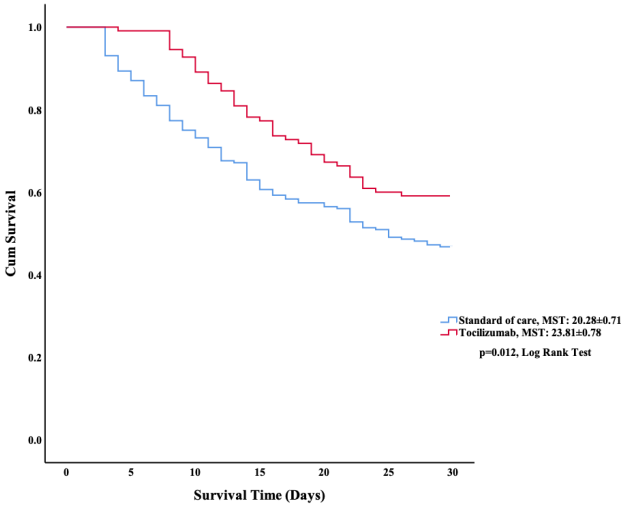

Figure 1. *Kaplan–Meier curves of survival for severe COVID-19 pneumonia patients. Effect of TCZ therapy on 30-days mortality. P values calculated using the Log-rank test. MST: mean survival time*

General characteristics of tocilizumab therapy patients and predictors of 30-days mortality in TCZ therapy

Patients treated with TCZ were grouped as survivors (n=65) and non-survivors (n=45). The mean age of the patients significantly higher in non-survivors, and proportion of the patients 65 years old higher in non-survivors (p=0.010, p=0.048). Percentage of hypertension and diabetes mellitus were significantly higher in non-survivor patients (p=0.003, p=0.007, respectively). In addition, we found that SOFA and APACHE-II scores were significantly higher in those who died within 30 days (p<0.001, p<0.001, respectively). Comparison of the baseline demographic characteristics of the two groups are summarized in Table III.

Table III. *Baseline demographic characteristics of the tocilizumab therapy*

		All patients (n=110)	Survivors (n=65)	Non-survivors (n=45)	P value
Mean age, years (Mean±SD)		63.02±11.58	57.66±12.06	63.04±8.09	**0.010**
Age	≥ 65 years	44 (40.0%)	21 (32.3%)	23 (51.1%)	**0.048**
	< 65 years	66 (60.0%)	44 (67.7%)	22 (49.9%)	
Gender	Male, (%)	75 (68.1%)	45 (69.2%)	32 (71.1%)	0.832
	Female, (%)	35 (31.9%)	20 (30.8%)	13 (29.9%)	
At least one comorbidity	Yes, (%)	83 (75.4%)	44 (67.6%)	39 (86.6%)	**0.023**
	No, (%)	27 (24.6%)	21 (32.4)	6 (13.4%)	
Comorbid diseases	DM, (%)	49 (44.5%)	22 (33.8%)	27 (60.0%)	**0.007**
	COPD (%)	15 (13.6%)	8 (12.3%)	7 (15.5%)	0.626
	HT, (%)	73 (66.3%)	36 (55.3%)	37 (82.2%)	**0.003**
	CHF, (%)	6 (5.4%)	4 (6.1%)	2 (4.4%)	0.698
	CAD, (%)	28 (25.4%)	14 (21.5%)	14 (31.1%)	0.257
Days between the onset of symptoms and TCZ treatment initiation		9.66±2.22	9.63±2.08	9.71±2.43	0.853
SOFA score, (Min-Max)		4 (3-9)	4 (3-7)	5 (3-9)	**<0.001**
APACHE-II score, (Mean±SD)		17.32±4.20	15.89±3.68	18.96±3.69	**<0.001**

DM: diabetes mellitus, COPD: chronic obstructive pulmonary disease, HT: hypertension, CHF: chronic heart failure, CAD: coronary artery disease, SOFA: sequential organ failure assessment, APACHE-II: acute physiology assessment and chronic health evaluation II, Min: minimum, Max: maximum, SD: standard deviation

Baseline laboratory parameters of the patients that were treated with TCZ were summarized in Table IV. We found that blood levels of LDH, D-dimer, procalcitonin and lactate on the day of TCZ admission were respectively significantly higher in non-survivors (p=0.030, p=0.038, p=0.034, p<0.001), And, counts of the lymphocytes were lower in non-survivors (p=0.030).

When we evaluated the prognostic factors affecting the 30-days mortality in the patients treated with TCZ therapy, we found that patients with diabetes mellitus and hypertension had significantly shorter survival times (p=0.029, p=0.047, respectively). In addition, patients with hyperlactatemia (blood lactate level >2 mmol/L) on the day of TCZ therapy admission had a significantly shorter survival time (p=0.002). Also, patients receiving invasive mechanical ventilation on the day of TCZ therapy admission had a significantly shorter survival time compared with patients receiving non-invasive mechanical ventilation (p=0.007).

The factors detected in the univariate survival analysis were included in the Cox regression analysis for the multivariate analysis of factors affecting the survival of patients. Univariate and multivariate survival analysis of the patients receiving TCZ therapy are presented in Table V. According to the Cox regression model, it was found that type of respiratory support (HR:2.19, CI:1.10-4.36, p=0.025) and hyperlactatemia on the day of TCZ therapy admission (HR:2.93 CI:1.53-5.64, p=0.001) were the significant and independent prognostic factors of survival in severe COVID-19 pneumonia patients treated with TCZ.

http://doi.org/10.5472/marumj.1191126
Marmara Med J 2022;35(3): 308-315

Table IV. *Baseline laboratory findings of the TCZ therapy patients*

	All patients (n=110)	Survivors (n=65)	Non-survivors (n=45)	P value
White blood cells, mm³ (Mean±SD)	12444±5255	12566±5029	12267±5618	0.771**
Neutrophils, mm³ (Mean±SD)	10833±4468	10528±3873	11274±5224	0.391**
Lymphocytes, mm³ (Mean±SD)	726±333	738±367	643±259	**0.030****
Urea, mg/dL (min-max)	49 (15-213)	48(15-98)	50 (17-213)	0.116*
Creatinine, mg/dL (min-max)	0.80 (0.36-3.64)	0.78 (0.36-1.60)	0.86 (0.55-3.64)	0.088*
AST, U/L (min-max)	49 (12-426)	45(12-146)	52 (12-426)	0.657*
ALT, U/L (min-max)	38 (7-265)	38 (12-178)	37 (7-265)	0.990*
LDH, IU/L (min-max)	617 (126-1604)	589 (281-1604)	718 (126-1339)	**0.030***
Albumin, g/dL (Mean±SD)	2.74±0.44	2.76±0.47	2.70±0.38	0.515**
Ferritin, ng/dL (Mean±SD)	1067±581	1039±572	1107±599	0.550**
D-Dimer, µg/mL (Mean±SD)	3.81±5.86	2.85±4.49	5.21±7.24	**0.038****
Fibrinogen, ng/dL (Mean±SD)	505±177	527±472	472±182	0.108**
CRP, mg/dL (Mean±SD)	14.98±7.16	14.96±7.17	15.02±7.23	0.963**
PCT, ng/mL (Mean±SD)	0.30±0.27	0.25±0.24	0.36±0.30	**0.034****
pH, (Mean±SD)	7.44±0.05	7.44±0.04	7.43±0.07	0.593**
pO_2, mmhg (min-max)	68.97 (47-195)	69.45 (52-146)	66.00 (47-195)	0.534*
pCO_2, mmhg (min-max)	36.60 (24-62)	37.65 (24-55)	36.00 (27-62)	0.114*
Lactate, mmol/L (Mean±SD)	2.10±0.95	1.83±0.26	2.50±1.06	**<0.001****

AST: aspartate aminotransferase, ALT: alanine transaminase, LDH: lactate dehydrogenase, CRP: C-reactive protein, PCT: procalcitonin, Min: minimum, Max: maximum, Std: standard deviation
** Mann–Whitney U test*
*** Independent samples t test*

Table V. *Univariate and multivariate survival analysis of the patients receiving TCZ therapy*

		Univariate analysis*		Multivariate analysis**	
		MST days	P value	HR (95% CI)	P value
Age	≥ 65 years	21.56±1.31	**0.029**	1.74 (0.93-3.25)	0.079
	< 65 years	25.31±0.92			
At least one comorbidity	Absent	25.85±1.54	**0.045**	0.49 (0.09-2.70)	0.416
	Present	23.15±0.89			
Diabetes mellitus	Absent	25.13±1.02	**0.012**	1.65 (0.81-3.34)	0.162
	Present	22.18±1.16			
Hypertension	Absent	26.32±1.22	**0.007**	2.16 (0.49-9.55)	0.307
	Present	22.54±0.96			
Type of respiratory support	IMV	20.23±1.71	**0.007**	**2.19 (1.10-4.36)**	**0.025**
	NIMV	24.66±0.85			
Blood lactate level	< 2 mmol/L	25.83±0.98	**0.002**	**2.93 (1.53-5.64)**	**0.001**
	≥ 2 mmol/L	21.56±1.16			

MST: mean survival time, HR: hazard ratio, CI: confidence interval, IMV: invasive mechanical ventilation, NIMV: non-invasive mechanical ventilation
** Log rank test*
*** Cox regression*

4. DISCUSSION

In the present study, we evaluated the efficacy of the TCZ therapy in severe COVID-19 pneumonia patients and prognostic factors affecting 30 days mortality in severe COVID-19 pneumonia patients treated with TCZ. We found that patients receiving TCZ therapy had a significantly longer survival time compared with the usual care (patients not received TCZ therapy). Also, type of respiratory support and hyperlactatemia on the day of TCZ therapy admission were the significant and independent prognostic factors of survival in severe COVID-19 pneumonia patients treated with TCZ.

http://doi.org/10.5472/marumj.1191126
Marmara Med J 2022;35(3): 308-315

Cytokine storm is the clinical picture characterized by excessive and uncontrolled release of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-7, IL-10, TNF-α, granulocyte-colony-stimulating factor (G-CSF), and interferon. Cytokine storm syndrome may be caused by a variety of infectious and rheumatic diseases or cancer immunotherapy. Since, cytokine storm is a life-threatening critical condition, it requires follow up in the intensive care unit and in clinical practice it is commonly characterized with increased systemic inflammation and may lead to multi-organ dysfunction [18-20].

In studies, it has been shown that a high cytokine profile can develop in some serious COVID-19 cases, which is also observed in severe acute respiratory syndrome associated coronavirus (SARS-CoV) and middle-east respiratory syndrome-coronavirus (MERS-CoV) infections. High serum levels of IL-6, TNF-α, IL-1β, IL-8, and IL-2R have been detected in cases of severe COVID-19 pneumonia. However, the levels of cytokines were found to be significantly higher in patients with severe disease compared to those with mild disease [18, 19, 21, 22]. In addition, it has been shown that the frequency of ARDS and mortality are higher in cases of COVID-19 pneumonia, in which inflammation-related biomarkers such as CRP and ferritin are elevated as well as cytokines [23-25].

In the studies conducted, it has been stated that in patients with COVID-19, a rapid deterioration of the clinical picture can be observed especially within the second week of the disease. This hyper-inflammation is usually manifested by an unexpected aggravation of symptoms such as high fever and respiratory distress and is associated with an increase in acute phase reactants (erythrocyte sedimentation rate (ESR), CRP, and ferritin), coagulation factors (D-dimer), and intracellular lysis markers (lactate dehydrogenase (LDH), creatine kinase (CK)) [2, 4, 26, 27]. Increased cytokine levels observed in the COVID-19-related cytokine storm, unlike other pneumonitis species, leads to the development of organ dysfunction and the need for intensive care [28]. In our study, in accordance with the literature, it was observed that cases of severe COVID-19 pneumonia, cytokine storm or COVID-19-associated hyperinflammatory syndrome developed within the second week of the disease. Similarly, in our study, the levels CRP, ferritin, LDH, CK, and D-dimer were well above the normal values.

Another important finding of cytokine storm is an increase in the number of neutrophils and a decrease in the number of lymphocytes, monocytes and basophils, in the peripheral blood. In the COVID-19-related cytokine storm, increased inflammatory cytokines generally cause a decrease in the number of all T cells and impair their functions. In the studies conducted, it has been shown that, as the number of lymphocytes decreases, the severity of the disease, the rate of the development of septic shock, and mortality increase, in COVID-19 cases [5, 29, 30]. Similarly, in our study, there was lymphopenia (852±669/mm^3) in cases with severe COVID-19 pneumonia associated cytokine storm. Also, in patients receiving TCZ therapy, the counts of the lymphocytes were lower in the non-survivors' group compared to the survivors' group, and the difference was statistically significant (738±367/mm^3 vs 643±259/mm^3, p=0.030).

In severe COVID-19 pneumonia, it has been shown that the host immune response is responsible from the cytokine storm or COVID-19-associated hyperinflammatory syndrome. Although, not specific for COVID-19-related cytokine storms, it is considered that anti-inflammatory treatment approaches may be useful. It was thought that blocking the overproduction of IL-6, which plays a key role especially in the cytokine storm, could be beneficial in the COVID-19-related cytokine storm [9, 12, 26, 27].

In studies evaluating the effects of TCZ treatment on clinical improvement, the need for mechanical ventilation, and survival in severe COVID-19 cases with cytokine storm conflicting results have been reported [10, 31, 32]. In a study conducted in China in the first period of the pandemic, it was found that TCZ treatment in severe COVID-19 pneumonia cases provided a decrease in oxygen demand in 75% of the patients, improvement in thoracic CT findings in 90%, and an increase in lymphocyte count in 52.6% of the patients. Moreover, it was found that all patients treated with TCZ were recovered [33]. Similarly, in two studies conducted in Italy, it has been shown that TCZ treatment provided a fast and permanent clinical improvement in severe COVID-19 pneumonia cases, decreased the need for invasive mechanical ventilation and more importantly reduced mortality [13, 34]. In the present study, we found that severe COVID-19 pneumonia patients treated with TCZ had a statistically significant longer survival time compared with patients treated with standard therapy (p=0.012).

In a systematic meta-analysis in which Tleyjeh et al., evaluated the efficacy and safety of TCZ treatment in COVID-19 cases, it was found that TCZ treatment did not reduce the risk of short-term mortality although, it reduced the need for mechanical ventilation in COVID-19 cases [35]. In addition, in a randomized placebo-controlled double-blind study, it was shown that TCZ therapy was not effective in preventing the need for invasive mechanical ventilation and mortality compared to standard therapy in patients hospitalized with moderate COVID-19 [15]. However, a recently published randomized controlled RECOVERY trial showed that tocilizumab improved survival and clinical outcomes in hospitalized COVID-19 pneumonia patients with hyperinflammation. In addition, it was shown that TCZ therapy was effective in preventing the need for invasive mechanical [36].

We think that it is very important for clinicians to predict which cases will benefit from treatment, before the decision for TCZ treatment in severe COVID-19 pneumonia patients. However, there are a limited number of studies evaluating prognostic factors in severe cases of COVID-19 pneumonia treated with TCZ. In a retrospective cohort study in which Lohse et al., evaluated prognostic factors in patients with severe COVID-19 pneumonia associated cytokine storm treated with TCZ, it was found that patients with severe lymphopenia, increased oxygen demand, low fibrinogen level, and increased serum aspartate aminotransferase (AST) levels did not benefit from TCZ treatment [27].

In the present study, among the patients treated with TCZ therapy, we found that levels of LDH, PCT, D-dimer and lactate

http://doi.org/10.5472/marumj.1191126
Marmara Med J 2022;35(3): 308-315

were significantly higher in non-survivors' patients. And counts of the lymphocytes were significantly lower in non-survivor patients. We also found that the presence of comorbidities such as HT and DM were other risk factors for poor prognosis in patients receiving TCZ therapy. More importantly, patients receiving invasive mechanical ventilation on the day of TCZ therapy admission had a significantly shorter survival time compared with patients receiving non-invasive mechanical ventilation.

The present study has some limitations. First, this is a retrospective, small sample size cohort study, conducted in a single center. Second, IL-6, which is the most important biomarker of cytokine storms, was not evaluated in the study.

In conclusion, TCZ therapy improved 30-days survival in critically ill COVID-19 pneumonia patients with hyperinflammation. And, patients receiving invasive mechanical ventilation and hyperlactatemia on the day of TCZ admission were less likely to benefit from TCZ treatment. We believe that more clinical studies are needed to predict which patients will benefit from TCZ therapy.

Compliance with Ethical Standards

Ethical Approval: The present study protocol was approved by the Clinical Ethics Committee of Inonu University School of Medicine (2020/164). Due to the retrospective nature of the study, the written informed consent form was not obtained.

Financial Support: This manuscript did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Authors' contributions: OC, LD, USK, AG, BT and EO: Concept and design, USK, EO, LD and AG: Analysis and interpretation, LD, USK and AG: data acquisition, writing: LD, USK and AG: Writing, clinical revision: BT, USK, LD and EO: Clinical revision. final approval: all of authors.

REFERENCES

[1] Chakraborty C, Sharma AR, Sharma G, Bhattacharya M, Lee SS. SARS-CoV-2 causing pneumonia-associated respiratory disorder (COVID-19): diagnostic and proposed therapeutic options. Eur Rev Med Pharmacol Sci 2020;24:4016-26. doi:10.26355/eurrev_202004_20871

[2] Grasselli G, Zangrillo A, Zanella A, et al. Baseline characteristics and outcomes of 1591 patients ınfected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. JAMA 2020;323:1574-81. doi:10.1001/jama.2020.5394

[3] Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. JAMA 2020;323:2052-9. doi:10.1001/jama.2020.6775

[4] Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054-62. doi:10.1016/s0140-6736(20)30566-3

[5] Bonam SR, Kaveri SV, Sakuntabhai A, Gilardin L, Bayry J. Adjunct immunotherapies for the management of severely ıll COVID-19 patients. Cell Rep Med 2020;1:100016. doi:10.1016/j.xcrm.2020.100016

[6] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506. doi:10.1016/s0140-6736(20)30183-5

[7] Odabasi Z, Cinel I. Consideration of severe coronavirus disease 2019 as viral sepsis and potential use of ımmune checkpoint ınhibitors. Crit Care Explor 2020;2:e0141. doi:10.1097/CCE.000.000.0000000141

[8] Sun X, Wang T, Cai D, et al. Cytokine storm intervention in the early stages of COVID-19 pneumonia. Cytokine Growth Factor Rev 2020;53:38-42. doi:10.1016/j.cytogfr.2020.04.002

[9] Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. J Infect 2020;80:607-13. doi:10.1016/j.jinf.2020.03.037

[10] Kewan T, Covut F, Al-Jaghbeer MJ, Rose L, Gopalakrishna KV, Akbik B. Tocilizumab for treatment of patients with severe COVID-19: A retrospective cohort study. EClinicalMedicine 2020;24:100418. doi:10.1016/j.eclinm.2020.100418

[11] Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: A single center experience. J Med Virol 2020;92:814-8. doi:10.1002/jmv.25801

[12] Mehta N, Mazer-Amirshahi M, Alkindi N, Pourmand A. Pharmacotherapy in COVID-19; A narrative review for emergency providers. Am J Emerg Med 2020;38:1488-93. doi:10.1016/j.ajem.2020.04.035

[13] Toniati P, Piva S, Cattalini M, et al. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in Brescia, Italy. Autoimmun Rev 2020;19:102568. doi:10.1016/j.autrev.2020.102568

[14] Furlow B. COVACTA trial raises questions about tocilizumab's benefit in COVID-19. Lancet Rheumatol 2020;2:e592. doi:10.1016/s2665-9913(20)30313-1

[15] Stone JH, Frigault MJ, Serling-Boyd NJ, et al. Efficacy of Tocilizumab in Patients Hospitalized with Covid-19. N Engl J Med 2020;383:2333-44. doi:10.1056/NEJMoa2028836

[16] Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985;13:818-29.

[17] Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med 1996;22:707-10. doi:10.1007/bf01709751

[18] Qin C, Zhou L, Hu Z, et al. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis 2020;71:762-8. doi:10.1093/cid/ciaa248

[19] Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. Front Immunol 2020;11:1446. doi:10.3389/fimmu.2020.01446

[20] Wang W, Liu X, Wu S, et al. Definition and risks of cytokine release syndrome in 11 critically ıll COVID-19 patients with pneumonia: analysis of disease characteristics. J Infect Dis 2020;222:1444-51. doi:10.1093/infdis/jiaa387

[21] Lau SKP, Lau CCY, Chan KH, et al. Delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the novel Middle East respiratory syndrome coronavirus: implications for pathogenesis and treatment. J Gen Virol 2013;94:2679-90. doi:10.1099/vir.0.055533-0

[22] Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol 2017;39:529-39. doi:10.1007/s00281.017.0629-x

[23] Liu F, Li L, Xu M, et al. Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. J Clin Virol 2020;127:104370. doi:10.1016/j.jcv.2020.104370

[24] Liu T, Zhang J, Yang Y, et al. The role of interleukin-6 in monitoring severe case of coronavirus disease 2019. EMBO Mol Med 2020;12:e12421. doi:10.15252/emmm.202012421

[25] Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 2020;180:934-43. doi:10.1001/jamainternmed.2020.0994

[26] Borrega J G, Godel P, Rüger MA, et al. In the eye of the storm: ımmune-mediated toxicities associated with CAR-T cell therapy. Hemasphere. 2019;3:e191. doi:10.1097/HS9.000.000.0000000191

[27] Lohse A, Klopfenstein T, Balblanc JC, et al. Predictive factors of mortality in patients treated with tocilizumab for acute respiratory distress syndrome related to coronavirus disease 2019 (COVID-19). Microbes Infect 2020;22:500-3. doi:10.1016/j.micinf.2020.06.005

[28] McElvaney OJ, McEvoy NL, McElvaney OF, et al. Characterization of the Inflammatory Response to Severe COVID-19 Illness. Am J Respir Crit Care Med 2020;202:812-21. doi:10.1164/rccm.202.005.1583OC

[29] Tan L, Wang Q, Zhang D, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduct Target Ther 2020;5:33. doi:10.1038/s41392.020.0148-4

[30] Zheng M, Gao Y, Wang G, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol 2020;17:533-5. doi:10.1038/s41423.020.0402-2

[31] Alattar R, Ibrahim TBH, Shaar SH, et al. Tocilizumab for the treatment of severe coronavirus disease 2019. J Med Virol 2020;92:2042-9. doi:10.1002/jmv.25964

[32] Salama C, Han J, Yau L, et al. Tocilizumab in patients hospitalized with covid-19 pneumonia. N Engl J Med 2021;384:20-30. doi:10.1056/NEJMoa2030340

[33] Zhao M. Cytokine storm and immunomodulatory therapy in COVID-19: Role of chloroquine and anti-IL-6 monoclonal antibodies. Int J Antimicrob Agents 2020;55:105982. doi:10.1016/j.ijantimicag.2020.105982

[34] Guaraldi G, Meschiari M, Cozzi-Lepri A, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. Lancet Rheumatol 2020;2:e474-e84. doi:10.1016/s2665-9913(20)30173-9

[35] Tleyjeh IM, Kashour Z, Damlaj M, et al. Efficacy and safety of tocilizumab in COVID-19 patients: a living systematic review and meta-analysis. Clin Microbiol Infect 2021;27:215-27. doi:10.1016/j.cmi.2020.10.036

[36] RECOVERY Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 : a randomised, controlled, open-label, platform trial. Lancet 2021;397:1637-45. doi:10.1016/s0140-6736(21)00676-0

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Systemic immune-inflammation index and other inflammatory parameters in patients receiving biological or targeted synthetic DMARDs for inflammatory rheumatic disease

Halise Hande GEZER[1], Ozlem PEHLIVAN[1]

[1] Rheumatology Clinic, Umraniye Training and Research Hospital, Istanbul, Turkey.

Corresponding Author: Halise Hande GEZER
E-mail: hande_snc@hotmail.com

Submitted: 02.03.2022 **Accepted:** 23.06.2022

ABSTRACT

Objective: To investigate the short – and long-term dynamics in inflammation markers [systemic immune-inflammation index (SII), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and lymphocyte/ monocyte ratio (LMR)] before and after biological disease-modifying antirheumatic drugs (bDMARD) or targeted synthetic DMARD (tsDMARD) treatment.

Patients and Methods: Two hundred twenty-six patients (115 women, 47±13.8 years) were included. Age, gender, disease duration, and treatments were recorded retrospectively. Complete blood counts including neutrophil, lymphocyte, platelet, monocyte and acute phase reactants were noted at the visit before the biological treatment, at the 3rd month, 6th month, and the last visit on medication. SII, NLR, PLR and LMR were calculated, and their dynamics over time were compared.

Results: Significant changes were observed over time in all parameters reflecting inflammation (SII, NLR, PLR, LMR, ESR, and CRP) ($p<0.05$). In the correlation analysis of changes at baseline and six months, significant correlations with ΔESR were observed with ΔCRP, ΔPLR, ΔSII and ΔNLR ($p<0.05$), but no correlation with LMR was detected. Also, significant correlations with ΔCRP were noted with ΔNLR, ΔSII, ΔPLR, and ΔLMR ($p<0.05$).

Conclusion: Significant and favourable changes were observed in all inflammatory parameters after treatment, and this variation remained stable as long as the drug was continued.

Keywords: Inflammation, Systemic immune inflammation index, Biological treatment

1. INTRODUCTION

In inflammatory rheumatic diseases, acute phase reactants such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) increase with cytokines, including TNF-α and IL-6. ESR and CRP are valuable markers for discriminating inflammatory rheumatic disease from non-inflammatory conditions and also have an essential role in the follow-up of the treatment response and disease activity [1].

In the presence of inflammation, leukocytes, platelets, ferritin and CRP increase, while parameters such as albumin decrease. With the release of cytokines, chemokines, and reactive oxygen derivatives, neutrophils, monocytes, and macrophages migrate to the site of inflammation, and the platelets are displaced from the bone marrow to the periphery, so the proportions of hemogram parameters change [2]. In recent years, new parameters reflecting inflammation apart from ESR and CRP were proposed from the blood cell counts ratio based on the knowledge that blood cell interactions are essential in the pathogenesis of inflammation and immune responses [3]. Of these, the neutrophil-lymphocyte ratio (NLR) is the most commonly used parameter, that was first proposed as an inflammatory marker after recognising that cancer patients sustained neutrophilia with lymphocytopenia, and then several studies showed its association with the poor prognosis in inflammatory rheumatic diseases, cancers, and neurological disease [4-6]. It has been stated that NLR regresses with inflammatory disease treatment and is an independent cardiovascular risk factor in patients with psoriasis [7]. In addition to NLR, platelet-lymphocyte ratio (PLR), lymphocyte-monocyte ratio (LMR), and mean platelet volume (MPV) are the other parameters reflecting inflammation [8]. Although, these parameters reflect inflammation, different results have been seen in various diseases. In renal disease, PLR was a better marker than NLR in terms of inflammation [9].

How to cite this article: Gezer HH, Pehlivan O. Systemic immune-inflammation index and other inflammatory parameters in patients receiving biological or targeted synthetic DMARDs for inflammatory rheumatic disease. Marmara Med J 2022; 35(3): 316-322, doi: 10.5472/marumj.1186732

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1186732
Marmara Med J 2022;35(3): 316-322

Recently, the composite systemic immune-inflammation index (SII), which was developed to include most of these parameters and is thought to reflect inflammation better, has been created. Geng et al., suggested that SII is a new and simple prognostic predictor for cancer patients and is superior to the other systemic inflammation index, including PLR, NLR, and LMR, reflecting the balance between host inflammatory and immune response status [10].

This study aimed to seek i) the short – and long-term change of NLR, PLR, LMR, and SII with the biological disease-modifying antirheumatic drugs (bDMARD) or targeted synthetic disease-modifying antirheumatic drugs (tsDMARD), ii) the relationship of these parameters with the most commonly used inflammatory parameters including ESR and CRP, and iii) the predictive role of these scores concerning the treatment switch.

2. PATIENTS and METHODS

Study design and patients

We retrospectively reviewed the medical records of 226 patients with bDMARD or tsDMARD treatment for at least six months from January 2015 to April 2022 at Umraniye Training and Research Hospital, Istanbul. Patients with incomplete data, pregnancy, malignancy and acute and chronic infection during the investigation were excluded from the study.

The study protocol was approved by the Ethical Committee of Umraniye Training and Research Hospital (Number:21/04/2022.146). Informed consent was waived because of the retrospective nature of the study.

Clinical Data and Medications

Patient characteristics were recorded, including age, gender, body mass index (BMI, kg/m2), disease duration, and treatment duration. The inflammatory rheumatic disease was noted and categorised as rheumatoid arthritis (RA), psoriatic arthritis (PsA), other spondyloarthritis (SpA) (axial SpA, peripheral SpA, enteropathic SpA), vasculitis (Behcet's disease, Takayasu's arteritis, Cogan's syndrome), autoinflammatory diseases [familial Mediterrean fever (FMF)], connective tissue diseases [systemic lupus erytematosus (SLE), sjogren, systemic sclerosis)], and adult Still's disease.

Infliximab, adalimumab, etanercept, certolizumab pegol, and golimumab were classified as anti-TNF-α treatments that inhibit the activity of central proinflammatory cytokines TNF-α. Tocilizumab is an inhibitor of the cytokine interleukin-6, and abatacept is a selective modulator of the T-lymphocyte activation pathway. Rituximab was classified as B-cell-targeted therapy, secukinumab as an IL-17 inhibitor, and anakinra and canakinumab as IL-1 inhibitors [11]. Tofacitinib is a targeted, small-molecule inhibitor of JAK1 and JAK3 [12]. TNF-α inhibitors, tocilizumab, abatacept, rituximab, and secukinumab were classified as bDMARD and tofacitinib as tsDMARD. The patients' treatments at the first two visits and, if treatment changed because of unresponsiveness or side effects, at the last visit on medication were noted.

Laboratory Data and Inflammatory Markers

We collected laboratory data, including complete blood counts [white blood cell (WBC), neutrophil, lymphocyte, monocyte, and platelet counts], ESR, and CRP before the administration of bDMARD or tsDMARD treatment (baseline), at 3rd month, 6th month, and the last visit on medication.

The NLR and PLR were calculated by dividing the neutrophils and platelets by the lymphocyte count, respectively. The normal NLR range was defined as an NLR score of 1-2, with higher scores indicating low inflammation (2-3), moderate inflammation (3-7), and severe inflammation (>7) [13]. LMR was also calculated according to the lymphocyte count ratio to monocyte count. The SII was calculated from the platelet, neutrophil, and lymphocyte counts using the following formula: SII = platelet×neutrophil/lymphocyte counts [14].

Changes between baseline and six months were analysed for all parameters (ΔSII, ΔNLR, ΔPLR, ΔLMR, ΔESR, ΔCRP). We defined Δ as the difference between inflammatory parameter levels on baseline and six months.

Statistical Analysis

Categorical variables were expressed as percentages, while continuous variables as means (±) or medians [interquartile range (IQR)]. Kolmogorov–Smirnov test was used to verify the normality of the distribution. The chi-squared test (Fischer's exact test when expected numbers were below five) was used for qualitative data. Friedman test was used to compare four consecutive measures of variables, followed by a Tukey posthoc test for pair-wise comparisons. The correlation of the changes between baseline and six months (ΔSII, ΔNLR, ΔPLR, ΔLMR, ΔESR, ΔCRP) with each other was examined by Spearman's test. Receiver operating characteristic (ROC) analysis was performed to evaluate the impact of SII on the treatment switch. The level of significance was set as $p < 0.05$. Data were processed using the Statistical Package for Social Sciences Software (SPSS v22.00. Armonk, IBM Corp).

3. RESULTS

Demographic and Clinical Data

A total of 226 patients with bDMARD or tsDMARD were included in the study. The mean age of the patients was 47±13.8 years, and 115 (51.1%) of the patients were female. While the mean disease duration was 120±8.9 months, the mean bDMARD or tsDMARD treatment duration were 49.8±30.3 months. While 43.8% of patients were using corticosteroids at baseline, this rate decreased 28.7% at the last visit. Table I represents the patient's demographic and clinical characteristics.

The mean NLR was 2.66±1.7, indicating normal and increased inflammation in 39.8% and 60.2% of the patients. Of 136 patients

http://doi.org/10.5472/marumj.1186732
Marmara Med J 2022;35(3): 316-322

with increased inflammation, 75 (33.2%) had mild inflammation, 55 (24.3%) moderate, and 6 (2.7%) severe inflammation. Of the six patients with severe inflammation, five were RA, and one was Takayasu arteritis; in terms of treatment, three were on anti-TNF, one was tofacitinib, and one was tocilizumab.

Table I. *Demographic and clinical characteristics of patients with biological treatment (n=226)*

Demographic data	Patients	
Age, years	47 (13.8)	
Female gender	115 (51.1%)	
Disease characteristics		
Disease duration, months	120 (8.9)	
Treatment duration, months	49.8 (30.3)	
Disease		
Rheumatoid arthritis	83 (36.7%)	
Psoriatic arthritis	29 (12.8%)	
Spondyloarthritis excluding PsA	101 (44.7%)	
Vasculitis	5 (2.2%)	
Connective tissue disease	4 (1.7%)	
Hereditary Mediterranean Fever	3 (1.3%)	
Adult onset Still's disease	1 (0.4%)	
bDMARD and tsDMARD received		
	First two visits	**Last visit**
Anti-TNF treatment	168 (74.3%)	167 (73.9%)
Infliximab	6 (2.7%)	9 (4%)
Etanercept	33 (14.6%)	29 (12.8%)
Adalimumab	55 (24.3%)	55 (24.3%)
Golimumab	44 (19.5%)	41 (18.1%)
Certolizumab pegol	30 (13.3%)	33 (14.6%)
Tocilizumab	6 (2.7%)	2 (0.9%)
Rituximab	11 (4.9%)	21 (9.3%)
Abatacept	7 (3.1%)	3 (1.3%)
Tofacitinib	23 (10.2%)	17 (7.5%)
Secukinumab	8 (3.5%)	13 (5.8%)
Anakinra	3 (1.3%)	3 (1.3%)

Data are presented as mean (SD) or n (%)

While there was no difference in NLR, SII, MLR, and CRP values between genders, females had higher ESR (P<0.001) and lower PLR (p=0.01) than males.

When we classified the patients as RA and SpA and compared the basal inflammatory parameters, ESR and NLR were significantly higher in patients with RA than in SpA (p <0.05). Table II represents the basal parameters according to the disease groups.

Table II. *Comparison of basal inflammatory parameters in rheumatoid arthritis and spondyloarthritis patients*

	Rheumatoid arthritis (n=83)	Spondyloarthritis (n=130)	P
SII (x109/L)	755.389 (476.135)	676.677 (492.469)	0.08
NLR	2.36 (1.78)	2.13 (1.1)	0.03
PLR	143 (80.9)	126 (62.8)	0.22
LMR	4.18 (2.75)	4.44 (1.92)	0.52
ESR (mm/h)	40 (36)	31 (35)	0.01
CRP (mg/L)	21 (36.5)	14 (32.5)	0.69

SII: Systemic immune inflammation index, PLR: platelet-neutrophil ratio, LMR: Lymphocyte-monosite ratio, NLR: neutrophil-lymphocyte ratio, ESR: erytrocyte sedimentation rate, CRP: C-reactive protein. Data are presented as median (IQR). Bold indicates statistically significant difference

Time-dependent variation was significantly different in all parameters (p<0.05). A significant change was observed after the treatment compared to the baseline, and no difference was observed in the follow-ups after the treatment (Table III). In Fig. I, the dynamics of inflammatory parameters are shown graphically.

Table III. *Inflammatory parameter dynamics in patients treated with bDMARD or tsDMARD*

Parameters	Pre-treatment 1	After treatment (3rd month) 2	After treatment (6th month) 3	Last visit 4	P	Pairwise comparisons* P
SII (x10⁹/L)	705.719 (876.458)	497.453 (323.540)	483.199 (362.803)	543.420 (381.318)	**<0.001**	1 vs 2: <0.001 1 vs 3: <0.001 1 vs 4: <0.001
NLR	2.25±1.39	1.84 (0.86)	1.9 (1.12)	1.95 (1.2)	**<0.001**	1 vs 2: <0.001 1 vs 3: <0.001 1 vs 4: 0.001
PLR	127 (69)	121 (50.3)	116 (50.2)	114 (52.9)	**<0.001**	1 vs 2: <0.001 1 vs 3: <0.001 1 vs 4: <0.001
LMR	4.36 (2.18)	4.34 (2)	4.35 (2.38)		**0.004**	1 vs 2: 0.004 1 vs 3: <0.001
ESR (mm/h)	35 (36)	20 (17)	20 (18)	23 (18.8)	**<0.001**	1 vs 2: p<0.001 1 vs 3: p<0.001 1 vs 4: p<0.001 2 vs 4: <0.001
CRP (mg/L)	16.5 (34)	6 (9.92)	5 (8.3)	9.7 (1.4)	**<0.001**	1 vs 2: p<0.001 1 vs 3: p<0.001 1 vs 4: p<0.001

SII: Systemic immune inflammation index, PLR: platelet-neutrophil ratio, LMR: Lymphocyte-monosite ratio, NLR: neutrophil-lymphocyte ratio, ESR: erytrocyte sedimentation rate, CRP: C-reactive protein.
Data are presented as median (IQR). Bold indicates statistically significant difference.
** Friedman Test with Tukey Multiple Pairwise Comparisons*

http://doi.org/10.5472/marumj.1186732
Marmara Med J 2022;35(3): 316-322

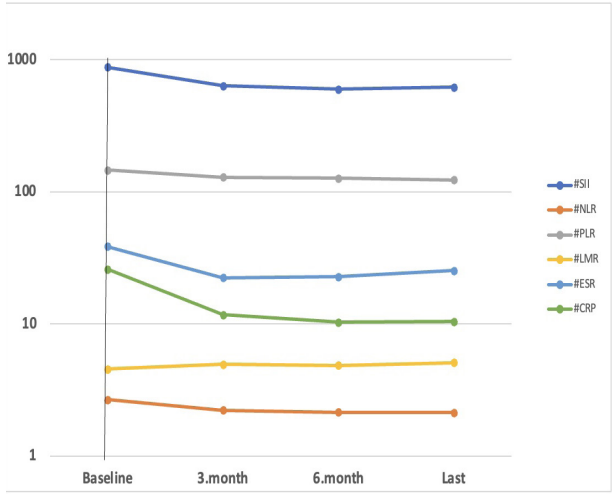

Figure 1. *SII, NLR, PLR, LMR, ESR, and CRP dynamics in patients receiving biological and targeted synthetic DMARDs*

Table IV shows the correlation of changes in inflammatory parameters in the first six months. ΔESR showed a positive correlation with ΔCRP, ΔSII, ΔPLR, and ΔNLR ($p<0.05$). There was no correlation between ΔESR and ΔLMR ($p>0.05$). Also, ΔCRP showed a positive correlation with ΔESR, ΔSII, ΔNLR, Δand PLR and a negative correlation with ΔLMR ($p<0.05$).

Table IV. *Correlations of change in inflammatory parameters at six months*

		ΔSII	ΔNLR	ΔPLR	ΔLMR	ΔESR	ΔCRP
ΔSII	Spearman' rho p-value	-					
ΔNLR	Spearman' rho p-value	**0.906** <0.001	-				
ΔPLR	Spearman' rho p-value	**0.703** <0.001	**0.607** <0.001	-			
ΔLMR	Spearman' rho p-value	**-0.487** <0.001	**-0.539** <0.001	**-0.498** <0.001	-		
ΔESR	Spearman' rho p-value	**0.316** <0.001	**0.249** <0.001	**0.337** <0.001	**-0.121** 0.06	-	
ΔCRP	Spearman' rho p-value	**0.467** <0.001	**0.385** <0.001	**0.292** <0.001	**-0.221** 0.001	**0.504** <0.001	-

SII: Systemic immune inflammation index, PLR: platelet-neutrophil ratio, LMR: Lymphocyte-monosite ratio, NLR: neutrophil-lymphocyte ratio, ESR: erytrocyte sedimentation rate, CRP: C-reactive protein

At least two bDMARD or tsDMARD were used in 79 patients, 147 continued with the first biologic therapy, and the median treatment switch number was 1 (range:1-4). The ROC curve evaluated the predictive value of the SII, NLR, PLR, and LMR for treatment switch by comparing the AUC area. While, no significant results were found in the whole patient group, the analysis was repeated only with RA and PsA patients since there may not be an acute phase elevation in disease activity in axial spondyloarthritis. The AUC of the SII, NLR, PLR, and LMR for treatment switch in RA and PsA were 0.531, 0.506, 0.512, and 0.480, respectively (Fig. II), indicating that SII is superior to other inflammatory parameters. The optimal cut-off value of baseline SII to predict treatment switch was>$648x10^9$, with 65% sensitivity and 52% specificity (95% confidence interval 1.013–1.986, p =0.04).

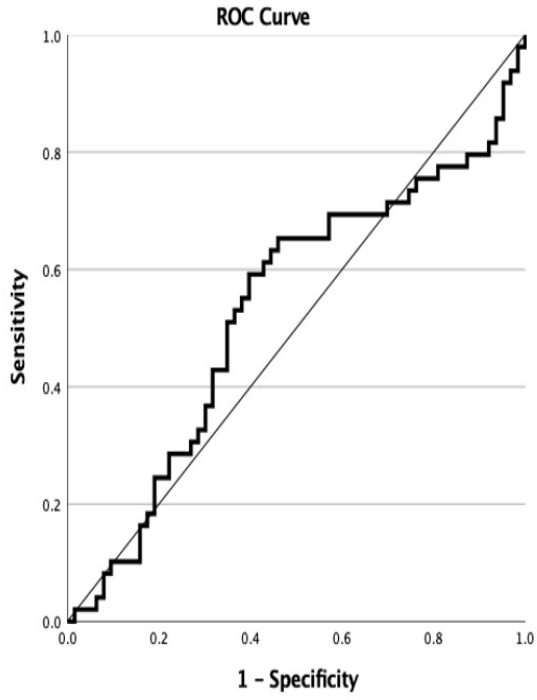

Figure 2. *Receiver operator characteristic (ROC) curve of systemic immune inflammation index (SII) for predicting treatment switch in patients with rheumatoid arthritis and psoriatic arthritis.*

4. DISCUSSION

Although, several clinical studies have investigated inflammatory parameters in rheumatic patients, information on the change of these parameters, especially SII, is lacking after the new treatments bDMARD and tsDMARD. Therefore, we performed a clinical retrospective study to evaluate the dynamics of these parameters in a population of different patient groups and found that NLR, SII, and PLR decreased within three months of initiation of treatment and remained stable at reduced levels for at 6^{th} months and as long as the drug was continued; similarly, LMR increased dramatically at three months and remained stable. These results demonstrate a rapid and significant reduction in inflammation parameters under bDMARD or tsDMARD treatment.

Rheumatic diseases are associated with systemic inflammation and elevated acute phase reactants. In clinical practice, ESR and CRP have frequently used parameters reflecting inflammation

http://doi.org/10.5472/marumj.1186732
Marmara Med J 2022;35(3): 316-322

and response to therapy [15]. Usually, high ESR, leukocytosis, left shift, anaemia, and thrombocytosis have diagnostic value in predicting inflammatory or infectious diseases. Apart from these tests, new markers have been developed recently from hemogram parameters. NLR was the first identified marker reflecting inflammation and was defined by the rapidly increasing neutrophils and the oppositely decreased lymphocytes in sepsis. The opposite changes in neutrophil and lymphocyte counts are a multifactorial and complex dynamic process depending on the regulation of various immunologic, neuroendocrine, humoral and biologic processes such as margination/emargination, mobilisation/redistribution, accelerated/delayed apoptosis, the influence of stress hormones and sympathetic/parasympathetic imbalance of the vegetative nervous system [13]. It has started to be used in many fields after its importance in the diagnosis and treatment follow-up in sepsis. High NLR values are associated with severe inflammation, cancer, injury, trauma or major surgery and mark the worsening prognosis regarding morbidity or mortality. Ahn et al., showed that NLR reflects vasculitis activity and suggest that physicians should pay more attention to patients with NRL at diagnosis ≥5.9 in terms of relapse [16]. In a study in RA, Uslu et al., showed that the mean NLR was 2.1, which was higher than healthy controls, and NLR was correlated with disease activity [17]. In our study, NLR was found to be 2.6 higher than these values since, our patient population was high disease activity requiring biological treatment. Although, no study was conducted on patients receiving biological therapy for the active rheumatic disease similar to our patient group, the mean NLR was 2.9 in psoriasis patients receiving biological treatment for severe disease [7].

As NLR is a parameter indicating inflammation, it is a risk factor in cardiovascular diseases. It has been shown that all-cause mortality and coronary heart disease increase above the cut-off point of ≥ 2.15 [18]. Although, we did not evaluate the cardiovascular morbidity and mortality of the patients, our baseline NLR ratio was 2.6. It decreased to 2.2 in the 3rd month and 2.1 in the 6th month, which means these patients are at risk for cardiovascular diseases. There is a significant decrease in NLR with biological therapy, which may benefit cardiovascular diseases.

Biological therapies provide both symptomatic relief and functional improvement by reducing inflammation. Change in ESR and CRP with biological treatment is known and expected, but NLR reflecting inflammation has recently been used frequently to evaluate the treatment effect. In a study showing the change of NLR after a biological therapy in psoriasis, a rapid and significant decrease was observed in the first three months, and significantly lower levels were sustained throughout the following treatment years, which is in agreement with our results [7].

In addition to neutrophils and lymphocytes, monocytes play an essential role in inflammation. They accumulate in the vessel wall, transform into macrophages, and contribute to the release of proinflammatory cytokines. Decreased lymphocytes and increased monocytes in inflammation cause a decline in the LMR ratio. With the reduction of inflammation with treatment, the LMR ratio is expected to increase, as in our study [2].

Platelet-lymphocyte ratio is another parameter revealing shifts in platelet lymphocyte counts in several conditions, including inflammatory disease, thrombotic states and malignancy. Undulations in platelet counts in rheumatic diseases imply nonspecific inflammatory thrombopoiesis, with the release of reactive cells from the bone marrow to the bloodstream, migration to and excessive consumption at inflammatory websites, and their destruction via binding to anti-platelet antibodies [3]. One of the areas where thrombocytosis and PLR are most useful for diagnosis is large-vessel vasculitis, especially temporal arteritis. In a study involving 537 patients, thrombocytosis rate was observed in patients diagnosed with temporal arteritis with positive temporal artery biopsy. It was stated that thrombocytosis is an essential clue in diagnosing temporal arteritis [19, 20]. The shift in this parameter generally correlates with other inflammatory markers reflecting systemic inflammation. When PLR and NLR were evaluated together, it showed an increase in platelets and neutrophils and a decrease in lymphocytes in the active RA [21]. In our study, PLR correlated with all other inflammatory parameters and had a high correlation with NLR, the most frequently investigated and used parameter for inflammation. Although, many studies have evaluated these parameters in inflammatory diseases, there are few researches on their fluctuations after anti-inflammatory treatment. There are only four small studies evaluating PLR. In one of these, a significant decrease in PLR and DAS28 was observed after rituximab treatment [22], and in the other research, a substantial reduction in both PLR, NLR and CRP was observed after bDMARD treatment in PsA [23]. In another study in ankylosing spondylitis, platelet count was correlated with disease activity, and anti-TNF treatment decreased the platelet count. In regression analysis, a high platelet count may predict a poorer response to anti-TNF-α therapy [24]. Similar to our results, in the last study conducted in 2020, NLR, PLR, MPV and CRP decreased after the biological treatment, including infliximab, etanercept, adalimumab and ustekinumab, and this result was independent of drug [8]. Our baseline PLR ratio was 146, which decreased significantly over time to 129 in the 3rd month and 127 in the 6th month. Platelet-lymphocyte ratio also reflects atherosclerosis by showing inflammation like NLR. Active platelets and interactions of platelets with other cells could initiate inflammation in the arterial wall, thus partially explaining atherosclerosis associated with chronic inflammation [25].

The systemic immune inflamation index is a novel biomarker including neutrophils, platelets, and lymphocyte count. Although, there are a few studies on rheumatic diseases, it is essential, especially in determining the prognosis in malignancies, including renal, lung, prostate and oesophagus cancer. SII is vital in prognosis because of neutrophils' role in regulating angiogenesis, chemokine and cytokine release, and the production of chemokines and cytokines from platelets in metastases of malignancies [26]. SII has been evaluated in rheumatic diseases, including RA, PsA, Still's disease, and

http://doi.org/10.5472/marumj.1186732
Marmara Med J 2022;35(3): 316-322

vasculitis, but post-treatment results were not analysed in these studies [14, 27-30]. To the best of our knowledge, this is the first study to evaluate SII in response to immunosuppressive therapy in rheumatic diseases. Similar to other parameters, SII decreased significantly at three months and remained stable at reduced follow-up levels. The change at six months was also significantly correlated with other parameters. The ROC analysis for its role in predicting switches in biological therapy was superior to other parameters. Although, low sensitivity and specificity, SII≥ $648x10^9$ at baseline can predict the treatment switch in RA and PsA. In Behçet disease, SII was significantly higher in the active patients and determined a cut-off value of 552.12 for SII with relatively high sensitivity and specificity. CRP is a commonly used test to evaluate disease activity in clinical practice. The marker most associated with a change in CRP at six months was ESR and then SII, followed by NLR. [30].

There may be some possible limitations in this study. The first was that it was a single-center, retrospective study. The other was that the confounding effect of additional rheumatic drugs could not be evaluated because patients in the SpA group generally received only bDMARD treatment, while other patients such as RA, PsA and SLE often received additional csDMARD or steroid treatment.

In conclusion, significant changes were observed in all inflammatory parameters after treatment, and this change remained stable as long as treatment continued. SII, NLR, LMR and PLR are simple and cheap markers. They may be seen as valuable markers for demonstrating systemic inflammation in rheumatic disease and may also indicate treatment response.

Compliance with Ethical Standards

Ethical Approval: The study protocol was approved by the Ethical Committee of Umraniye Training and ResearcHospital (Number:21/04/2022.146). Informed consent was waived because of the retrospective nature of the study.

Financial Support: No special funding was obtained.

Conflict of Interest Statement: There is no conflict of interest.

Authors' Contributions: HHG, OP: Idea/ Concept, HHG: Design, OP: Control/Supervision. HHG, OP: Data Collection and processin, . HHG, OP: Analysis, HHG, OP: Literature review. HHG, OP: Critical review. Both authors approved the final version of the article.

REFERENCES

[1] Kim MJ, Lee EB, Song YW, Park JK. Profile of common inflammatory markers in treatment-naïve patients with systemic rheumatic diseases. Clin Rheumatol 2020;39:2899-906.doi:10.1007/s10067.020.05049-9

[2] Kadiyoran C, Zengin O, Cizmecioglu HA, et al. Monocyte to lymphocyte ratio, Neutrophil to lymphocyte ratio, and red cell distribution width are the associates with gouty arthritis. Acta Medica (Hradec Kralove) 2019;62:99-104. doi:10.14712/18059.694.2019.132

[3] Gasparyan AY, Ayvazyan L, Mukanova U, et al. The platelet-to-lymphocyte ratio as an inflammatory marker in rheumatic diseases. Ann Lab Med 2019;39:345-57.doi:10.3343/alm.2019.39.4.345

[4] Walzik D, Joisten N, Zacher J, Zimmer P. Transferring clinically established immune inflammation markers into exercise physiology: focus on neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and systemic immune-inflammation index. Eur J Appl Physiol 2021;121:1803-14. doi:10.1007/s00421.021.04668-7

[5] Hemond CC, Glanz BI, Bakshi R, Chitnis T, Healy BC. The neutrophil-to-lymphocyte and monocyte-to-lymphocyte ratios are independently associated with neurological disability and brain atrophy in multiple sclerosis. BMC Neurol 2019;19:23.doi:10.1186/s12883.019.1245-2

[6] Han BK, Wysham KD, Cain KC, Tyden H, Bengtsson AA, Lood C. Neutrophil and lymphocyte counts are associated with different immunopathologiceal mechanisms in systemic lupus erythematosus. Lupus Sci Med 2020;7: e000382.doi:10.1136/lupus-2020-000382.

[7] Hoffmann JHO, Knoop C, Enk A, Hadaschik EN. Detailed Long-term dynamics of neutrophil-to-lymphocyte ratio under biologic treatment reveals differential effects of tumour necrosis factor-alpha and interleukin 12/23 antagonists. Acta Derm Venereol 2021;101:adv00568.doi:10.2340/actadv.v101.271

[8] An I, Ucmak D, Ozturk M. The effect of biological agent treatment on neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, mean platelet volume, and C-reactive protein in psoriasis patients. Postepy Dermatol Alergol 2020;37:202-6.doi:10.5114/ada.2020.94838

[9] Turkmen K, Erdur FM, Ozcicek F, et al. Platelet-to-lymphocyte ratio better predicts inflammation than neutrophil-to-lymphocyte ratio in end-stage renal disease patients. Hemodial Int 2013;17:391-6.doi:10.1111/hdi.12040

[10] Geng Y, Shao Y, Zhu D, et al. Systemic immune-inflammation index predicts prognosis of patients with esophageal squamous cell carcinoma: a propensity score-matched analysis. Sci Rep 2016;6:39482.doi:10.1038/srep39482

[11] Strand V, Kimberly R, Isaacs JD. Biologic therapies in rheumatology: lessons learned, future directions. Nat Rev Drug Discov 2007;6:75-92.doi:10.1038/nrd2196

[12] Boyle DL, Soma K, Hodge J, et al. The JAK inhibitor tofacitinib suppresses synovial JAK1-STAT signalling in rheumatoid arthritis. Ann Rheum Dis 2015;74:1311-6.doi:10.1136/annrheumdis-2014-206028

[13] Zahorec R. Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy 2021;122:474-88. doi:10.4149/BLL_2021_078

[14] Yorulmaz A, Hayran Y, Akpinar U, Yalcin B. Systemic immune-inflammation index (SII) predicts increased severity in psoriasis and psoriatic arthritis. Curr Health Sci J 2020;46:352-7.doi:10.12865/CHSJ.46.04.05

[15] Dayer E, Dayer JM, Roux-Lombard P. Primer: the practical use of biological markers of rheumatic and systemic

inflammatory diseases. Nat Clin Pract Rheumatol 2007;3:512-20.doi:10.1038/ncprheum0572.

[16] Ahn SS, Jung SM, Song JJ, Park YB, Lee SW. Neutrophil to lymphocyte ratio at diagnosis can estimate vasculitis activity and poor prognosis in patients with ANCA-associated vasculitis: a retrospective study. BMC Nephrol 2018;19:187. doi:10.1186/s12882.018.0992-4

[17] Uslu AU, Küçük A, Şahin A, et al. Two new inflammatory markers associated with Disease Activity Score-28 in patients with rheumatoid arthritis: neutrophil-lymphocyte ratio and platelet-lymphocyte ratio. Int J Rheum Dis 2015;18:731-5. doi:10.1111/1756-185X.12582

[18] Kim S, Eliot M, Koestler DC, Wu WC, Kelsey KT. Association of neutrophil-to-lymphocyte ratio with mortality and cardiovascular disease in the jackson heart study and modification by the duffy antigen variant. JAMA Cardiol 2018;3:455-62. doi:10.1001/jamacardio.2018.1042

[19] Walvick MD, Walvick MP. Giant cell arteritis: laboratory predictors of a positive temporal artery biopsy. Ophthalmology 2011;118:1201-4.doi:10.1016/j.ophtha.2010.10.002

[20] Oh LJ, Wong E, Andrici J, McCluskey P, Smith JEH, Gill AJ. Full blood count as an ancillary test to support the diagnosis of giant cell arteritis. Intern Med J 2018;48:408-13. doi:10.1111/imj.13713

[21] Zhang Y, Yin Y, Kuai S-g, Shan Z, Pei H, Wang J. Combination of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio as diagnostic biomarker for rheumatoid arthritis. Int J Clin Exp Med 2016;9:22076-22081

[22] Sargin G, Senturk T, Yavasoglu I, Kose R. Relationship between neutrophil-lymphocyte, platelet-lymphocyte ratio and disease activity in rheumatoid arthritis treated with rituximab. Int J Rheum Dis 2018;21:2122-7.doi:10.1111/1756-185X.13400

[23] Asahina A, Kubo N, Umezawa Y, Honda H, Yanaba K, Nakagawa H. Neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and mean platelet volume in Japanese patients with psoriasis and psoriatic arthritis: Response to therapy with biologics. J Dermatol 2017;44:1112-21.doi:10.1111/1346-8138.13875

[24] Qian H, Chen R, Wang B, et al. Associations of platelet count with inflammation and response to anti-tnf-α therapy in patients with ankylosing spondylitis. Front Pharmacol 2020;11:559593.doi:10.3389/fphar.2020.559593

[25] Boyraz I, Koç B, Boyacı A, Tutoğlu A, Sarman H, Ozkan H. Ratio of neutrophil/lymphocyte and platelet/lymphocyte in patient with ankylosing spondylitis that are treating with anti-TNF. Int J Clin Exp Med 2014;7:2912-5.

[26] Li C, Tian W, Zhao F, et al. Systemic immune-inflammation index, SII, for prognosis of elderly patients with newly diagnosed tumors. Oncotarget 2018;9:35293-9.doi:10.18632/oncotarget.24293

[27] Satis S. New inflammatory marker associated with disease activity in rheumatoid arthritis: the systemic immune-inflammation index. Curr Health Sci J 2021;47:553-7. doi:10.12865/CHSJ.47.04.11

[28] Kim JW, Jung JY, Suh CH, Kim HA. Systemic immune-inflammation index combined with ferritin can serve as a reliable assessment score for adult-onset Still's disease. Clin Rheumatol 2021;40:661-8. doi:10.1007/s10067.020.05266-2

[29] Chen JB, Tang R, Zhong Y, et al. Systemic immune-inflammation index predicts a reduced risk of end-stage renal disease in Chinese patients with myeloperoxidase-anti-neutrophil cytoplasmic antibody-associated vasculitis: A retrospective observational study. Exp Ther Med 2021;22:989. doi:10.3892/etm.2021.10421

[30] Tanacan E, Dincer D, Erdogan FG, Gurler A. A cutoff value for the Systemic Immune-Inflammation Index in determining activity of Behçet disease. Clin Exp Dermatol 2021;46:286-91. doi:10.1111/ced.14432

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

The effect of social support, depression, and illness perception on treatment adherence in patients with multiple sclerosis

Derya GIRGIN[1], Zeynep TOSUN[2], Nilda TURGUT[3], Sibel GULER[4]

[1] Neurology Clinic, Tekirdağ Namık Kemal University Hospital, Tekirdağ, Turkey.
[2] Department of Nursing, Tekirdag Namik Kemal University Health College, Tekirdağ, Turkey.
[3] Department of Neurology, School of Medicine, Tekirdag Namik Kemal University, Tekirdağ, Turkey.
[4] Department of Neurology, School of Medicine, Trakya University, Edirne, Turkey.

Corresponding Author: Zeynep Tosun
E-mail: ztosun@nku.edu.tr

Submitted: 21.01.2022 **Accepted:** 05.04.2022

ABSTRACT

Objective: This study was planned to determine the treatment adherence levels of multiple sclerosis (MS) patients and the factors affecting treatment adherence.

Patients and Methods: This descriptive and cross-sectional study was conducted with 211 people with MS. Data for this study was obtained through face-to-face interviews with MS patients who presented at the neurology outpatient clinics of two university hospitals between April and October 2018. The "Morisky, Green, and Levine Adherence Scale", "Beck Depression Inventory", "Multidimensional Perceived Social Support Scale", and the "Illness Perception Scale" were used in data collection.

Results: The mean age of the sample was 40.03±10.82, and 70.1% were female. Treatment adherence was not good in half of the patients (51.7%). Patients with good adherence were found to have higher Multidimensional Perceived Social Support Scale scores ($p<0.01$) and lower Beck Depression Inventory scores ($p<0.01$). The illness perceptions of the patients regarding MS did not affect treatment adherence ($p>0.05$).

Conclusion: Treatment adherence was insufficient in half of the MS patients. According to our findings, ensuring more cooperation with the families of patients, which constitute the strongest source of social support, increasing treatment adherence can be suggested as well as screening patients with regard to depressive symptomology during follow-up.

Keywords: Multiple sclerosis, Treatment adherence, Social support, Depression, Illness perception

1. INTRODUCTION

Multiple Sclerosis (MS) is the most widespread chronic neurological disorder causing disability among young adults, and it is estimated to affect 2.2 million people worldwide with a prevalence of 33 per hundred thousand [1]. MS prevalence has been calculated to be between 19 and 288 per hundred thousand in Turkey [2-5]. In recent years, the incidence of the disease has been increasing, especially among women [6].

Although, full recovery from MS is not possible today, medical treatment is important with regard to decreasing disability and slowing down progression. Obtaining the expected result from these medicines applied in differing forms is only possible through regular and continuous use of the medicines, in other terms, ensuring "treatment adherence" [7,8].

The fatigue and weariness brought about by long term use of medicines in chronic diseases is one of the most important obstacles for treatment adherence. Alongside this, problems that may vary with the disease and the medicine used may affect treatment adherence. The frequency and method of medication application, the logistical difficulties of obtaining medicine depending on social security status or place of residence, and regulations regarding the funding of medicine can also be listed as factors affecting treatment adaptation in MS, just as in any other chronic disease. In addition to those problems, the severity of the disease, emerging cognitive problems, and depressive mood in MS all make continuing treatment more difficult, and factors such as fear of injection, injection area reactions, and side effects of medications can affect treatment

How to cite this article: Girgin D, Tosun Z, Turgut N, Guler S. The effect of social support, depression, and illness perception on treatment adherence in patients with multiple sclerosis. Marmara Med J 2022; 35 (3): 323-329, doi: 10.5472/marumj.1192560

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1192560
Marmara Med J 2022;35(3): 323-329

satisfaction. Additionally, the treatment of MS being directed towards delaying poor prognosis and an insufficient perception of medicine efficiency can affect patient motivation negatively in MS in a manner differing from most chronic diseases [8-10].

The factors affecting treatment satisfaction in MS change alongside changing treatment options, changing treatment satisfaction levels in turn affect medication adherence, and a need to periodically evaluate medication adherence thus arises.

Treatment adaptation in MS is a multidimensional concept, and it is difficult to evaluate. While treatment adherence can be promptly evaluated using measurable objective parameters such as blood sugar, blood pressure, or HbA1C levels in other chronic diseases, there are no measurable parameters indicating treatment adherence in MS. Thus, the treatment adherence levels of individuals with MS can only be evaluated using standardized scales.

The aim of this study was to evaluate the attitudes of MS patients regarding medication adherence using standardized evaluation tools and to examine the effect of certain factors discussed in a small number of studies such as perceived social support and illness perception on treatment adherence. The findings of the study may contribute to planning efforts to increase treatment adherence.

2. PATIENTS and METHODS

This study was conducted as a cross-sectional research. Data for the study was obtained through face-to-face interviews with patients who presented at the neurology outpatient clinics of two university hospitals in the cities of Edirne and Tekirdag between April and October 2018.

Patients were invited to the study consecutively according to the time of admission to the outpatient clinic. Two hundred eleven patients who were 18 years of age or above, had been receiving treatment for MS for at least 6 months, had no communication problems, and signed the informed consent form were included in the study.

Data Collection Tools

Data were collected by use of a patient information form including socio-demographic, disease and treatment related characteristics of the patients, which was developed by the researchers. Also the four-item "Morisky, Green, and Levine Adherence Scale (MGLS)", the "Beck Depression Inventory (BDI)", the "Multidimensional Perceived Social Support Scale (MPSSS)", and the "Revised Illness Perception Scale (RIPS)" were used for data collection.

The Morisky, Green, and Levine Adherence Scale (MGLS)

This scale was developed by Morisky, Green, and Levine [11]. The scale consists of four closed ended items with two choices each. The questions are answered as "yes" or "no". Adherence was classified as high if all four questions were answered as "no", moderate if one or two questions were answered as "yes", and low if more than two questions were answered as "yes". Patients with moderate or low adherence were considered non-adherent [11]. In this study, the Cronbach alpha coefficient of the scale was calculated to be 0.829.

The Revised Illness Perception Questionnaire (IPQ-R)

The scale was developed by Weinmann et al. [12] and revised by Moss-Morris et al. [13]. The revised illness perception questionnaire (IPQ-R) was tested for the validity and reliability in Turkish by Kocaman et al. [14]. The IPQ-R consists of three sub-dimensions: illness identity, illness perception, and causes of the illness. The illness identity is questioned through 14 items, illness perception is questioned through 38 items, and the causes of the illness are questioned through 18 items. The identity of the illness is determined by asking the patients whether they have experienced the symptoms on the scale and whether they find these symptoms related to the illness. In the identity of the disease, each experienced and associated symptom is scored as "1". The illness perception comprises seven dimensions: timeline acute/chronic, timeline cyclical, consequences, personal control, treatment control, illness coherence, and emotional representations. The causes of the illness are sub divided into psychological characteristics, immune disorders, external risk factors, and accident/luck. Illness perception and the causes dimensions of the illness are evaluated with a 5-point Likert scale [13]. In our study, the Cronbach's alpha value of the illness perception dimension of the scale was calculated as 0.672.

The Multidimensional Scale of Perceived Social Support (MSPSS)

This scale was developed by Zimet et al. [15]. and tested for validity and reliability in Turkish by Eker et al. in 1995 [16]. The scale was revised for its cultural adaptation [17]. The scale consists of four items each in the family, friends, and significant other categories, for a total of 12 items. The scores that can be attained from the scale vary between 12 and 84, and higher scores indicate higher perceived social support. In this study, the Cronbach alpha value of the scale was found to be 0.897.

The Beck Depression Inventory (BDI)

This scale was developed to measure the bodily, emotional, cognitive, and motivational symptoms seen during depression [18]. There are 21 symptom categories in the scale, each scored between 0 and 3. The total depression score is obtained by the sum of these scores. Higher total scores indicate higher levels of depressive symptomology. The validity and reliability study of the Turkish form of the scale was performed by Hisli [19]. In this study, the Cronbach alpha value of the scale was found to be 0.919.

Local ethical board permission from Namık Kemal University, School of Medicine was taken before the study (Decision number: 2018/51/03/24). The volunteers who participated in the study were informed of the aim of the study by the researcher and gave consent. The principles of the Helsinki Declaration were upheld throughout the study.

Statistical Analysis

The SPSS (IBM, v.21,0) package program was used for statistical analyses. Descriptive statistical methods (numbers-percentages, mean, median, minimum and maximum) were used in the evaluation of study data. The internal consistency analyses of the scales used in the study were performed using the Cronbach alpha coefficient. The difference between nominal variables was determined using the Chi-square test. Since, Kolmogorov Smirnov test showed that data did not comply with normal distribution, the difference between continuous variables was calculated using Mann Whitney U or Kruskal Wallis test. The level of statistical significance was taken as $p<0.05$.

3. RESULTS

The mean age of the 211 individuals with MS who constituted the sample was 40.03±10.82, and 70.1% were female. In 88.2% of the patients (n=186), the clinical type of the disease was Relapsing-Remitting Multiple Sclerosis (RRMS). The mean time from diagnosis in patients was calculated as 8.05±6.29 years. More than half of the patients (54.3%) were using subcutaneous (SC) medications. 68.7% of the patients considered their treatment schedule as easy regarding the frequency and type of application (Table I).

Table I. *The effect of clinical variables on treatment adherence*

Variables	All groups	Perfect (high) adherence (MGLS=0)	Insufficient (medium andl ow) adherence (MGLS=1-4)	P-Value
Age, year				
Mean±SD	40.03±11.11	39.16±10.46	39.39±11.55	0.514[a]
Median (Min-Max)	40.0 (18.0-71.0)	40.0 (20.0-69.0)	40.0 (18.0-71.0)	
Gender				
Female, n (%)	148 (70.1)	73 (49.3)	75 (50.7)	0.387[b]
Male, n (%)	63 (29.1)	29 (46)	34 (54)	
MS type				
RRMS, n (%)	186 (88.2)	91 (48.9)	95 (51.1)	0.402[b]
SPMS/PPMS, n (%)	25 (11.8)	11 (44.0)	14 (56.0)	
Disease duration, year				
Mean±SD	8.05±6.29	6.45±5.11	8.72±6.70	0.052[a]
Median (Min-Max)	5.0 (0.6-29.0)	5.0 (0.6-20.0)	7.0 (1.0-29.0)	
Relapses within last year				
Mean±SD	0.89±0.99	0.87±0.94	0.92±1.08	0.932[a]
Median (Min-Max)	1.0 (0.0-5.0)	1.0 (0.0-5.0)	1.0 (0.0-5.0)	
Attendance at outpatient clinic				
Regular, n (%)	200 (94.8)	100 (50)	100 (50)	0.038*[b]
Irregular, n (%)	11 (5.2)	2 (18.2)	9 (81.8)	
Number of outpatient clinic visit (last year)				
Mean±SD	5.37±3.32	5.30±3.37	5.51±3.47	0.245[a]
Median (Min-Max)	4.0 (0.0-12.0)	4.0 (0.0-12.0)	4.0 (1.0-12.0)	
Hospitalization for MS				
Yes, n (%)	193 (91.5)	88 (45.6)	105 (54.4)	0.008**[b]
No, n (%)	18 (8.5)	14 (77.8)	4 (22.2)	
Route of medication administration				
Oral,n (%)	87 (41.2)	43 (49.4)	44 (50.6)	0.451[b]
Parenteral, n (%)	124 (58.8)	59 (47.6)	65 (52.4)	
Frequency of s.c.injection				
3-4 times a week, n (%)	95 (82.6)	37 (38.9)	58 (61.1)	0.011*[b]
Once a week, n (%)	20 (17.4)	14 (70.0)	6 (30.0)	
Person applying s.c. injection				
Self-injection, n (%)	88 (77.2)	39 (44.3)	49 (55.7)	0.938[c]
By someone else, n (%)	19 (16.7)	9 (47.4)	10 (52.6)	
Changeable, n (%)	7 (6.1)	3 (42.9)	4 (57.1)	
Perception of treatment program				
Complicated, n (%)	66 (31.3)	26 (34.9)	40 (60.6)	0.054[b]
Simple, n (%)	145 (68.7)	76 (48.3)	69 (51.7)	
The last major treatment change, year				
Mean±SD	3.79±3.76	3.18±3.29	4.35±4.20	0.048*[a]
Median (Min-Max)	2.0 (0.0-20.0)	2.0 (0.0-19.0)	3.00 (0.5-20)	

**p<0.05; **p<0.01; SD: Standart deviation; Min: Minimum; Max: Maximum;MGLS:Morisky, Green, and Levine Adherence Scale; [a]Mann-Whitney U test; [b]Chi-square test; [c]Fisher chi-square test;*

http://doi.org/10.5472/marumj.1192560
Marmara Med J 2022;35(3): 323-329

Treatment adherence was not good in half of the patients (51.7%) (Fig. 1). The mean score of the MS patients from the MGLS was 0.78±0.90.

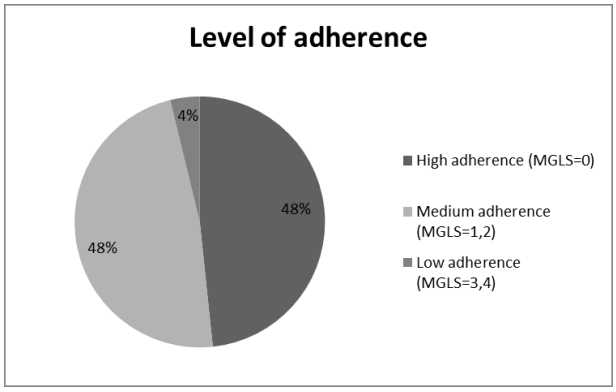

Figure 1. *The patients` level of adherence; MGLS: Morisky, Green, and Levine Adherence Scale*

Treatment adherence did not vary according to age or sex among the patients, and neither was it affected by type of MS, disease duration, number of attacks, number of outpatient clinic visit, route of medicationadministration,person applying the SC injection, or perception of difficulty in the treatment program. The application frequency of SC injections, on the other hand, was seen to be an important variable in treatment adherence ($p<0.05$). Patients with poor adherence were found to miss out on their outpatient clinic visit more compared to patients with good adherence, and have to be hospitalized more ($p<0.05$), (Table I).

Multiple sclerosis patients with poor treatment adherence stated forgetfulness (46%) to be the most important reason behind this situation (Fig 2).

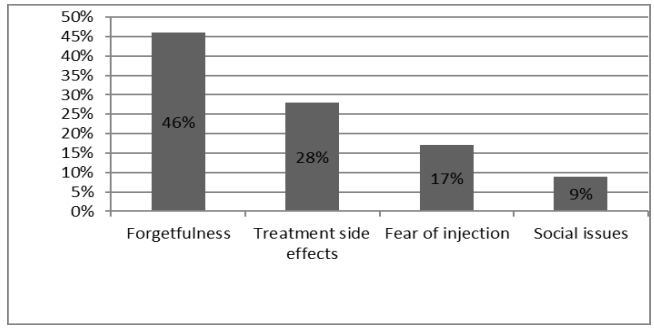

Figure 2. *Causes of non-adherence to treatment ;Note:More than one reason has been chosen*

In the illness perception evaluation, the MS patients were found to complain most about fatigue (87.2%), and hold psychological reasons responsible for the presence of their disease (Not included in Tables).

The effect of illness perception on treatment adherence among MS patients could not be shown ($p>0.05$). The strongest source of social support among the MS patients was "family". Patients with good treatment adherence had higher MSPSS scores ($p<0.01$) and lower BDI scores ($p<0.01$), (Table II).

Table II. *Depression, social support, and illness perception according to treatment adherence*

	All groups	High adherence (MGLS=0) n=102	Medium adherence (MGLS=1-2) n=101	Low adherence (MGLS=3-4) n=8	P Value
IPQ-R					
Consequences					
Mean±SD	19.06±5.26	18.49±5.11	19.73±5.22	18.00±7.19	0.211[d]
Median (Min-Max)	19.0(6.0-30.0)	18.0 (6.0-30.0)	2.0 (9.0-29.0)	18.5 (10.0-30.0)	
Timelineacute/chronic					
Mean±SD	22.56±4.75	22.45±4.55	22.89±4.76	19.87±7.03	0.315[d]
Median (Min-Max)	23.0 (6.0-30.0)	22.5 (11.0-30.0)	24.0 (6.0-30.0)	21.5 (10.0-30.0)	
Personal control					
Mean±SD	20.19±3.82	19.84±3.76	20.56±3.96	20.12±3.44	0.567[d]
Median (Min-Max)	20.0 (10.0-30.0)	20.0 (10.0-27.0)	21.0 (10.0-30.0)	20.0 (16.0-27.0)	
Treatment control					
Mean±SD	17.82±3.89	17.96±3.88	17.85±3.75	15.87±5.51	0.634[d]
Median (Min-Max)	18.0 (7.0-25.0)	18.0 (8.0-25.0)	18.0 (7.0-25.0)	17.0 (7.0-23.0)	
Illness coherence					
Mean±SD	17.31±4,07	17.45±4,09	17.11±4.00	18.00±4.95	0.745[d]
Median (Min-Max)	17.0 (7.0-25.0)	17.0 (7.0-25.0)	17.0 (9.0-25.0)	18.0 (9.0-25.0)	
Timelinecyclical					
Mean±SD	13.78±3.46	13.59±3.67	13.93±3.29	14.25±3.05	0.677[d]
Median (Min-Max)	14.0 (4.0-20.0)	14.0 (4.0-20.0)	14.0 (4.0-20.0)	15.5 (7.0-16.0)	
Emotional representations					
Mean±SD	18.91±5.71	19.05±5.73	18.90±5.68	17.37±6.30	0.629[d]
Median (Min-Max)	20.0 (6.0-30.0)	19.0 (6.0-30.0)	20.0 (6.0-29.0)	16.5 (10.0-30.0)	

http://doi.org/10.5472/marumj.1192560
Marmara Med J 2022;35(3): 323-329

Table II. *(Contued)*

MSPSS					
MSPSS total					
Mean±SD	64.79±16.53	69.52±14.20	60.48±17.33	58.87±19.18	0.000**d¥
Median (Min-Max)	69.0 (18.0-84.0)	74.0 (27.0-84.0)	61.0 (18.0-84.0)	61.5 (36.0-81.0)	
MSPSS-FA					
Mean±SD	24.87±4.76	25.68±4.16	24.01±5.12	25.25±6.25	0.007*d¥
Median (Min-Max)	27.0 (5.0-28.0)	28.0 (7.0-28.0)	26.0 (5.0-28.0)	28.0 (10.0-28.0)	
MSPSS-FR					
Mean±SD	20.65±7.28	22.40±6.36	19.15±7.61	17.37±9.41	0.002*d¥
Median (Min-Max)	23.0 (4.0-28.0)	25.0 (6.0-28.0)	21.0 (4.0-28.0)	19.5 (4.0-28.0)	
MSPSS-SO					
Mean±SD	19.20±8.13	21.37±7.23	17.25±8.42	16.25±8.90	0.001**d¥
Median (Min-Max)	21.0 (4.0-28.0)	24.0 (4.0-28.0)	17.0 (4.0-28.0)	19.0 (4.0-28.0)	
BDI					
BDI score					
Mean±SD	13.10±10.28	9.00±8.74	16.73±10.40	20.33±3.77	0.003*d¥
Median (Min-Max)	11.0 (0.0-42.0)	7.5 (0.0-42.0)	17.0 (0.0-41.0)	19.0 (17.0-25.0)	

**p<0.05; **p<0.01; SD: Standart deviation; Min: Minimum; Max: Maximum; [d]Kruskal-Wallis Test; ¥the difference was between patients with high adherence and medium adherence according to Tukey's post hoc analysis; MGLS:Morisky, Green, and Levine Adherence Scale; MSPSS: Multidimensional Scale of Perceived Social Support; MSPSS-FA: MSPSS Family; MSPSS-FR: MSPSS Friends; MSPSS-SO: MSPSS Significant Other; BDI: Beck Depression Inventory;IPQ-R: Revised Illness Perception Questionnaire*

4. DISCUSSION

Adherence with treatment is important in slowing the progression of MS and improving the patient's quality of life, and therefore factors that may affect adherence should be investigated.

In our study, 48.3% of the individuals with MS were seen to exhibit good treatment adherence. While only patients receiving parenteral treatments are included in some studies evaluating treatment adherence, other studies include all administration methods including oral and parenteral administration in a manner similar to our study. In a study conducted with 198 patients in Turkey using the Multiple Sclerosis Treatment Adherence Questionnaire, 59.6% of the patients were found to adhere to their disease-modifying therapy. In that particular study, oral medication adherence was not evaluated [10]. In another study conducted in the Turkish society with 219 MS patients, the facts that SC interferon treatments were skipped four times a month, glatiramer acetate treatments were skipped six times a month, and IM interferon treatments skipped once a month were considered poor treatment adherence by the authors. In that particular study, while 53% of the RRMS group and 52% of the SPMS group had good treatment adherence, 24.9% of the patients completely abandoned their treatments [9]. In a multinational evaluation, 75% of 2648 MS patients receiving SC and IM treatments were found to have good treatment adherence [20], while 71% of 157 MS patients receiving oral and/or parenteral treatments were found to have good treatment adherence in a study conducted in Spain [21], and 48% of 188 patients receiving immune modulators were found to have good treatment adherence in a study conducted in Brazil [22].

The difference in the methods of evaluating treatment adherence may be held responsible for the variance in results to a degree. For example, although 3.8% of the patients were categorized as having poor treatment adherence and 47.9% were categorized as having medium treatment adherence according to the MGLS in our study, 27.5% of the patients answered "yes" when the researchers asked the question "Do you think you have poor treatment adherence?" As it can be seen from this example, the use of standardized evaluation scales can contribute to more objective and comparable results.

The effects of MS type, disease duration, number of attacks, outpatient clinic visit frequency, the person performing the SC injection, or the perceived difficulty of the treatment schedule of the patient on treatment adherence could not be shown in this study. While Rio et al., did report higher rates of low treatment adherence among SPMS patients [23], most studies could not find a relationship between treatment adherence and type of MS [9,10,21]. Treatment adherence in subcutaneously administered mediations has been found to be lower compared to medications administered orally or through IV infusion [24]. In our study, while whether the medication was administered through oral or parenteral means did not affect treatment adherence, the frequency of SC injections did. Treatment adherence was better among those receiving weekly SC injections compared to those receiving 3-4 SC injections a week.

Our results Show that patients who missed their outpatient clinic visits were found to have low treatment adherence. The importance of regular outpatient clinic attendance for treatment adherence has been previously emphasized in the literature [22,25]. Since, the reasons behind missing outpatient clinic visits and having poor treatment adherence may overlap, questioning the reasons behind missing outpatient clinic appointments may contribute to increasing treatment adherence.

In our study, patients hospitalized were shown to have lower treatment adherence, and this finding was interpreted as low treatment adherence negatively affecting disease progression.

The cost of inpatient treatment to the health care system is another important dimension of low treatment adherence.

In this study, 46% of the patients stated forgetfulness to be the primary reason behind poor treatment adherence. Forgetfulness has been shown as an important reason behind low treatment adherence in MS in the literature as well [10,20,21,26]. A third of MS patients experience injection related problems such as exasperation caused by long term injection applications, pain in the injection area, injection related anxiety, and skin reactions [20]. In our study, 28% of the patients stated that they experienced treatment adherence difficulties because of the side effects of medications.

Treatment adherence among MS patients is a multifactorial concept. The chronic nature of the disease, the prognosis of the disease, variances in disease progression all constitute the component of illness perception [12,13]. It is known that MS patients who accept their disease are better in terms of adherence to treatment [27]. Ilness perception was considered as a predictor for treatment adherence. However, illness perception was found not to affect treatment adherence among MS patients in this study.

Social support is very important in coping with chronic diseases and ensuring treatment adherence. In our study, the importance of social support for treatment adherence among MS patients was shown. While the strongest source of social support for our sample was "family", the weakest source was "significant others". In another study examining the role of social support among MS patients in Turkey, family was found to be the strongest source of social support [28]. The importance given to the MSPSS components of social support, namely family, friends, and significant others, may vary from society to society. For example, support from friends was found to be a more effective type of social support compared to familial support in a study conducted with MS patients in the USA [29].

Comorbid conditions that are considered basic factors affecting treatment adherence, such as depression, can be found among substantial number of patients as a result of MS and/or its treatment [24,30-32]. The effect of depression on treatment adherence is unclear. Although, some studies have reported depression to not affect treatment adherence [7,8], depressive symptomology was shown to negatively affect treatment adherence in our study, in a manner similar to the study by Higuera et al. [24].

Limitations

The fact that the results of this study conducted in two centers cannot be generalized to all MS patients constitutes the limitation of this study.

5. CONCLUSION

Half of the MS individuals in the study (52%) did not have good treatment adherence. Treatment adherence should be evaluated using standardized evaluation tools during patient follow up and the reasons behind poor treatment adherence should be explored. Since, forgetfulness was stated to be the most important reason behind poor treatment adherence, considering the cognitive disruption in individuals with MS, planning should be performed to ensure the patients do not forget taking medications on time. More cooperation with families, which constitute the strongest source of social support in the treatment process, should be achieved. Since, depression is an important factor in the treatment adherence of MS patients, screening tools should be used for the early detection of depressive symptoms. Various precautions should be taken to ensure regular outpatient clinic visits to increase treatment adherence. The treatment adherence of patients should be comprehensively evaluated in cases of frequent hospitalizations.

Acknowledgments

The authors thank all the patients and their care givers who took the time to participate in this study.

Compliance with Ethical Standards

EthicalApproval: Local ethical board permission was taken from Namık Kemal University, School of Medicine before the study (Decision number: 2018/51/03/24). The volunteers who participated in the study were informed of the aim of the study by the researcher and gave consent. The principles of the Helsinki Declaration were upheld throughout the study. In order to use the MGLS, permission was taken from Donald E. Morisky through e-mail.

Financial support: This research received no specific grant from any funding agency in the public, commercial, or non-profit sectors.

Conflict of Interest Statement: There is no conflict of interest.

Authors' Contributions: DG, ZT, NT and SG: Concept, DG and ZT: Design, ZT, NT, SG: Supervision, DG and ZT: Data Collection and/ or processing, DG and ZT: Analysis and / or interpretation, DG and ZT: Literature search, DG and ZT: Writing, ZT, NT and SG: Critical review. All authors read and approved the final version of the aticle.

REFERENCES

[1] GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2019; 18:459-80. doi:10.1016/S1474-4422(18)30499-X

[2] Celik Y, Birgili O, Yılmaz H, et al. Prevalence of multiple sclerosis in the metropolitan area of Edirne City, Turkey. Balkan Med J 2011; 28:193-6 doi: 10.5174/tutfd.2010.04089.0

[3] Türk Börü Ü, Duman A, Kulualp AŞ, et al. Multiple sclerosis prevalence study: The comparison of 3 coastal cities, located in the black sea and mediterranean regions of Turkey. Medicine (Baltimore) 2018;97:e12856. doi:10.1097/MD.000.000.0000012856

[4] Akdemir N, Terzi M, Arslan N, Onar M. Prevalence of multiple sclerosis in the Middle Black Sea Region of Turkey

and demographic characteristics of patients. Noro Psikiyatr Ars 2017;54:11-4. doi:10.5152/npa.2016.12451

[5] Gökçe ŞF, Çiğdem B, Nemmezi Karaca S, et al. Prevalence of multiple sclerosis in an urban population of Sivas province in Turkey. Turk J Med Sci 2019;49:288-94. doi:10.3906/sag-1808-112

[6] Magyari M, Sorensen PS. The changing course of multiple sclerosis: rising incidence, change in geographic distribution, disease course, and prognosis. Curr Opin Neurol 2019;32:320-26. doi:10.1097/WCO.000.000.0000000695

[7] McKay KA, Tremlett H, Patten SB, et al. Determinants of non-adherence to disease-modifying therapies in multiple sclerosis: A cross-Canada prospective study. Mult Scler 2017;23:588-96. doi:10.1177/135.245.8516657440

[8] Hao J, Pitcavage J, Jones JB, Hoegerl C, Graham J. Measuring adherence and outcomes in the treatment of patients with multiple sclerosis. J Am Osteopath Assoc 2017;117:737-47. doi:10.7556/jaoa.2017.145

[9] Köşkderelioğlu A, Gedizlioğlu M, Ortan P, Öcek Ö. Evaluation of the adherence to immunmodulatory treatment in patients with multiple sclerosis. Noro Psikiyatr Ars 2015;52:376-9. doi:10.5152/npa.2015.8825

[10] Erbay Ö, Usta Yeşilbalkan Ö, Yüceyar N. Factors affecting the adherence to disease-modifying therapy in patients with multiple sclerosis. J Neurosci Nurs 2018;50:291-7. doi:10.1097/JNN.000.000.0000000395

[11] Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care 1986;24:67-74. doi:10.1097/00005.650.198601000-00007

[12] Weinman J, Petrie K, Moss-Morris R, Horne R. The illness perception questionnaire: a new method for assessing the cognitive representation of illness. Psychol Health 1996; 11:431-45. doi: 10.1080/088.704.49608400270

[13] Moss-Morris R, Weinman J, Petrie K, et al. The revised illness perception questionnaire (IPQ-R). Psychol Health 2002;17:1-16. doi:10.1080/088.704.40290001494

[14] Kocaman N, Ozkan M, Armay Z, Ozkan S. The reliability and the validity study of Turkish adaptation of the revised Illness Perception Questionnaire. Anatolian Journal of Psychiatry 2007; 8:271-280.

[15] Zimet GD, Dahlem NW, Zimet SG, Farley GK. The Multidimensional Scale of Perceived Social Support. J Pers Assess 1988; 52 :30-41. doi:10.1207/s15327752jpa5201_2

[16] Eker D, Arkar H. Perceived social support: psychometric properties of the MSPSS in normal and pathological groups in a developing country. Soc Psychiatry Psychiatr Epidemiol 1995;30:121-6. doi:10.1007/BF00802040

[17] Eker D, Arkar H, Yaldız H. Factorial structure, validity, and reliability of revised form of the Multidimensional Scale of Perceived Social Support. Türk Psikiyatri Dergisi 2001; 12: 17-25.

[18] Beck AT, Ward CH, Mendelson M, et al. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561-71. doi:10.1001/archpsyc.1961.017.10120031004

[19] Hisli N. Use of the Beck Depression Inventory with Turkish uni versity students: reliablity, validity and factor analysis. J Psychol1989;:3-13.

[20] Devonshire V, Lapierre Y, Macdonell R, et al. The Global Adherence Project (GAP): a multicenter observational study on adherencet o disease-modifying therapies in patients with relapsing-remitting multiple sclerosis. Eur J Neurol 2011;18:69-77. doi:10.1111/j.1468-1331.2010.03110.x

[21] MorilloVerdugo R, Ramírez Herráiz E, Fernández-Del Olmo R, et al. Adherence to disease-modifying treatments in patients with multiple sclerosis in Spain. Patient Prefer Adherence 2019;13:261-72. doi:10.2147/PPA.S187983

[22] Câmara NAAC, Gondim APS. Factors associated with adherence to immunomodulator treatment in people with multiple sclerosis. Braz J Pharm Sci 2017;53:e16132.doi.org/10.1590/s2175.979.0201700.011.6132

[23] Río J, Porcel J, Téllez N, et al. Factors related with treatment adherence to interferon beta and glatiramer acetate therapy in multiple sclerosis. Mult Scler 2005;11:306-9. doi:10.1191/135.245.8505ms1173oa

[24] Higuera L, Carlin CS, Anderson S. Adherence to disease-modifying therapies for multiple sclerosis. J Manag Care Spec Pharm 2016;22:1394-401. doi:10.18553/jmcp.2016.22.12.1394

[25] Gromisch ES, Turner AP, Leipertz SL, et al. Who is not coming to clinic? A predictive model of excessive missed appointments in persons with multiple sclerosis. Mult Scler Relat Disord 2020;38:101513. doi:10.1016/j.msard.2019.101513

[26] Ožura A, Kovač L, Sega S. Adherence to disease-modifying therapies and attitudes regarding disease in patients with multiple sclerosis. Clin Neurol Neurosurg 2013;115 Suppl1:S6-S11. doi:10.1016/j.clineuro.2013.09.013

[27] Kołtuniuk A, Rosińczuk J. The levels of depression, anxiety, acceptance of ıllness, and medication adherence in patients with multiple sclerosis –descriptive and correlational study. Int J Med Sci 2021;18:216-25. doi:10.7150/ijms.51172

[28] Zengin O, Erbay E, Yıldırım B, Altındag O. Quality of life, coping, and social support in patients with multiple sclerosis: A pilot study. Turk J Neurol 2017; 23:211-8.

[29] Kasser SL, Kosma M. Social cognitive factors, physical activity, and mobility ımpairment in adults with multiple sclerosis. Behav Med 2018;44:306-13. doi:10.1080/08964.289.2017.1368441

[30] Soyuer F, Unalan D, Mirza M. Depressive symptoms in multiple sclerosis and the association with sociodemographic factors and functional status. Turk J Neurol 2010;16:31-5.

[31] Bilgi E, Ozdemir HH, Bulut S. Determining the frequency of depression and cognitive dysfunction in patients with multiple sclerosis. TJN 2013; 19:11-4.

[32] Gottberg K, Einarsson U, Fredrikson S, et al. A population-based study of depressive symptoms in multiple sclerosis in Stockholm county: association with functioning and sense of coherence. J Neurol Neurosurg Psychiatry 2007;78:60-5. doi:10.1136/jnnp.2006.090654

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Unilocular and multilocular thymic cysts: A study on the possible histomorphological and/or clinical differences

Emine BOZKURTLAR

Department of Pathology, School of Medicine, Marmara University, Istanbul, Turkey

Corresponding Author: Emine BOZKURTLAR
E-mail: eminebash@gmail.com

Submitted: 30.04.2022 **Accepted:** 23.07.2022

ABSTRACT

Objective: Thymic cysts are rare mediastinal cystic pathologies and have two subtypes namely unilocular and multilocular. This study aims to investigate the clinicopathological characteristics of thymic cysts and to compare the clinical and histopathologic features of multilocular thymic cysts (MTCs) and unilocular thymic cysts (UTCs).

Patients and Methods: Twenty-three patients with the diagnosis of thymic cyst between 2012 and 2020 were included. We compared the clinicopathological characteristics of unilocular and multilocular thymic cysts.

Results: The mean age of patients was 43 years, ranging from 6 to 80 years. Fourteen cases were UTCs and 9 cases were MTCs. MTCs were found to be statistically more common in younger patients, and have much more histomorphological changes pointing to the complicated cysts ($p<0.05$). Moreover, we noted that patients with MTCs were found to be more symptomatic before diagnosis. Furthermore, in our study when we compared MTCs and UTCs, UTCs were slightly (64.2%) more frequent in female patients.

Conclusion: According to our results, thymic cysts may also be seen at younger ages. MTC and UTC can have different histomorphological characteristics, such as foreign body reaction and hemorrhage. Moreover, there can be different clinical features, such as age, gender, and symptoms, between these two subtypes.

Keywords: Thymus, Cyst, Multilocular, Unilocular, Pathology, Histomorphology

1. INTRODUCTION

Thymus is a specialized lymphoid organ located in the anterior mediastinum. Thymic cysts (TCs) are uncommon benign lesions arising in the anterior mediastinum or cervical region. Either congenital or acquired in origin they comprise 1-3% of all mass lesions in the anterior mediastinum. However, they should be kept in mind in the differential diagnosis of mediastinal lesions. The two subtypes of TC are multilocular thymic cysts (MTCs) and unilocular thymic cysts (UTCs). According to the reports, UTCs are congenital lesions with thin walls that do not exhibit inflammatory changes, whereas, MTCs are acquired lesions that develop as a result of inflammatory conditions. These inflammatory conditions have thick fibrous walls [1, 2]. Neoplastic and other pathologies originating from the thymus or non-thymic tissues may be linked to TCs, particularly to MTC [4-8]. Therefore, it is crucial to examine these lesions in detail and make a precise diagnosis.

The purpose of this study is to describe the clinicopathological characteristics of thymic cysts and to compare the clinical and histomorphological characteristics of MTCs and UTCs, as well as to investigate possible pathologies that could coexist or be related to thymic cysts.

2. PATIENTS and METHODS

The demographic and clinical features of 23 cases with thymic cysts admitted to Marmara University Hospital, Istanbul between 2012 and 2020, were obtained from the data of our hospital management system. All patients underwent resection by video-assisted thoracic surgery, and the diagnoses were confirmed by histopathological examination. All hematoxylin and eosin (H&E) stained slides were evaluated by the researcher. Histomorphological findings including the diameter of cysts,

How to cite this article: Bozkurtlar E. Unilocular and multilocular thymic cysts: A study on the possible histomorphological and/or clinical differences. Marmara Med J 2022; 35 (3): 330-334, doi: 10.5472/marumj.1192193

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1192193
Marmara Med J 2022;35(3): 330-334

unilocular or multilocular structure of lesions, the epithelial lining of cysts, and the structure/contents of the cyst wall and lumen were examined in detail.

The present study was approved by the Marmara University, School of Medicine, Clinical Research Ethics Committee (dated, Jan 2017; Approval No. 09.2017.69).

Statistical Analysis

One sample t-test and chi-square test were used for statistical analysis. A p value of < 0.05 was considered statistically significant. Data were analyzed with *Jamovi* (The jamovi project 2021-Version 1.6) [3].

3. RESULTS

The main demographic and clinical features of the patients with TCs are listed in Table I. Of the 23 patients, 10 (43.4%) were male and 13 (56.5%) were female. The mean age of the patients was 43 years (ranging from 6 to 80 years).

Table I. *Demographic and clinical features of patients with thymic cyst.*

Case number	Age	Gender	Symptoms	Initial clinical/ radiological diagnosis related to thymic cysts
1	72	F	Not known	AMM
2	48	F	Screening for MG	MG/AMM
3	40	F	Dyspnea	Cervical mass
4	39	F	Screening for MG	MG/AMM
5	7	M	Mass lesion protruding below sternum with cough	Thymic hyperplasia
6	21	F	Screening for MG	MG/AMM
7	37	F	Dry cough	Pericardial mass
8	53	F	Dyspnea	AMM/Cyst
9	9	M	Servical swelling	Thymic cyst
10	66	M	Dry cough	AMM/Cyst-Thymic cyst
11	32	M	Incidental	AMM/Cyst
12	6	F	Chest pain	Mediastinal cyst
13	11	M	Servical swelling	Cervical mass
14	70	F	Incidental	Pericardial cyst
15	53	F	Incidental	AMM
16	62	M	Incidental	Pericardial cyst
17	55	F	Incidental	Thymoma
18	39	M	Screening for MG	MG/Thymoma
19	48	F	Dorsalgia	AMM/Cyst
20	57	M	Screening for MG	MG/AMM
21	49	M	Dyspnea	AMM
22	80	M	Chest pain	AMM/Cyst
23	36	F	Incidental	AMM/Cyst

M: Male, F: Female, AMM: Anterior mediastinal mass, MG: Myasthenia gravis.

Patients presented to the clinic with a wide range of symptoms; 3 (13%) with dyspnea, 2 (8.6%) with dry cough, 1 (4.3%) with cough and protruding mass lesion below the sternum, 2 (8.6%) with chest pain, 2 (8.6%) with cervical swelling and 1 (4.3%) with dorsalgia. TCs were noticed incidentally on radiological imaging in six (26%) patients and five (21.7%) of 23 were detected during the screening of thymus by virtue of Myasthenia gravis. No clinical data were obtained for one patient and patients with known clinical history had no previous trauma or thoracic surgery. Median follow-up was 4.7 years for TCs.

Ten (43.4%) of 23 patients had cystic lesions initially diagnosed by radiological evaluation. Only one patient had a diagnosis of a thymic cyst according to his radiological report. Two of ten (20%) were described as having a pericardial cystic lesion. Other than cystic lesions, 13 (56.5%) anterior mediastinal masses, 2 (8.6%) cervical masses, 1 (4.3%) pericardial mass, 2 (8.6%) thymomas and 1 (4.3%) thymic hyperplasia were detected on radiological imaging.

Histomorphologically, two of 23 (8.6%) were diagnosed as cervical TCs. The rest of the cases had mediastinal TCs.

The mean diameter of thymus and the largest cystic lesion were 5.8 cm (min:0.5, max:14 cm) and 2.7 cm (min:0.5, max:8 cm), respectively (Table II). In 7 (30.4%) cases, the cyst walls were single-layered and two-layered epithelioid cells, and in 9 (39.1%) cases, the cyst walls were lined with ciliated pseudostratified epithelium (Figure 1). Cholesterol cleft, histiocyte, hemorrhage, and foreign body reaction pointing to the complicated cysts were found in the cyst wall and lumen in 6 (26%) and 9 (39.1%) cases, respectively, (Figure 1). Fifteen (65.2%) residual thymus tissue, 1 (4.3%) thymic hyperplasia, 4 (17.2%) thymic tissue with reactive follicular hyperplasia, and 3 (13%) thymomas were detected in the thymic tissue adjacent to the thymic cysts.

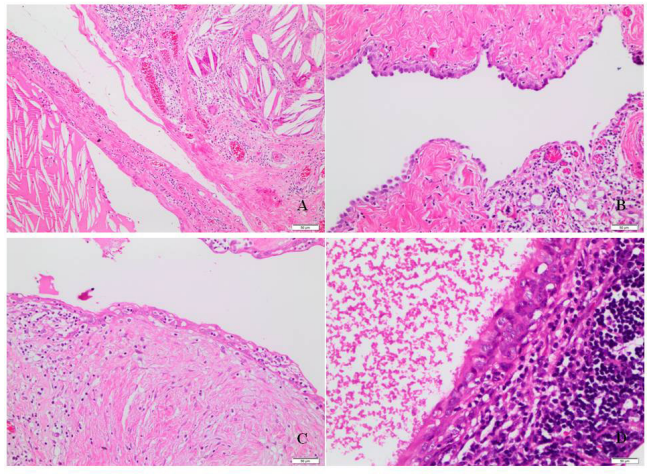

Figure 1. A: Foreign body type reaction with cholesterol clefts, H&E-4x. **B:** One-layered epithelium of cyst wall, H&E-10x. **C:** Two-layered epithelium of cyst wall, H&E-10x. **D:** Pseudostratified epithelium of cyst wall, H&E-20x.

http://doi.org/10.5472/marumj.1192193
Marmara Med J 2022; 35(3): 330-334

Table II. *Histomorphological findings of thymic cysts of patients.*

Case number	Size of total lesion [cm]	Diameter of largest cystic lesion [cm]	M/U	LE of cyst	Fibrous wall of cyst	Complication on wall of cyst	Complication in lumen of cyst	Accompanying thymic tissue
1	1.5	1.5	U	OE	Present	Absent	Absent	RT
2	6	3	U	CP	Absent	Absent	Absent	RFH
3	4.6	1.5	M	CP	Present, focal	Absent	Absent	Thymic hyperplasia
4	10	1	M	CP	Present	Absent	Absent	Thymoma, B1
5	5.5	1	M	TE	Present	FBR, CC	FBR, CC	RT
6	5	2	U	OE	Absent	Absent	Absent	RFH
7	3	3	U	OE	Present	Absent	Absent	RT
8	4.5	4.5	U	TE	Present	Absent	Absent	RT
9	4	1.5	M	TE	Present	FBR, CC	FBR, CC	RT
10	6.5	6.5	U	TE	Absent	Absent	Absent	RT
11	5	5	U	CP	Present	LK , H, HI	LK, H, HI	RT
12	6.5	2	M	TE	Present, focal	FBR, CC	FBR, CC	RT
13	6	2.5	M	OE	Present	FBR, CC	FBR, CC, H	RT
14	8	8	U	OE	Present, focal	Absent	Absent	RT
15	4.5	4.5	U	CP	Present, focal	Absent	Absent	RT
16	0.5	0.5	U	CP	Present	Absent	Absent	RT
17	3.5	3.5	U	CP	Present	Absent	Absent	RT
18	7	0.8	U	TE	Present, focal	Absent	Absent	Thymoma, B2
19	2	2	U	CP	Present	Absent	Absent	RT
20	6	1.5/0.8	M	OE	Present	Absent	Histiocytes	Thymoma, B2
21	16	7/3.5	M	OE	Absent	Absent	Focal FBR, CC	RFH
22	4	4	U	TE	Absent	Absent	Absent	RT
23	14	8/1.5	M	CP	Absent	Absent	CC, H	RFH

Multiloculation: M, Uniloculation: U, LE: Lining epithelium, OE: One-layered epitheloid, CP: Ciliated pseudostratified, TE: Two-layered epitheloid, FBR: Foreign body type reaction, CC: Cholesterol clefts, LK: Lamellar keratin, H: Hemorrhage, HI: Histiocytes, RT: Residual thymus, RFH: Reactive follicular hyperplasia.

When we grouped the cases as MTC and UTC, the number of cases was 9 and 14, the mean age was 28.2 years (ranging from 6 to 49 years) and 52.6 years (ranging from 21 to 80 years), and the ratio between the male and female was 4/5 and 9/5, respectively (Table II). In terms of mean age, MTC cases were found to be statistically more common in younger patients ($p<0.05$). Moreover, UTC cases were more frequent in female patients. The total lesion diameters of MTC and UTC cases (6 and 5.4 cm, respectively) were identical. Morphologically, the ciliated pseudostratified epithelium was slightly more frequently seen in UTCs. In MTC cases, histomorphological features pointing to the complicated cysts, such as foreign body type reaction, cholesterol clefts, lamellar keratin, hemorrhage, and histiocytes, in the cyst wall and lumen were seen more frequently ($p<0.05$) (Table II). It was discovered that cases with the diagnosis of MTC were more symptomatic ($p=0.193$). Regarding the presence of Myasthenia gravis disease and other accompanying thymus pathologies, there was no statistically significant difference between the MTC and UTC groups.

4. DISCUSSION

In our study, the mean age of cases with TC was 43 years, and the most common symptom of in these cases was dyspnea. Clinical or radiological cystic lesions at initial diagnosis were defined in almost half (43.4%) of TCs. When we compared features of MTCs and UTCs of our cases, MTCs were detected in younger age groups, were more symptomatic, and represented more histomorphological features pointing to the complicated cysts than UTCs.

Thymic cysts are rare lesions arising from embryonic remnants along the course of thymic migration or acquired lesions in the neck or the anterior mediastinum. Cervical TCs represent 1% of all cervical cystic masses. Cervical TC is a rare lesion, mostly described in pediatric patients, and much more rarely seen in adults [4-8]. We discovered that cervical TCs were not that infrequent since two (8.6%) of our 23 cases were cervical TC. In addition, the ratio of adult cervical TC of our TCs was 4.3% (a patient at 40 years) which may represent adult patients with cervical TC are more common than known. But the last inference is uncertain because of the limited number of our cases.

Thymic cysts and conditions characterized by cystic changes in the thymus can cause non-specific symptoms such as chest pain, dyspnea, and cough [9, 10]. Cervical swelling, sternal swelling, and dorsalgia were observed in our patients and the most common symptom of TCs was dyspnea. Statistically, significant difference was not found between UTC and MTC, even though MTC cases were more frequently symptomatic in terms of the symptoms that led patients to apply to the clinic, in our study.

Mediastinal cystic lesions are infrequent but have a variety of histological types, not only thymic but also bronchogenic, pericardial, enteric, Müllerian, lymphatic, and parathyroid types [11, 12]. Several radiological researches study the differential diagnosis of thymic cystic and thymic epithelial tumors, but it has not been well understood yet [13, 14]. The radiological or clinical diagnosis of the cystic mediastinal or thymic lesion could be described for approximately only half of our thymic cysts (43.4%). Histomorphological evaluation of mediastinal/thymic lesions is mandatory for precise diagnosis. Diagnosis for our patients were precise as all of our cases were evaluated histomorphologically.

Although, in some researches, it has been shown that the mean age of patients with TCs was 50 years and older, the mean age of our patients was 43 years [15]. We divided our cases into two groups as UTCs and MTCs. The mean age for UTCs (52.6 years) was statistically older than that of MTCs (28.2 years) . No gender difference was observed in our patients, which is consistent with studies in the literature [14]. On the other hand, in females, UTCs were seen slightly more frequently when compared with males, but there was no statistical difference between the genders.

In one of the earliest studies on MTC cases, Suster et al., defined histomorphological findings in MTCs, such as cholesterol granulomas and hemorrhage accompanying acute and chronic inflammation, and revealed that this inflammation played a role in the development of MTCs [1]. In contrast, UTCs are known to be congenital lesions with thin walls that do not exhibit inflammatory changes [2]. In our study, MTC cases had more frequent morphological findings that indicated complications/inflammation in the cyst wall and lumen, such as foreign body reaction and hemorrhage. However, not all of our MTC cases exhibited the histomorphological changes suggestive of this complication, but also some of UTCs represented these complicated features with inflammation. This might imply that not all MTCs are necessarily caused by acquired inflammation, and vice versa for UTC but to be certain, further clinical follow-up and histomorphological analysis of larger series are required.

Although, they are not very common, concomitant lesions such as thymoma in the thymus tissue, and thymic and reactive follicular hyperplasia have been described in the literature [10, 16-18]. In fact, in one instance, no solid component was seen, and all of the thymoma areas surrounding the thymic cyst had a cystic structure [19]. Furthermore, it is important to keep in mind cystic morphology when making a differential diagnosis of thymic cysts, since, it may play a crucial role in the development of mediastinal seminoma and micronodular thymoma [9, 20]. In addition, it should be noted that malignant neoplastic proliferations originating from the thymus or other origins may especially develop in MTCs [21-23]. In our study, thymoma, or thymic or reactive follicular hyperplasia was found accompanying thymic cysts in 8 (34.8%) cases.

According to the previous studies, it has been revealed that the incidence of Sjögren's syndrome and similar autoimmune diseases was high in MTC [16, 24-26]. However, no myasthenia gravis, Sjögren's syndrome, or a similar autoimmune disease was observed during preoperative or postoperative follow-up in MTCs with reactive follicular hyperplasia in our study. This situation may be related to short mean follow-up time.

Due to its retrospective nature and focus on cases from a single center, our study has some limitations. Additionally, the small number of cases prevented us from producing results from some statistical analyses.

Conclusion

As a result, thymic cysts are rare mediastinal or cervical lesions that can be unilocular or multilocular. They should be kept in mind in the differential diagnoses during the evaluation of any thymic cyst. Detailed and careful sampling is important to make a definite diagnosis. MTCs and UTCs have clinical differences such as age and gender of the patient, being symptomatic, and histomorphological differences such as hemorrhage and foreign body reaction.

The only radical treatment is the complete surgical removal of the cyst, which can suppress symptoms, provide a formal diagnosis, and prevent complications.

Compliance with Ethical Standards

Ethical Approval: This study was approved by Marmara University The School of Medicine Clinical Research Ethics Committee with the approval number 09.2017.69, dated Jan 2017.

Financial Support: No specific funding was received.

Conflict of Interest: There are no conflicting interests.

Author Contribution: EB: Drafting of the work, data acquisation, critical revision, concept, design of the study and statistical analysis.

REFERENCES

[1] Suster S, Rosai J. Multilocular thymic cyst: an acquired reactive process. Study of 18 cases. Am J Surg Pathol 1991;15:388-98. PubMed PMID: 2006719.

[2] Indeglia RA, Shea MA, Grage TB. Congenital cysts of the thymus gland. Arch Surg 1967;94:149-52. doi: 10.1001/archsurg.1967.013.30070151030. PubMed PMID: 6017453.

[3] Jamovi. The jamovi project. 1.6 ed2021. Accessed on December 11,2021, from http://www.jamovi.org.

[4] Almofada HS, Almedemgh NI, Othman EO. Adult Cervical Thymic Cysts: A Narrative Review. Ear Nose Throat J 2022:145.561.3221111490. doi: 10.1177/014.556.13221111490. PubMed PMID: 35763329.

[5] Cigliano B, Baltogiannis N, De Marco M, et al. Cervical thymic cysts. Pediatr Surg Int 2007;23:1219-25. doi: 10.1007/s00383.006.1822-5.

[6] Chang A, Nataraja RM, Pudel E, Stunden R, Baré S, Pacilli M. Diagnosis and management of ectopic cervical thymus in children: Systematic review of the literature. J Pediatr Surg 2021;56:2062-8. doi: 10.1016/j.jpedsurg.2021.03.003.

[7] Sturm JJ, Dedhia K, Chi DH. Diagnosis and management of cervical thymic cysts in children. Cureus 2017;9:e973. doi: 10.7759/cureus.973.

[8] Michalopoulos N, Papavramidis TS, Karayannopoulou G, et al. Cervical thymic cysts in adults. Thyroid 2011;21:987-92. doi: 10.1089/thy.2010.0142.

[9] Oramas DM, Moran CA. Micronodular thymomas with prominent cystic changes: A clinicopathological and immunohistochemical study of 25 cases. Int J Surg Pathol 2021;29:352-7. doi: 10.1177/106.689.6920963803.

[10] Yasuda K, Kidokoro Y, Makishima K, et al. A rare case of combined thymoma and a multilocular thymic cyst discovered due to chest pain. Surg Case Rep 2021;7:158. doi: 10.1186/s40792.021.01243-2.

[11] Syred K, Weissferdt A. non-neoplastic mediastinal cysts. Adv Anat Pathol 2020;27:294-302. doi: 10.1097/pap.000.000.0000000261.

[12] Roden AC, Fang W, Shen Y, et al. Distribution of Mediastinal Lesions Across Multi-Institutional, International, Radiology Databases. J Thorac Oncol 2020;15:568-79. doi: 10.1016/j.jtho.2019.12.108.

[13] Kim H, Yoon SH, Kim J, et al. Growth of thymic epithelial tumors and thymic cysts: Differential radiological points. Thorac Cancer 2019;10:864-71. doi: 10.1111/1759-7714.13016.

[14] Zhonggao J, YiJiao W, Yongfeng W, et al. Multislice computed tomography performance in differential diagnosis of high-density thymic cyst and thymoma in lesions less than 3 cm. Thorac Cancer 2018;9:1300-4. doi: 10.1111/1759-7714.12840.

[15] Nam JG, Goo JM, Park CM, Lee HJ, Lee CH, Yoon SH. Age–and gender-specific disease distribution and the diagnostic accuracy of CT for resected anterior mediastinal lesions. Thorac Cancer 2019;10:1378-87. doi: 10.1111/1759-7714.13081.

[16] Minato H, Kinoshita E, Nakada S, et al. Thymic lymphoid hyperplasia with multilocular thymic cysts diagnosed before the Sjögren syndrome diagnosis. Diagn Pathol 2015;10:103. doi: 10.1186/s13000.015.0332-y.

[17] Scharifker D. True thymic hyperplasia associated with a unilocular thymic cyst: an unusual combination not previously reported. Ann Diagn Pathol 2006;10:32-5. doi: 10.1016/j.anndiagpath.2005.04.005.

[18] Izumi H, Nobukawa B, Takahashi K, et al. Multilocular thymic cyst associated with follicular hyperplasia: clinicopathologic study of 4 resected cases. Hum Pathol 2005;36:841-4. doi: 10.1016/j.humpath.2005.05.004.

[19] Furuya T, Kato D, Yamazaki S, et al. Microthymoma and microscopic thymomas associated with a thymic cyst without solid component. Gen Thorac Cardiovasc Surg 2018;66:303-6. doi: 10.1007/s11748.017.0808-7

[20] Moran CA, Suster S. Mediastinal seminomas with prominent cystic changes. A clinicopathologic study of 10 cases. Am J Surg Pathol 1995;19:1047-53. doi: 10.1097/00000.478.199509000-00008

[21] Moran CA, Suster S, El-Naggar A, Luna MA. Carcinomas arising in multilocular thymic cysts of the neck: a clinicopathological study of three cases. Histopathology 2004;44:64-8. doi: 10.1111/j.1365-2559.2004.01767.x.

[22] Moran CA, Suster S, Silva EG. Low-grade serous carcinoma of the ovary metastatic to the anterior mediastinum simulating multilocular thymic cysts: a clinicopathologic and immunohistochemical study of 3 cases. Am J Surg Pathol 2005;29:496-9. doi: 10.1097/01.pas.000.015.5147.37800.e1.

[23] Shen X, Jin Y, Shen L, Sun Y, Chen H, Li Y. Thymoma and thymic carcinoma associated with multilocular thymic cyst: a clinicopathologic analysis of 18 cases. Diagn Pathol 2018;13:41. doi: 10.1186/s13000.018.0719-7.

[24] Kondo K, Miyoshi T, Sakiyama S, Shimosato Y, Monden Y. Multilocular thymic cyst associated with Sjögren's syndrome. Ann Thorac Surg 2001;72:1367-9. doi: 10.1016/s0003-4975(00] 02706-5.

[25] Gorospe L, García-Villanueva MJ, García-Cosío-Piqueras M, García-Gómez-Muriel I. Multilocular thymic cyst in a patient with Sjögren syndrome. Rheumatology (Oxford] 2019;58:369. doi: 10.1093/rheumatology/key221.

[26] Matsumoto S, Mori Y, Takiya H, Iwata H, Shirahashi K. Multilocular thymic cyst associated with rheumatoid arthritis. Kyobu Geka 2012;65:205-8.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Comparison of respiratory morbidity in late preterm infants and intrauterine growth retarded infants at school-age

Mahmut Caner US[1], Ulfet VATANSEVER[2], Ridvan DURAN[2], Betul ACUNAS[2]

[1] Department of Pediatrics, Haseki Research and Education Hospital, Istanbul, Turkey.
[2] Department of Pediatrics, School of Medicine, Trakya University, Edirne, Turkey.

Corresponding Author: Mahmut Caner US
E-mail: mcanerus@gmail.com

Submitted: 31.03.2022 **Accepted:** 17.06.2022

ABSTRACT

Objective: We aimed to determine respiratory morbidity of late preterm infants versus infants with intrauterine growth retardation (IUGR) at school-age.

Patients and Methods: Late preterm appropriate for gestational age (AGA) infants (34-36, 6/7 weeks) (Group 1), IUGR infants (Group 2), extremely preterm AGA (Group 3) and term AGA infants (Group 4) born between 2004 and 2008 were included in this case-control study and assessed for respiratory morbidity at school-age. We evaluated the impact of late preterm compared with IUGR and term gestation on respiratory morbidity by using validated American Thoracic Society – Division of Lung Diseases (ATS-DLD-78-C) and the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaires. Questionnaires on wheezing, infectious respiratory morbidity, and physician-diagnosed asthma panels were constituted and groups were compared.

Results: A total of 160 patients were enrolled in the study and 97 (60.6%) of them were boys. Respiratory morbidities at school-age were found to be significantly higher in both late preterm and IUGR groups when compared to term controls. Each weekly increase in gestational age reduced the risk for wheezing episodes (OR perGW:0.82,95%CI:0.71-0.97, p:0.02). Regarding infectious respiratory morbidities, there was a significant increase when the number of people living at home increased (OR perperson:1.79,95%CI:1.12-2.87, p:0.01), and a decrease in female gender (OR:0.41,95%CI:0.17-0.99, p:0.04) and in the week of gestation (OR perGW:0.84,95%CI:0.71-1.00, p:0.04). Atopic dermatitis (OR:5.26,95%CI:1.57-17.69, p<0.01) and maternal asthma (OR:5.38,95%CI:1.17-24.60, p:0.03) history were found to be risk factors for asthma.

Conclusion: Being IUGR may be an important risk factor for respiratory morbidity at school-age. Further studies are needed on this subject.

Keywords: Premature Birth, Late preterm, Fetal Growth Retardation, Asthma, School-age

1. INTRODUCTION

Preterm births have increased from 9.5% to 12.7% in the last 3 decades and 60-70% of this change is due to the increased prevalence of late preterm births [1]. Until recently, protocols prepared for term babies were used for late preterm infants, which include the most important part of this increase observed in preterm births. However, it was shown that these babies face increased morbidity and mortality, especially in respiratory and neurodevelopmental aspects both in the neonatal period and in school-age period when compared to term infants [2]. Neonatal morbidities such as respiratory distress syndrome (RDS), transient tachypnea of the newborn and neonatal pneumonia were more common in preterm infants than terms; and at school-age decreased lung capacity, increased bronchial asthma episodes and respiratory tract infections were more frequently found. In a study by Kotecha et al., respiratory morbidity was found to be 10 times higher in this group compared to term infants [1].

On the other hand, IUGR causes respiratory problems in later life by giving rise to decreased lung function and early respiratory morbidity, as well as impaired lung function in infancy [3]. However, few studies have been conducted on the comparative evaluation of school-age respiratory morbidity of late preterm infants and newborns with IUGR [4]. The presence of prematurity in IUGR infants causing respiratory morbidity is important to determine the risk group for long-term follow-up.

Our hypothesis was that those children with late preterm and IUGR birth history have increased respiratory morbidity at school age compared to their healthy term birth history peers. We

How to cite this article: Us MC, Vatansever U, Duran R, Acunas B. Respiratory morbidity of late preterm vs intrauterine growth retarded infants at school-age. Marmara Med J 2022; 3; 35(3):335-342. doi: 10.5472/marumj.1195309

ISSN: 1309-9469

aimed to evaluate respiratory morbidity of children who have late preterm and IUGR history and compare them with healthy peers at school age and point out the effects of risk factors encountered in the neonatal period for respiratory morbidity at school age.

2. PATIENTS and METHODS

Our study is a case-control study in which the school-age respiratory morbidity of newborns with late preterm and IUGR birth history who were followed and treated in the neonatology department between January 2006 and December 2008 were evaluated.

A total number of 160 children were included in the study. Children who were admitted to the neonatal intensive care unit and/or those who were born in our hospital and followed up with their mothers between January 2006 – December 2008 and reevaluated at school age (5-7 years old) during the study, were divided into three main groups: a) Late preterm appropriate for gestational age (AGA) infants (n:50): according to the new BALLARD scoring system [5] with gestational age 34-36 6/7 and were at the 10-90th percentile according to the Fenton growth curves [6] (Group 1) b) Newborns with IUGR (n:50): term or preterm infants below 10th percentile according to the values calculated according to Fenton growth curves [6] (Group 2) and, c) the healthy term (gestation week 38-42 weeks), AGA newborns (n:50) born between January 2006 and December 2008, were included in the study as the control group (Group 4). In addition to these main groups, group 2 was divided into two subgroups to compare the effect of IUGR in different gestational ages: preterm IUGR infants (<34 weeks of gestation) (n:10) (Group 2a), late preterm (between 34-36 6/7 gestational age) IUGR infants (n:19) (Group 2b) were divided into subgroups. An equal number of preterm AGA infants (<34 weeks of gestation) was added as the last group to compare the effect of being IUGR in preterms' (Group 3) (n:10) (Figure 1 Flow Chart of the study). Patients with major anomalies, patients who did not come to the school-age assessment or /and rejected to sign the informed consent form were excluded from the study.

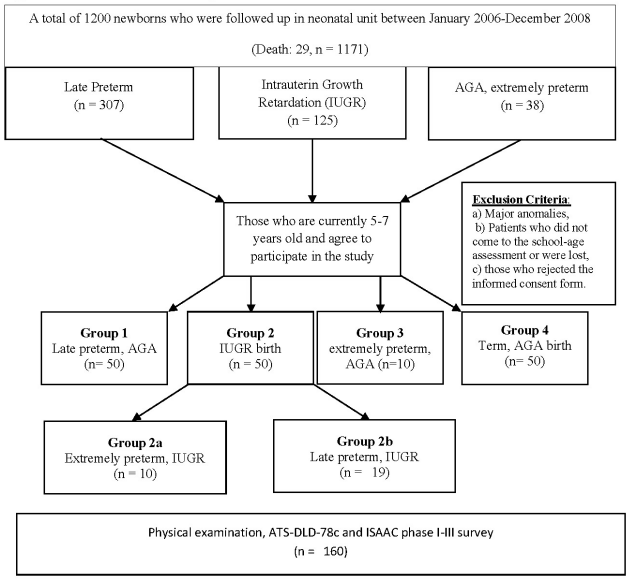

Figure 1. Flow chart of the study

Early (perinatal-neonatal), natal and postnatal period data were recorded from the hospital database system and patient files. The morbidities observed during the follow-up of the newborns, the duration of hospitalization, need for oxygen, surfactant administration, positive pressure ventilation requirement, and need for mechanical ventilation support and duration were documented from patient file records. During the study, the study and control group cases that were of school age and had perinatal parameters as defined above were contacted by telephone and invited to the outpatient clinic. Anthropometric measurements of all patients were taken (TBS model, scale with serial number 1212430 and height meter, Tartımsan, Istanbul, Turkiye). Demographic information, socioeconomic level, mother/father education level, mother's smoking status and medical history were questioned, and physical examinations of the patients were performed. The validated Turkish forms of American Thoracic Society – Division of Lung Diseases (ATS-DLD-78-C) [7] and International Study of Asthma and Allergies in Childhood (ISAAC) questionnaires [8] were tested on 10 randomly selected patients by 2 different investigators to evaluate their intelligibility for screening respiratory diseases. The ATS-DLD-78-C questionnaire consisted of 46 questions with 3 options (yes, no, unknown) in 7 sections (socio-demographic characteristics, cough, sputum, wheezing, chest diseases, other diseases, and family history). The ISAAC questionnaire consisted of 31 questions in 2 parts (environmental factors, wheezing) most of which had 2 answer options (yes, no). In the wheezing section of the questionnaire the presence of wheezing was questioned in the first 2 questions, the number of symptom days in the following 3 multiple-choice questions, and the exercise and symptoms, nighttime symptoms, and asthma in the last 3 questions. The questionnaires were applied separately to both mother and father. After comparing the answers, it was decided that the questions were understandable. After the physical examination, the questionnaire forms were filled in face-to-face by the research doctor who did not know the patient's medical history (M.C.U).

Responses with "yes" to the survey questions include; the presence of wheezing/wheezing in the chest independent of having a cold, presence of wheezing in the chest day/night, presence of two or more wheezing episodes causing shortness of breath, the need of using medication during wheezing attacks causing shortness of breath, one or more of the presence of wheezing episodes after exercise or playing games "Respiratory morbidity 1 – wheezing panel", presence of hospitalization due to severe respiratory infection under 2 years of age, having had more than 1 previous bronchiolitis/bronchitis/pneumonia or two or three of them, more than 3 days in the last 3 years of chest disease, cough/sputum attacks lasting at least for a week or longer, sputum, breast fullness, cough present for more than 3 months of the year "Respiratory morbidity 2 – infectious respiratory morbidity panel", presence of physician-diagnosed asthma history, current asthma, and the presence of a history of being followed up with a diagnosis of asthma "Respiratory morbidity 3 – physician-diagnosed asthma panel" [9-13].

Ethics committee approval was obtained for the study from the Trakya University Faculty of Medicine Local Ethics Committee numbered 18/5 and protocol number TÜTF-GOKAEK 2013/150, and registered with Clinical Trials (NCT04849494).

Statistical Analysis

SPSS version 19 license number: 10240642 was used as a statistics program in the study. The minimum sample size required to obtain statistically significant results using the random sampling method was 160 in total, and it was determined a minimum of 50 for each group in late preterm, IUGR and the control groups (Pi1: 0.198, Pi2: 0.028, Alpha: 0.05, n: 50. power 80%). There was no missing data. While evaluating the study data, T-test or Mann-Whitney U test were used in samples independent of one-way analysis by evaluating the normal distribution of the data in comparing the quantitative data. A Chi-square test was used for comparing qualitative data between the two groups. Logistic regression analysis (Likelihood Ratio) method was used, taking respiratory morbidity as the dependent variable and risk factors as the independent variables. A value of p <0.05 was accepted as the limit for a significant difference.

3. RESULTS

When the early demographic data of the study and control groups were compared, no statistically significant difference was found between groups in terms of gender. Cesarean birth and multiple pregnancy rates in the study groups (Group 1, Group 2 and Group 3) were found to be statistically significantly higher when compared to Group 4 (respectively p <0.01, p <0.01). In terms of the week of gestation there was a statistically significant difference between the study Groups and Group 3 was significantly lower (p<0.001). As expected, it was found that the mean birth weight, birth length and birth head circumference of Group 1 cases were statistically significantly higher than Group 2 and Group 3 (p <0.01 for all). The 1st minute Apgar score was found significantly higher in Group 1 cases (p: 0.04). No statistically significant difference was found between the groups in the 5-minute Apgar median (Table I). There was no statistically significant difference between the groups in terms of anthropometric measurements at school-age. The socioeconomic levels of the groups were found to be similar (Table I).

Table I. *Comparison of demographic, socioeconomic and perinatal data of groups*

		Group 1 (n: 50)		Group 2 (n: 50)		Group 3 (n: 10)		Group 4 (n: 50)	p*
		n (%)		**n (%)**		**n (%)**		**n (%)**	
Gender	**Male**	29 (58)		32 (64)		7 (70)		30 (60)	0.82
	Female	21 (42)		18 (36)		3 (30)		20 (40)	
Type of Delivery	**Normal Birth**	9 (18) [a]		8 (16) [a]		2 (20) [a]		38 (76) [b]	**<0.01**
	Caesarean section	41 (82)		42 (84)		8 (80)		12 (24)	
Multiple pregnancy		24 (48) [a]		25 (50) [a]		6 (60) [a]		0 (0) [b]	**<0.01**
Mechanical ventilator requirement		3 (6) [a]		11 (22) [b]		4 (40) [c]			**0.02**
		Mean, SD	**Median**	**Mean, SD**	**Median**	**Mean, SD**	**Median**		**p****
Gestational age (week±SD)		35.2± 0.9	35	34.8± 2.1	35	30.3±1.9	30		**<0.01**
Birth weight (gram±SD)		2210.4±533.7	2105	1662.2±341.1	1620	1353.3±245.5	1347		**<0.01**
Birth height (cm±SD)		44.9±2.8	44	42.6±3.3	43.5	39.6±2.2	38.5		**<0.01**
Birth head circumference (cm±SD)		31.5±1.8	31.25	29.9±2.3	30.1	27.9±2.2	27.5		**<0.01**
Apgar score	**Minute 1**	8.3±1.1	9	7.6±1.7	8	6.8±1.8	6.5		**0.04**
	Minute 5	9.5±0.8	10	9.2±1.2	9	8.6±1.1	8.5		0.10
Number of days of mechanical ventilation		2.0±1.4	1	3.6±2.3	5	4.1±3.3	4		0.44
Total number of days with oxygen		2.3±5.8	0	2.8±8.3	1	8.6±17.6	4.5		0.33
Length of hospital stay (day)		10.1±8.9	9	14.9±16.2	9	27.8±23.2	24		0.16
		Mean,SD		**Mean,SD**		**Mean,SD**		**Mean,SD**	**p****
Age (month)[a]		75.3±8.4		79.6±7.7		74.9±6.3		76.4±8.3	0.20
Weight (kg)[a]		22.5±5.1		23.0±4.8		22.4±4.2		21.6±3.5	0.29
Height (cm)[a]		119±7		122±6		120±6.2		116±7	0.10
BMI (kg/m²)[a]		15.5±2.5		15.1±1.8		15.3±1.0		15.7±1.7	0.33
Number of people living in the house		4.3±0.6		4.3±0.7				4.36±0,7	0.93
		n (%)		**n (%)**		**n (%)**		**n (%)**	**p***
Socioeconomic Level	**Good**	3 (6)		2 (4)		1 (10)		3 (6)	0.95
	Avarage	45 (90)		47 (94)		8 (80)		46 (92)	
	Low	2 (4)		1 (2)		1 (10)		1 (2)	
Physician diagnosed GOR		14 (28) [a]		13 (26) [a]		2 (20) [a]		5 (10) [b]	**0.04**
History of atopic dermatitis		10 (20) [a]		11 (22) [a]		3 (40) [a]		1 (2) [b]	**0.02**
Having a pet in the house		11 (22)		8 (16)		0 (0)		15 (30)	0.24
Maternal smoking		14 (28)		19 (38)		3 (30)		20 (40)	0.40
Maternal asthma history		9 (18) [a]		15 (30) [a]		1 (10) [b]		5 (10) [b]	**0.03**

*Chi-square; ** Kruskal-Wallis test; PPV: Positive pressure ventilation, same letters indicates no significant relationship between groups and each different letter indicates a statistically significant difference at the p<0.05 level; MAS: Meconium aspiration syndrome, BMI: Body mass index, GOR: gastroesophageal reflux.[a] measurements and sociodemographic data has assessed at school-age

http://doi.org/10.5472/marumj.1195309
Marmara Med J 2022;35(3): 335-342

Table II. *Respiratory morbidity questionnaires of study and control groups*

	Group 1 (n: 50)	Group 2 (n: 50)	Group 4 (n: 50)	p*
	n (%)	n (%)	n (%)	
Congested in chest or bring up phlegm apart from colds	9 (18)	4 (8)	1 (2)	**0.01**[a,b]
Congested or bring up phlegm, sputum or mucus from his/her chest 3 months a year	6 (12)	4 (8)	0 (0)	**0.04** [a,b]
Attacks of cough, chest congestion or phlegm lasting for ≥1 week /year	22 (44)	23 (46)	9 (18)	**<0.01** [a,b]
Wheezy or whistling occasionally apart from cold	6 (12)	11 (22)	0 (0)	**<0.01** [a,b]
Attack of wheezing causing short of breath	13 (26)	16 (32)	3 (6)	**<0.01** [a,b]
Attacks of wheezing after exercising	9 (18)	8 (16)	2 (4)	**0.04** [a,b]
During past 3 years any chest illness keeping him/her from usual activities for as much as 3 days	15 (30)	23 (46)	1 (2)	**<0.01** [a,b]
More congested than usual with any of these illness	15 (30)	20 (40)	0 (0)	**0.02** [a,b]
Doctor diagnosed asthma	9 (18)	15 (30)	5 (10)	**0.03** [a,b]
Hospitalization for severe chest illness/cold <age 2	10 (20)	16 (32)	4 (8)	**<0.01** [a,b]
Severe chest illness/cold <age 2	14 (28)	25 (50)	8 (16)	**<0.01** [a,b]
Doctor diagnosed allergic reaction to pollen or dust	8 (16)	8 (16)	1 (2)	**0.01** [a,b]
Hospitalization for severe chest illness /cold <age 2	10 (20)	16 (32)	-	**0.01**
Any other severe chest illness or chest cold < age 2	14 (28)	25 (50)	-	**0.02**
≥2 attack of wheezing causing shortness of breath	13 (26)	16 (32)	-	**0.02**

*Chi-square, a: Significant relationship between Group1 and Group 4, b: Significant relationship between Group 2 and Group 4, a,b: Significant relationship between both Group 1 and 4 and Group 2 and 4

Table III. *Comparison of the groups in terms of Respiratory morbidity panels*

	Group 1 (n: 50)	Group 2 (n: 50)	Group 4 (n: 50)	p*	Group 1 (n: 50)	Group 2b (n: 19)	p*	Group 2a (n: 10)	Group 3 (n: 10)	p*
	n (%)	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
Respiratory morbidity 1	27 (54)[a]	24 (48)[b]	13 (26)[a,b]	**0.01**	27 (54)	7 (36.8)	0.20	9 (90)	1 (10)	**0.002**
Respiratory morbidity 2	16 (32)	13 (26)	9 (18)	0.27	16 (32)	2 (10.5)	0.07	6 (60)	5 (50)	0.46
Respiratory morbidity 3	9 (18)[a]	15 (30)[b]	4 (8)[a,b]	**0.01**	9 (18)	3 (15.8)	0.82	5 (50)	1 (10)	**0.05**
Total respiratory morbidity	28 (56)[a]	24 (48)[b]	18 (36)[a,b]	**0.05**	28 (56)	7 (36.8)	0.15	9 (90)	5 (50)	0.18

*Chi-square, a: Significant relationship between Group1 and Group 4, b: Significant relationship between Group 2 and Group 4, a,b: Significant relationship between both Group 1 and 4 and Group 2 and 4; Respiratory morbidity 1 wheezing panel, Respiratory morbidity 2 infectious respiratory morbidity panel, Respiratory morbidity 3 physician-diagnosed asthma panel.

Compared with term gestation, both late preterm gestation and being IUGR were associated with a significant increase in most of the respiratory morbidities at school-age such as physician-diagnosed asthma, allergic rhinitis, wheezing and whistling episodes, exercise-induced wheezing, and episodes of shortness of breath, etc. Furthermore, the presence of IUGR was significantly associated with an increase in >2 episodes of shortness of breath (p=0.02), hospital admission for severe respiratory infection before age 2 (p=0.01), and severe chest disease before the age of 2 (p=0.02) (Table II).

Respiratory morbidity 1-3 and total respiratory morbidity were significantly higher in infants with both late preterm and IUGR history compared to the control group. When late preterm AGA and IUGRs were compared, no statistically significant difference was found between the groups. Respiratory morbidity 1 and 3 were found to be statistically significantly higher in extremely preterm patients with IUGR compared to AGA controls (Table III).

Logistic regression analysis was performed to show the effects of factors on wheezing phenotype, infectious respiratory morbidity, and physician-diagnosed asthma. When the effect of variables on wheezing (respiratory morbidity 1) was examined, gestational age (p: 0.02, OR: 0.83, 95% confidence interval: 0.71-0.97) was found to be significant. When the effect of variables on infectious respiratory morbidity (respiratory morbidity 2) was examined, gestational age (p: 0.04, OR: 0.84, 95% confidence interval: 0.71-1.00) and the number of people living in the house (p: 0.01, OR: 1.79, 95% confidence interval: 1.12-2.87) were found to be significant. Accordingly, infectious respiratory morbidity decreased 0.84 times with each weekly increase in the week of gestation, while the number of people living at home increased 1.79 times. Examining the effect of variables on the physician-diagnosed asthma panel (respiratory morbidity 3), maternal asthma diagnosis (p: 0.03, OR: 5.38, 95% confidence interval: 1.17-24.60) and the presence of atopic dermatitis in the patient (p: <0.01, OR: 5.26, 95% confidence interval: 1.57-17.69) were found to be significant. The probability of physician-diagnosed asthma increased by 5.38 times in the mother, while the history of atopic dermatitis increased by 5.26 times (Table IV).

http://doi.org/10.5472/marumj.1195309
Marmara Med J 2022;35(3): 335-342

Table IV. *Logistic regression analysis of respiratory morbidity panels*

	Respiratory morbidity 1			Respiratory morbidity 2				Respiratory morbidity 3			
Variables	Odds ratio	95% CI	p*	Variables	Odds ratio	95% CI	p*	Variables	Odds ratio	95% CI	p*
Gender Female	0.69	0.34-1.42	0.32	Gender Female	**0.41**	**0.17-0.99**	**0.04**	Gender Female	0.53	0.19-1.49	0.23
Having a pet in the house	1.47	0.63-3.40	0.36	Having a pet in the house	0.60	0.21-1.73	0.35	Having a pet in the house	0.47	0.12-1.87	0.28
Maternal smoking	1.34	0.65-2.79	0.42	Maternal smoking	0.84	0.34-2.03	0.70	Maternal smoking	1.18	0.42-3.27	0.74
Maternal asthma history	0.98	0.23-4.19	0.98	Maternal asthma history	2.17	0.45-10.33	0.32	Maternal asthma history	**5.38**	**1.17-24.60**	**0.03**
IUGR	1.20	0.53-2.69	0.65	IUGR	1.60	0.62-4.11	0.32	IUGR	0.46	0.16-1.33	0.15
Gestation week	**0.83**	**0.71-0.97**	**0.02**	Gestation week	**0.84**	**0.71-1.00**	**0.04**	Gestation week	0.89	0.73-1.10	0.30
Number of people living in the house	0.81	0.53-1.22	0.31	Number of people living in the house	**1.79**	**1.12-2.87**	**0.01**	Number of people living in the house	1.09	0.63-1.91	0.73
History of atopic dermatitis	1.81	0.61-5.37	0.28	History of atopic dermatitis	1.70	0.52-5.56	0.37	History of atopic dermatitis	**5.26**	**1.57-17.69**	**<0.01**
Physician diagnosed GOR	1.62	0.12-20.89	0.71	Physician diagnosed GOR	0.80	0.05-12.1	0.87	Physician diagnosed GOR	2.40	0.17-33.56	0.51
Constant	**7.30**		**0.01**	Constant	3.95		0.23	Constant	3.57		0.36

*Stepwise lojistic regression model (enter), GOR: gastroesophageal reflux, CI: confidence interval; Respiratory morbidity 1 wheezing panel, Respiratory morbidity 2 infectious respiratory morbidity panel, Respiratory morbidity 3 physician-diagnosed asthma panel.

Respiratory morbidity increased 1.8 times with an increase on the day of hospitalization (p: 0.003, OR 1.80, 95% confidence interval: 1.02-1.14), it was seen 0.02 times less in infants requiring mechanical ventilation (p: 0.04, OR: 0.01-0.75, 95% confidence interval: 0.01-0,75). Accordingly, each daily increase on the day of hospitalization caused a 1.8 times increase in respiratory morbidity, while it was 0.002 times less in those who did not require a mechanical ventilator other factor, especially IUGR, did not have statistically significant effects on respiratory morbidity (Table V).

Table V. *Logistic regression analysis of total respiratory morbidity panel*

Variables	Odds ratio	%95 %CI	p*
Gender Male	1.42	0.69-2.94	0.33
Total number of days with oxygen	1.05	0.91-1.21	0.44
Length of hospital stay (day)	**1.80**	**1.02-1.14**	**0.003***
Presence of physician diagnosed GOR	2.32	0.10-51.44	0.59
Presence of history of atopic dermatitis	1.67	0.54-5.14	0.37
Presence of mechanical ventilator requirement	**0.002**	**0.01-0.75**	**0.04***
Constant	0.95		0.004

*Stepwise lojistic regression model (enter), GOR: gastroesophageal reflux, CI: confidence interval

4. DISCUSSION

Late preterm birth and IUGR birth, have increased due to many etiological factors, like assisted reproductive techniques and the increase in risky pregnancies, bring many problems. Few studies have been conducted on the comparative evaluation of school-age respiratory morbidities of late preterm infants and newborns with IUGR, as well there is no study comparing the effects of late preterm birth and IUGR [4]. The frequency of wheezing due to airway obstruction during follow-up was observed in the first two years of life in prematurely born infants [14].

Studies have reported that there is a significant increase in admissions because of lower respiratory tract infections (LRTI) or bronchiolitis during infancy and early childhood in late preterms compared with term infants [15-19]. Similarly, studies including early and late preterm born adolescents have shown that admissions due to both infectious and non-infectious respiratory tract diseases are significantly increased compared to those who were term infants [20, 21]. Hoo et al., found that the frequency of wheezing and/or LRTI in the first year of life in late preterm infants was significantly higher than in term babies in which male gender and low gestational age were independent risk factors [22]. In the study of Coathup et al., it was stated that the rates of infection-related (acute bronchitis/bronchiolitis, pneumonia, upper and LRTI) hospital admissions from birth to 10 years of age were significantly higher in late preterm infants compared to term peers [23]. Similar to the literature, in our study, the frequency of upper respiratory tract infections in late preterm infants was found to be significantly higher than in term infants. Late preterm infants got sick more frequently and had more LRTIs' in the first 2 years of life, therefore hospitalization rates were found to be significantly higher. Also, respiratory morbidity due to infection, significantly increased in parallel with a decrease in the week of gestation, male gender and with an increase in number of people living at home.

The relationship between late preterm birth and asthma has not been clarified yet. It has been shown that the frequency of admission to the hospital in the first 6 years of life due to asthma is significantly higher in late preterms compared to term infants [18, 24]. The risk of asthma increased 2 times in late preterm infants compared to terms and male gender, low gestational age, maternal smoking during pregnancy and maternal asthma history were risk factors for asthma [25, 26]. In a recent study, it was shown that children with a history of late preterm labour had more frequent medication use and a need for hospitalization for asthma compared to their term-born controls when followed

http://doi.org/10.5472/marumj.1195309
Marmara Med J 2022;35(3): 335-342

up until the age of 7 [27]. However, it has been shown that there is no significant relationship between the use of treatment for asthma in young adults and the presence of a history of late preterm labour [28]. In the meta-analysis of Jaakkola et al., it was found that the asthma risk in individuals with a birth history below the 37th week of gestation was 1.07 times higher [29]. Contrastly, Voge et al., also found no relationship between late preterm birth and the risk of asthma [30]. In our study, similar to the literature, infectious wheezing at school-age was found to be significantly higher in late preterm infants. Also, the presence of wheezing attacks causing shortness of breath, wheezing attacks triggered by exercise and the presence of physician-diagnosed asthma were found to be statistically significantly higher in the late preterm group. Furthermore, the frequency of physician-diagnosed dust and pollen allergy, allergic rhinitis, allergic conjunctivitis, and atopic dermatitis in late preterm infants was significantly higher than in term controls.

IUGR has long-term consequences such as asthma and bronchiolitis [31]. Recent studies have shown that there is a relationship between IUGR and the development of wheezing, bronchial reactivity, asthma, LRTI and develop lower lung function at school-age [32], regardless of the gestational age [33-36]. In the study by Barker et al., it was shown that each unit's decrease in birth weight and birth height was associated with bronchitis, pneumonia, or cough in the first 5 years [37]. Contrastly, Sonnenschein-van der Voort et al., stated that the main reason for an increased risk factor for asthma and its symptoms was preterm delivery, not low birth weight [38]. In the study conducted by Lopuhaa et al., on adults, the frequency of obstructive airway disease and respiratory symptoms increased 1.7 times in those who were exposed to famine during the midgestation period [39]. In another study by Kotecha et al., the frequency of previous or ongoing asthma and wheezing was found to be similar between those who had an age-appropriate growth catch-up and those who did not and control groups [10]. In the study of Rona et al., it has been shown that the risk of wheezing decreases by 10% with each increased week of gestation [11]. In our study, the frequency of wheezing, physician-diagnosed asthma and other allergic conditions increased in IUGRs. Although, we found that wheezing, asthma and respiratory morbidity were significantly higher in both the late preterm group and the IUGR group compared to term controls; there was no significant difference between the late preterm AGA and IUGR groups. We concluded that IUGR may negatively affect allergic respiratory morbidity for advanced preterms, but not for late preterm infants and that the main determinant factor for respiratory morbidity for later periods of pregnancy is the gestational age. We found that the presence of maternal asthma and atopic dermatitis history in children increases the frequency of asthma at school age, and also each weekly increase in the gestational age reduces the risk for wheezing attacks.

In the study, which is based on parental reports, IUGRs were more likely to have upper respiratory tract infections [40]. With a 2-year follow-up of IUGRs, 50% of children coughed and approximately 58% of the children used treatments such as a bronchodilator, inhaled corticosteroid, and antibiotics due to chest diseases. It has been shown that there is a negative correlation between the birth weight Z score and hospital admission because of respiratory illness [13]. It was shown that being IUGR was significantly related to the increased infection-related hospital admissions during the first 10 years of age and modified the effect of gestational age; specifically, among the history of being both preterm and IUGRs [23]. In our study, although the respiratory infections and therefore hospitalization requirements in patients with a history of IUGR were found significantly increased compared to term controls, there was no significant difference between both late preterm AGA and late preterm IUGR groups, extremely preterm AGA and IUGR groups.

Since, spirometric measurements were not performed in our study, the negative effect of IUGR shown in the literature on lung volume and flows could not be shown. And low patient follow-up rates were also limitations of our study. As a result, it was thought that the decrease in gestational age was effective in respiratory diseases and symptoms. We concluded that IUGR negatively affects respiratory morbidity for extreme preterms, although, not for late preterm infants, this relationship cannot be demonstrated for later periods of pregnancy.

Late preterm births and IUGR, which are on the rise today, can bring many problems both in perinatal, neonatal, infancy and later life. We think that the follow-up during pregnancy, perinatal period, delivery room, and neonatal period should be reviewed, and detailed guidelines should be prepared to start with birth planning. For these children, long-term follow-up protocols should be determined, and follow-up timelines should be established instead of risk-free routine healthy term child follow-up. The effect of late preterm birth and IUGR history on negative respiratory outcomes in school-age and later should be investigated with further studies.

Acknowledgements: The authors acknowledge Prof. Necdet Sütcü for statical analyses.

Compliance with Ethical Standards

Ethical Approval: Ethics committee approval was obtained for the study from the Trakya University Faculty of Medicine Local Ethics Committee numbered 18/5 and protocol number TÜTF-GOKAEK 2013/150, and registered with Clinical Trials (NCT04849494).

Financial Support: The authors report no involvement in the research by the sponsor that could have influenced the outcome of this work.

Conflict of Interest Statement: The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the article.

Authors' contributions: MCU and BA: Concept and design of the study, MCU and UV: Acquisition and analysis of data, MCU, UV, RD and BA: Drafting the manuscript, tables and figures. All authors read and approved the final version of the article.

REFERENCES

[1] Kotecha SJ, Watkins WJ, Paranjothy S, Dunstan FD, Henderson AJ, Kotecha S. Effect of late preterm birth on longitudinal lung spirometry in school age children and adolescents. Thorax 2012;67:54-61. doi: 10.1136/thoraxjnl-2011-200329.

[2] Melamed N, Klinger G, Tenenbaum-Gavish K et al. Short-term neonatal outcome in low-risk, spontaneous, singleton, late preterm deliveries. Obstet Gynecol 2009;114(2 Pt 1):253-60. doi: 10.1097/AOG.0b013e3181af6931.

[3] Pike K, Jane Pillow J, Lucas JS. Long term respiratory consequences of intrauterine growth restriction. Semin Fetal Neonatal Med 2012;17:92-8. doi: 10.1016/j.siny.2012.01.003.

[4] Lima RdC, Victora CG, Menezes AMB, Barros FC. Respiratory function in adolescence in relation to low birth weight, preterm delivery, and intrauterine growth restriction. Chest 2005;128:2400-7. doi: 10.1378/chest.128.4.2400.

[5] Marin Gabriel MA, Martin Moreiras J, Lliteras Fleixas G et al. [Assessment of the new Ballard score to estimate gestational age]. An Pediatr (Barc) 2006;64:140-5. doi: 10.1157/13084173.

[6] Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatrm2013;13:59. doi: 10.1186/1471-2431-13-59.

[7] Esteves A, Solé D, Ferraz M. Adaptation and validity of the ATS-DLD-78-C questionnaire for asthma diagnosis in children under 13 years of age. Braz Ped News 1999;1:5.

[8] Mallol J, Crane J, von Mutius E et al. The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three: a global synthesis. Allergol Immunopathol (Madr) 2013;41:73-85. doi: 10.1016/j.aller.2012.03.001.

[9] Bayram I, Guneser-Kendirli S, Yilmaz M, Altintas DU, Alparslan N, Bingol-Karakoc G. The prevalence of asthma and allergic diseases in children of school age in Adana in southern Turkey. Turk J Pediatr 2004;46:221-5. PMID: 15503474.

[10] Kotecha SJ, Watkins WJ, Heron J, Henderson J, Dunstan FD, Kotecha S. Spirometric lung function in school-age children: effect of intrauterine growth retardation and catch-up growth. Am J Respir Crit Care Med 2010;181:969-74. doi: 10.1164/rccm.200.906.0897OC.

[11] Rona R, Gulliford M, Chinn S. Effects of prematurity and intrauterine growth on respiratory health and lung function in childhood. BMJ 1993;306(6881):817. doi: 10.1136/bmj.306.6881.817.

[12] Wjst M, Popescu M, Trepka MJ, Heinrich J, Wichmann H. Pulmonary function in children with initial low birth weight. Pediatr Allergy Immunol 1998;9:80-90. doi: 10.1111/j.1399-3038.1998.tb00308.x

[13] Peacock JL, Lo JW, D'Costa W, Calvert S, Marlow N, Greenough A. Respiratory morbidity at follow-up of small-for-gestational-age infants born very prematurely. Pediatr Res 2013;73(4 Pt 1):457-63. doi: 10.1038/pr.2012.201.

[14] Greenough A. Late respiratory outcomes after preterm birth. Early Hum Dev 2007;83:785-8. doi: 10.1016/j.earlhumdev.2007.09.006.

[15] Helfrich AM, Nylund CM, Eberly MD, Eide MB, Stagliano DR. Healthy Late-preterm infants born 33–36+ 6 weeks gestational age have higher risk for respiratory syncytial virus hospitalization. Early Hum Dev 2015;91:541-6. doi: 10.1016/j.earlhumdev.2015.06.009.

[16] Lanari M, Prinelli F, Adorni F et al. Risk factors for bronchiolitis hospitalization during the first year of life in a multicenter Italian birth cohort. Ital J Pediatr 2015;41:40. doi: 10.1186/s13052.015.0149-z.

[17] Odibo IN, Bird TM, McKelvey SS, Sandlin A, Lowery C, Magann E. Childhood Respiratory Morbidity after Late Preterm and Early Term Delivery: a Study of Medicaid Patients in South Carolina. Paediatr Perinat Epidemiol 2016;30:67-75. doi: 10.1111/ppe.12250.

[18] Haataja P, Korhonen P, Ojala R et al. Hospital admissions for lower respiratory tract infections in children born moderately/late preterm. Pediatr Pulmonol 2018;53:209-17. doi: 10.1002/ppul.23908.

[19] Kotecha SJ, Lowe J, Kotecha S. Does the sex of the preterm baby affect respiratory outcomes? Breathe (Sheff) 2018;14:100-7. doi: 10.1183/20734.735.017218.

[20] Srinivasjois R, Slimings C, Einarsdóttir K, Burgner D, Leonard H. Association of gestational age at birth with reasons for subsequent hospitalisation: 18 years of follow-up in a Western Australian population study. PloS One 2015;10:e0130535. doi: 10.1371/journal.pone.0130535.

[21] Hoo AF, Dezateux C, Henschen M, Costeloe K, Stocks J. Development of airway function in infancy after preterm delivery. J Pediatr 2002;141:652-8. doi: 10.1067/mpd.2002.128114.

[22] Coathup V, Carson C, Kurinczuk JJ, et al. Associations between gestational age at birth and infection-related hospital admission rates during childhood in England: Population-based record linkage study. PLoS One 2021;16:e0257341. doi:10.1371/journal.pone.0257341

[23] Leung JY, Lam HS, Leung GM, Schooling CM. Gestational Age, Birthweight for Gestational Age, and Childhood Hospitalizations for Asthma and Other Wheezing Disorders. Paediatr Perinat Epidemiol 2016;30:149-59. doi: 10.1111/ppe.12273.

[24] Abe K, Shapiro-Mendoza CK, Hall LR, Satten GA. Late preterm birth and risk of developing asthma. J Pediatr 2010;157:74-8. doi: 10.1016/j.jpeds.2010.01.008.

[25] Harju M, Keski-Nisula L, Georgiadis L, Raisanen S, Gissler M, Heinonen S. The burden of childhood asthma and late preterm and early term births. J Pediatr 2014;164:295-9 e1. doi: 10.1016/j.jpeds.2013.09.057.

[26] Haataja P, Korhonen P, Ojala R et al. Asthma and atopic dermatitis in children born moderately and late preterm. Eur J Pediatr 2016;175799-808. doi: 10.1007/s00431.016.2708-8.

[27] Crump C, Winkleby MA, Sundquist J, Sundquist K. Risk of asthma in young adults who were born preterm: a Swedish national cohort study. Pediatrics. 2011;127:e913-20. doi: 10.1542/peds.2010-2603.

[28] Jaakkola JJ, Ahmed P, Ieromnimon A, et al. Preterm delivery and asthma: a systematic review and meta-analysis. J Allergy Clin Immunol 2006;118:823-30. doi: 10.1016/j.jaci.2006.06.043.

[29] Voge GA, Carey WA, Ryu E, King KS, Wi CI, Juhn YJ. What accounts for the association between late preterm births and risk of asthma? Allergy Asthma Proc 2017;38:152-6. doi: 10.2500/aap.2017.38.4021.

[30] Eriksson L, Haglund B, Odlind V, Altman M, Ewald U, Kieler H. Perinatal conditions related to growth restriction and inflammation are associated with an increased risk of bronchopulmonary dysplasia. Acta Paediatrica 2015;104:259-63. doi: 10.1111/apa.12888.

[31] Arigliani M, Stocco C, Valentini E et al. Lung function between 8 and 15 years of age in very preterm infants with fetal growth restriction. Pediatr Res 2021;90:657-63. doi: 10.1038/s41390.020.01299-0.

[32] Pike KC, Crozier SR, Lucas JS et al. Patterns of fetal and infant growth are related to atopy and wheezing disorders at age 3 years. Thorax 2010;65:1099-106. doi: 10.1136/thx.2010.134742.

[33] Ronkainen E, Dunder T, Kaukola T, Marttila R, Hallman M. Intrauterine growth restriction predicts lower lung function at school age in children born very preterm. Arch Dis Child Fetal Neonatal Ed 2016;101:F412-F7. doi: 10.1136/archdischild-2015-308922.

[34] Kotecha SJ, Watkins WJ, Henderson AJ, Kotecha S. The effect of birth weight on lung spirometry in white, school-aged children and adolescents born at term: a longitudinal population based observational cohort study. J Pediatr 2015;166:1163-7. doi: 10.1016/j.jpeds.2015.01.002.

[35] Chan KN, Noble-Jamieson CM, Elliman A, Bryan EM, Silverman M. Lung function in children of low birth weight. Arch Dis Child 1989;64:1284-93. doi: 10.1136/adc.64.9.1284.

[36] Barker DJ, Godfrey KM, Fall C, Osmond C, Winter PD, Shaheen SO. Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airways disease. BMJ 1991;303(6804):671-5. doi: 10.1136/bmj.303.6804.671.

[37] Sonnenschein-van der Voort AM, Jaddoe VW, Raat H, et al. Fetal and infant growth and asthma symptoms in preschool children: the Generation R Study. Am J Respir Crit Care Med 2012;185:731-7. doi: 10.1164/rccm.201.107.1266OC.

[38] Lopuhaa CE, Roseboom TJ, Osmond C, et al. Atopy, lung function, and obstructive airways disease after prenatal exposure to famine. Thorax 2000;55:555-61. doi: 10.1136/thorax.55.7.555.

[39] Hoo AF, Stocks J, Lum S, et al. Development of lung function in early life: influence of birth weight in infants of nonsmokers. Am J Respir Crit Care Med 2004;170:527-33. doi: 10.1164/rccm.200.311.1552OC.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Prevalence of correct face mask usage among general public during COVID-19 pandemic in Denizli, Turkey

Suleyman Utku UZUN[1], Ozgur SEVINC[2], Ahmet ERGIN[3]

[1] Division of Epidemiology, Department of Public Health, School of Medicine, Pamukkale University, Denizli, Turkey.
[2] Department of Public Health, School of Medicine, Pamukkale University, Denizli, Turkey.
[3] Department of Public Health and Division of Social Pediatrics, Department of Child Health and Pediatrics, School of Medicine, Pamukkale University, Denizli, Turkey.

Corresponding Author: Suleyman Utku UZUN
E-mail: utkuuzun402@gmail.com

Submitted: 27.02.2022 **Accepted:** 17.04.2022

ABSTRACT

Objective: Objective: We aimed to evaluate correct facemask usage prevalence among the general public in marketplaces in Denizli, Turkey and also aimed to determine the effects of the announcement of asymptomatic coronavirus disease 2019 (COVID-19) cases and curfew decisions on compliance rates of appropriate mask use.

Materials and Methods: In this population-based study, a total of 6749 observations were made in the 8 different marketplaces between 22 November – 3 December 2020. Multiple logistic regression analysis was used to assess the effect of age, gender, observation time, and intervention type (announcement of asymptomatic COVID-19 case numbers and, announcement of nationwide curfew decisions) on correct facemask use prevalence.

Results: Correct facemask usage prevalence in customers and sellers was 84.3% and 46.3%, respectively. The announcement of the number of asymptomatic COVID-19 cases had no statistically significant effect on correct facemask usage prevalence both among customers and sellers (p>0.05). After the announcement of the curfew decisions, correct facemask usage prevalence increased among customers (OR:1.24 (1.02-1.52)) and sellers (OR:1.64 (1.32-2.03)).

Conclusion: The correct use of facemasks is not sufficient, especially among sellers. The announcement of curfew decisions has increased the correct use of masks although, the announcement of asymptomatic COVID-19 case numbers has no effect.

Keywords: COVID-19, Correct facemask use, Marketplaces, Turkey.

1. INTRODUCTION

Since, the beginning of the coronavirus disease 2019 (COVID-19) pandemic, several non-pharmaceutical public health interventions have been implemented to prevent the spread of the disease [1]. One of these interventions was to wear a facemask to prevent transmission through droplets.

There have been opposing views about who should and should not use masks, and there have been different examples of practices between countries. According to the recommendation issued by World Health Organization (WHO) on December 1, 2020; it is recommended that everyone use masks in public settings such as crowded open-air markets, schools, mosques, and hospitals where there is an increased risk of widespread transmission and social distancing cannot be maintained [2].

From the start of the COVID-19 outbreak in Turkey, different applications have been made between provinces on this issue. As of May 11, 2020, by the decision of the Denizli Provincial Public Health Council, it has been decided that facemask usage in all kinds of areas is mandatory in Denizli for all citizens [3].

If at least 80% of the population uses facemasks regularly in public, this could help eliminate the pandemic [4]. Pandemics can be prevented by determining whether society complies with compulsory mask use and related factors. Apart from individual factors, wide-ranging decisions by health authorities and governments can be much more effective in adherence to mask use. Revealing the effects of public health implementation decisions taken by authorities through scientific research will provide a basis for making decisions to be taken more quickly

How to cite this article: Uzun SU, Sevinc O, Ergin A. Prevalence of correct face mask usage among general public during COVID-19 pandemic in Denizli, Turkey. Marmara Med J 2022; 35(3): 343-349, doi: 10.5472/marumj.1191644

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1191644
Marmara Med J 2022;35(3): 343-349

to prevent rapidly developing public health problems such as communicable diseases. One of the methods to be used in the evaluation of public health practices is the evidence to be obtained from natural intervention research.

The objectives of this study were to (i) determine correct facemask usage prevalence and related factors in marketplaces in the city center of Denizli and (ii) to evaluate the effect of the announcement of asymptomatic COVID-19 case numbers and the effect of the announcement of nationwide curfew decisions on mask use status.

2. MATERIALS and METHODS

Type of Study

A population-based cross-sectional study was designed to determine the prevalence of correct face mask usage in the marketplaces in Denizli. The study was conducted between 22 November and 3 December 2020. After the data collection began, on 25 November 2020, the Ministry of Health of Turkey began to announce the number of asymptomatic COVID-19 cases for the first time since July 2020. Turkish Minister of Health admitted that they did not include asymptomatic COVID-19 cases in the daily announced tally since July 29, 2020 [5]. This practice ended on November 25, 2020. The Ministry of Health of Turkey began to announce the number of asymptomatic COVID-19 cases [6]. Also a curfew was announced on 30 November 2020. A nationwide partial curfew during the week (between 9 p.m. – 5 a.m.) and a weekend-long curfew (from Friday at 9 p.m. to Monday at 5 a.m.) were decided to be imposed in Turkey. Implementation of the weekend-long curfew started on 4 December 2020. So, this study is also a natural intervention study in which the effect of the announcement of the number of asymptomatic COVID-19 cases and the effect of the announcement of curfew decisions on the usage of facial masks is evaluated.

Study setting

Denizli is an industrial city with textile production and exports, It is a tourism destination in the southwestern part of Turkey due to the various types of thermal waters such as world heritage site Pamukkale and Karahayıt. According to the census of 2021, the population of Denizli is 1.051.056 [7]. Denizli is one of the warmest cities in Turkey, it has semi-humid climate with warm winter, very hot and dry summer.

Data Collection, Sample Size and Sampling Method

An observational data collection method was employed in order not to increase the risk of COVID-19 disease for both participants and observers. Also, observing participants' behaviors provides more reliable data as self-report data may create biases such as social desirability bias. The data were collected between 22 November – 3 December 2020. There are 48 marketplaces in the city center of Denizli [8]. Eight different marketplaces were selected by simple random sampling from these 48 marketplaces. Marketplaces were visited between the opening and closing hours of markets. The Hawthorne effect is a change in behavior as a response to observation [9]. It was important for participants to be unaware that they were being observed, so observations were performed by different students every hour in order to minimize the Hawthorne effect (especially for market sellers). For these observations, 6th-grade medical faculty students were trained before data collection. Students received a two-hour training from an associate professor (researcher O.S.) on the topic and substance of the research, how to make observations and how to fill in the observation forms, and possible problems they may encounter. Monitoring and evaluation of students' data collection were conducted by the supervisors. An observation form prepared by the researchers was used as data collection tool. This form was used for recording the data which includes the observee's gender, estimated age group (recorded as <10 years, 10-17 years, 18-64 years, and ≥65 years), the situation of using a mask (yes/no) and in/appropriate use of mask, type of mask (surgical mask, cloth mask, filtered mask) and information about whether they are a customer or a seller. Correct use of masks was evaluated according to WHO recommendations [10]. Appropriate mask usage was defined as if the mask covers the mouth and nose correctly. Inappropriate mask usage was defined as if the mask was under the nose and chin or on the hand. Since, the number of people using filter masks is low, those who use filter masks and those who use surgical masks were combined in the analysis.

The prevalence of facemask usage in different countries during the COVID-19 epidemic varied between 80-95% [11–16]. Based on these previous studies, the prevalence of facemask usage was estimated to be 80%. Considering α=0.05, p=0.80, and d=0.01, the required minimum sample size was found to be 6147 for this study.

Permissions and Ethics

The required permissions for conducting the research were obtained from the Turkey Ministry of Health's COVID-19 Scientific Research Platform, and the relevant municipalities (from both Pamukkale and Merkezefendi Metropolitan Municipality Administrations) before the study. The study was approved by Pamukkale University Ethics Committee (decision date: 13.10.2020, approval number:19).

Statistical Analysis

Statistical analysis was performed with R for Windows (version 3.6.2), and descriptive data were presented in numbers and percentages for categorical variables. Chi-square analysis was used to compare the prevalence of facemask usage according to variables. Multiple logistic regression analysis (backward LR method) was used to assess the effect of age, gender, observation time, and intervention type on correct facemask use. All comparisons were two-sided, and a p-value <0.05 was considered significant.

http://doi.org/10.5472/marumj.1191644
Marmara Med J 2022;35(3): 343-349

3. RESULTS

In this study, a total of 6749 observations were made in the marketplaces in Denizli, and 41.0% of them (n=2270) were women. 77.7% of those observed (n=5243) were in the 18-64 years age group. 71.9% of the observed people (n=4852) were customers, and 56% of the observations were made in the afternoon. It was observed that 4.0% of the people in the marketplaces did not have any kind of mask, while 84.0% of them used surgical masks. Correct face mask usage prevalence was 73.6%. It was found that 45.0% of the observed people were wearing their masks under their chins, and 39.1% of them were wearing their masks under their noses. Table I shows the participant characteristics and the prevalence of mask usage in marketplaces in Denizli.

Table 1. *Demographic characteristic and frequency of facemask use among general population observed in marketplaces in Denizli*

	n	%
Total number of observations	6749	100
Gender		
Female	2770	41.0
Male	3979	59.0
Age group		
<10 years	212	3.1
10-17 years	311	4.6
18-64 years	5243	77.7
≥65 years	983	14.6
Observation time		
a.m.	2960	43.9
p.m.	3789	56.1
Observed person		
Customer	4852	71.9
Seller	1897	28.1
Intervention type		
Before any intervention (22-25 November)	2096	31.1
Announcement of the number of asymptomatic coronavirus cases (26-30 November)	1678	24.9
Announcement of the curfew decisions (1-3 December)	2975	44.1
Facemask practice		
Yes	6476	96.0
No	273	4.0
Mask type		
Surgical mask	5670	84.0
Cloth mask	749	11.1
Filtered mask	57	0.9
No mask	273	4.0
Mask usage		
Correct Use	4968	73.6
Incorrect Use + No Mask	1781	26.4
Form of incorrect facemask usage (n=1781)		
Under the nose	697	39.1
Under the chin	801	45.0
In her/his hand	10	0.6
No mask	273	15.3

The correct mask usage prevalence was 84.3% among 4852 customers observed in the marketplaces in Denizli. The correct use of mask prevalence was found to be higher in female customers (90.9%) than in men (77.9%) (p<0.001). It has been observed that correct mask use increases as age increases (trend p <0.001) and the highest correct facemask usage prevalence was among customers over the age of 65 and was 87.9%. It was observed that the correct mask use prevalence was higher in customers who visited the marketplace in the morning than in the afternoon (86.1% vs 82.9%, p<0.001). When analyzed according to the type of intervention, it was found that the correct facemask usage prevalence in customers increased after the curfew decisions were announced (p<0.001). Post-hoc analysis showed that the decrease in correct mask use after the announcement of the number of asymptomatic COVID-19 cases was not statistically significant. Table II shows correct facemask usage prevalence among customers.

Table 2. *Correct facemask usage prevalence among customers observed in marketplaces in Denizli, Turkey*

	Incorrect Mask Usage + No Mask n (%)	Correct Mask Usage n (%)	p value
Total (n=4852)	762 (15.7)	4090 (84.3)	-
Gender			
Female	216 (9.1)	2161 (90.9)	**<0.001**
Male	546 (22.1)	1929 (77.9)	
Age group			
<10 years	77 (39.3)	119 (60.7)	**<0.001**
10-17 years	37 (18.7)	161 (81.3)	
18-64 years	547 (15.1)	3075 (84.9)	
≥65 years	101 (12.1)	735 (87.9)	
Observation time			
a.m.	292 (13.9)	1804 (86.1)	**0.003**
p.m.	470 (17.1)	2286 (82.9)	
Intervention type			
Before any intervention (22-25 November)	235 (16.8)	1163 (83.2)	**<0.001**
Announcement of the number of asymptomatic coronavirus cases (26-30 November)	260 (19.7)	1057 (80.3)	
Announcement of the curfew decisions (1-3 December)	267 (12.5)	1870 (87.5)	
Mask type			
Surgical+Filtered mask	518 (12.3)	3681 (87.7)	**<0.001**
Cloth mask	125 (23.4)	409 (76.6)	
No mask	119 (100)	0 (0)	
Form of incorrect facemask usage (n=762)			
Under the nose	348 (45.7)	-	-
Under the chin	289 (37.9)		
In her/his hand	6 (0.8)		
No mask	119 (15.6)		

http://doi.org/10.5472/marumj.1191644
Marmara Med J 2022;35(3): 343-349

Among sellers, the correct facemask usage prevalence was 46.3%. Correct facemask use of women was higher than men (58.3% vs 43.2%, p<0.001). As the age increases, the prevalence of correct mask use increases (trend p=0.002). Interestingly, it was found that the correct use of masks among sellers was higher in the afternoon (49.2% vs 42.8%, p<0.006). Before any intervention, correct facemask usage prevalence among sellers was 42.3%. The prevalence increased to 44.0% after the announcement of the number of asymptomatic COVID-19 cases, and to 50.6% after the curfew decisions were announced (p=0.003). Table III shows correct facemask usage prevalence among sellers.

Table 3. *Correct facemask usage prevalence among sellers observed in marketplaces in Denizli, Turkey*

	Incorrect Mask Usage + No Mask n (%)	Correct Mask Usage n (%)	p value
Total (n=1897)	1019 (53.7)	878 (46.3)	-
Gender			
Female	164 (41.7)	229 (58.3)	**<0.001**
Male	855 (56.8)	649 (43.2)	
Age group			
<10 years	12 (75.0)	4 (25.0)	
10-17 years	76 (67.3)	37 (32.7)	**0.002**
18-64 years	858 (52.9)	763 (47.1)	
≥65 years	73 (49.7)	74 (50.3)	
Observation time			
a.m.	494 (57.2)	370 (42.8)	**0.006**
p.m.	525 (50.8)	508 (49.2)	
Intervention type			
Before any intervention (22-25 November)	403 (57.7)	295 (42.3)	
Announcement of the number of asymptomatic coronavirus cases (26-30 November)	202 (56.0)	159 (44.0)	**0.003**
Announcement of the curfew decisions (1-3 December)	414 (49.4)	424 (50.6)	
Mask type			
Surgical+Filtered mask	740 (48.4)	788 (51.6)	**<0.001**
Cloth mask	125 (58.1)	90 (41.9)	
No mask	154 (100)	0(0)	
Form of incorrect facemask usage (n=762)	349 (34.3)		
Under the nose	512 (50.3)	-	-
Under the chin	4 (0.4)		
In her/his hand	154(15.0)		
No mask			

According to the results of multiple logistic regression analysis, the prevalence of correct facemask use in female customers was 2.86 times higher than male customers and 1.86 times higher among female sellers than male sellers. Although, there was no statistically significant difference between sellers, it was found that the prevalence of facemask usage among customers aged ≥65 was 4.89 times higher, 3.43 times higher for those aged between 18-64, and 3.01 times higher for those aged between 10-17 years, when the customers under 10 years of age were taken as reference. Among customers, the odds of correct facemask usage prevalence in the morning was 21% higher than in the afternoon. But among sellers, the odds of correct facemask usage prevalence in the afternoon was 50% higher than in the morning. Logistic regression analysis revealed that the announcement of the number of asymptomatic COVID-19 cases had no statistically significant effect on facemask usage prevalence both in customers and sellers, but after the announcement of the curfew decisions, correct facemask usage prevalence increased among customers (OR:1.24 (1.02-1.52)) and sellers (OR:1.64 (1.32-2.03)). The multiple logistic regression results are presented in Table IV.

Table 4. *Multiple logistic regression results of correct facemask usage prevalence among customers and sellers observed in marketplaces in Denizli, Turkey*

	Customers		Sellers	
Variables	OR (95% CI)	p value	OR (95% CI)	p value
Gender				
Male	Reference	<0.001	Reference	
Female	2.88 (2.43-3.43)		1.86 (1.48-2.34)	<0.001
Age Group				
<10 years	Reference		Reference	
10-17 years	3.01 (1.88-4.82)	<0.001	1.47 (0.44-4.95)	0.529
18-64 years	3.43 (2.50-4.71)	<0.001	2.52 (0.80-7.96)	0.114
≥65 years	4.89 (3.36-7.11)	<0.001	2.83 (0.86-9.30)	0.086
Observation Time				
a.m.	1.21 (1.02-1.43)	0.029	Reference	
p.m.	Reference		1.50 (1.24-1.83)	<0.001
Intervention Type				
Before any intervention	Reference		Reference	
Announcement of the number of asymptomatic coronavirus cases	0.88 (0.75-1.04)	0.146	1.10 (0.85-1.43)	0.443
Announcement of the curfew decisions	1.24 (1.02-1.52)	0.028	1.64 (1.32-2.03)	<0.001

4. DISCUSSION

This observational study was set out to investigate the correct use of facemasks of people in the marketplaces in Denizli. After the data collection began, we also had the chance to examine the

http://doi.org/10.5472/marumj.1191644
Marmara Med J 2022;35(3): 343-349

effects of some practices implemented by the Ministry of Health on mask use. Therefore, this study is also a natural intervention study.

During the COVID-19 pandemic, facemask practice in marketplaces in Denizli was quite high (96.0%). Previous studies about mask usage were much more focused on self-report facemask practice. The number of observational studies about face mask use is limited. In observational studies conducted in different countries at different times of the pandemic, it has been found that the use of masks varies considerably (3%-99.7%) [11,12,14,15,17–19]. Mask usage behavior is multifactorial but, since this study was carried out 10 months after the outbreak started in Turkey and the use of masks has been mandatory in Denizli since May 2020, this high mask use result is expected in our study.

The rate of correct facemask use in marketplaces in Denizli was high among customers (84.3%) but quite low among sellers (46.3%). A few studies have reported the correct use of masks. Results of observational mask usage practice from an observational study conducted among pedestrians in Iran, it was found that the prevalence of correct face-mask usage was 75.6% [16]. A high proportion of acceptable facemask use (95.7%) was observed among Malaysian individuals who were visiting the wet market [12]. Mask use practice is closely related to the presence of a legal obligation. However, a previous research showed that the correct use of the mask was mostly associated with individuals' knowledge level about appropriate mask use [20]. To increase the knowledge level and correct mask use practice, educational campaigns are needed. Poor correct face mask usage among sellers in our study was a notable observation. As sellers in marketplaces are in close contact with hundreds of people throughout the day, they have the potential to be a major source of virus transmission. For this reason, besides planning extensive educational programs for sellers, rigorous controls for correct mask use compliance should be implemented by police officers.

Both in customers and sellers, women's acceptable facemask usage was higher than men. Mask use was higher among women than men in previous observational studies [13,16–18]. Women were more risk averse, which influenced their behavioral responses, and as a result, females were more likely to adopt preventive measures [21]. Also, men may have convictions related to manliness and see themselves as more resistant to diseases, so they are less likely to take preventive health steps [22].

The compliance rate of correct facemask usage in customers increased with age and children had the lowest correct facemask prevalence. Our study supported previous studies [16–18,23,24], which reported older age were associated with higher mask use. This may be the result of the fact that older people have been reported to be at higher risk of COVID-19 mortality since the beginning of the COVID-19 pandemic and also younger individuals perceive themselves as strong against COVID-19 disease.

The correct use of facemasks among customers was higher in the morning, while among sellers it was higher in the afternoon. It may be possible to explain this situation as follows: Vegetable and fruit prices are higher in the morning hours in the marketplaces, and prices decrease in the afternoon, especially after 5 p.m. For this reason, the marketplace is very crowded in the evening hours close to the closing time of the markets. Customers with higher socioeconomic status who are aware of this situation and probably customers with higher knowledge of the COVID-19 disease shop in the early hours before the marketplaces are crowded. Sellers pay more attention to the correct use of facemasks when the marketplace is crowded in the afternoon, possibly due to customers with lower knowledge of the COVID-19 and more careless about preventative measures. Therefore, in marketplaces, there is more need for correct mask use control by police officers, especially in the afternoon.

It can be difficult to ensure compliance with public facemask wearing and especially correct mask use. Besides individual risk factors such as socio-demographic factors, COVID-19 risk perception, and trust in science and authorities, different governmental policies predict adherence to COVID-19 preventive measures [25-28]. Moreover, a study found that the scale of COVID-19 graphs used in mass media affected mask use preference [29]. So, it is very critical to understand the effect of different public health policies adopted by governments on public mask usage compliance. In this observational study, we had the chance to examine the effect of the announcement of the number of asymptomatic COVID-19 cases. We also examined the effect of the announcement of curfew decisions on the usage of the facemask. The use of facemask prevalence increased to 50.6% after the curfew decisions were announced.

Turkish Minister of Health said that the term "patients" referred to those who had tested positive for the disease and displayed symptoms. People who had a positive test but were asymptomatic were not included in the tally [5]. This practice ended on November 25, 2020. The Ministry of Health of Turkey began to announce the number of asymptomatic COVID-19 cases [6]. The announcement of asymptomatic COVID-19 cases had no statistically significant effect on the correct facemask use in both customers and sellers. In the early period of the pandemic, media reporting about COVID-19, elevated public health awareness and altered the community's behaviors and people began to take appropriate precautions such as wearing facemasks and washing their hands frequently [30]. We conducted this study in the 10th month of the outbreak in Turkey, so it is possible that people may now be desensitized to statistics and not able to understand or be less able to comprehend the severity of the COVID-19 pandemic. In the late period of the pandemic, the announcement of daily statistical parameters on mass media appeared to be ineffective in wearing the correct facemasks. However, after the announcement of the curfew decisions, correct facemask usage prevalence increased both among customers and sellers. Strict government interventions probably affect individuals' COVID-19 risk perception, and as a result, people begin to pay more attention to using proper facemasks. Previous studies showed that rather than voluntary policies, strict widespread

http://doi.org/10.5472/marumj.1191644
Marmara Med J 2022;35(3): 343-349

governmental steps are more effective to ensure mask-wearing compliance [17,24,31]. Without government action, the general public does not follow public health recommendations [17]. It is reported that compliance with preventive measures is higher when authorities take serious control measures with laws and regulations [27,31,32]. It is critical to correctly comprehend the factors that influence compliance with public health measures and to choose the right and effective implementation strategies.

Strengths and Limitations

This study's results depended on observational data. Age and gender information were collected based on observation. Also, the assessment of correct facemask use was evaluated only based on ensuring the mask covers the mouth and nose. Other aspects of proper mask use (touching the front of the mask, taking off, discarding and re-using the mask…etc.) have not been evaluated. But in terms of measuring the participants' proper mask use, observation outperforms self-reporting. Also, observations were made by different trained medical students every hour to minimize the Hawthorne effect. Since, results from natural experimental studies are urgently needed on this issue [32], this study provides results of the effects of two different governmental decisions on public correct facemask use.

Conclusion

Although, the use of masks is high in marketplaces in Denizli, Turkey, the correct use of facemasks is not sufficient, especially among sellers, the correct mask usage prevalence is quite low. Correct use of facemasks is lower in men and the young population. While, customers pay more attention to the correct use of masks in the morning hours, sellers pay more attention in the afternoon. The announcement of curfew decisions has increased the correct use of masks although the announcement of asymptomatic COVID-19 case numbers has no effect. Since, millions of people have not been vaccinated, people should follow coronavirus precautions. It is recommended that even fully vaccinated people should also maintain preventive measures such as correct mask wearing [33]. Informative training about correct facemask use targeted at specific populations is required. We urge health authorities and policymakers to consider serious regulations such as lockdowns to be implemented for the public to adopt and implement non-pharmaceutical protective interventions necessary to take control of the COVID-19 spread. Since, the COVID-19 pandemic is still ongoing and likely to continue around the world, using the correct face mask is essential.

Compliance with the Ethical Standards

Ethical Approval: This study was approved by the Pamukkale University Ethics Committee (decision date: 13.10.2020, approval number:19).

Financial Support: The authors have no relevant financial information to close.

Conflict of Interest: The authors have no potential conflicts of interest to disclose.

Authors' Contributions: SUU and OS: Concept and design, AE: Supervision, SUU and OS: Data collection and processing, SUU, OS and AE: Data analysis and interpretation, SUU: Literature search and drafting the article, SUU, OS and AE: Critical review. All authors approved the final version of the article.

REFERENCES

[1] Cheng VCC, Wong SC, Chuang VWM, et al. The role of community-wide wearing of face mask for control of coronavirus disease 2019 (COVID-19) epidemic due to SARS-CoV-2. J Infect 2020;81:107-14. doi: 10.1016/j.jinf.2020.04.024.

[2] WHO. Mask use in the context of COVID-19. Available from: https://www.who.int/publications/i/item/advice-on-the-use-of-masks-in-the-community-during-home-care-and-in-healthcare-settings-in-the-context-of-the-novel-coronavirus-(2019-ncov)-outbreak Accessed December 22, 2020.

[3] Republic of Turkey Governorship of Denizli. Denizli provincial general hygiene assembly decision 68 (COVID-19). [68 nolu (COVID-19) Denizli il umumi hıfzıssıhha meclis kararı] Available from: http://www.denizli.gov.tr/68-nolu-covid-19-denizli-il-umumi-hifzissihha-meclis-karari Accessed December 22, 2020. (Turkish.)

[4] Ngonghala CN, Iboi E, Eikenberry S, et al. Mathematical assessment of the impact of non-pharmaceutical interventions on curtailing the 2019 novel coronavirus. Math Biosci 2020;325:108364. doi: 10.1016/j.mbs.2020.108364.

[5] Financial Times. Turkey admits publishing incomplete coronavirus tally. Available from: https://www.ft.com/content/5307a438-f083-4f7f-a1da-5b0a0eaa6047 Accessed April 12, 2021.

[6] Wikipedia. COVID-19 pandemic in Turkey. Available from: https://en.wikipedia.org/wiki/COVID-19_pandemic_in_Turkey Accessed April 12, 2021.

[7] TURKSTAT. The results of address based population registration system, 2021. Available from: https://data.tuik.gov.tr/Bulten/Index?p=The-Results-of-Address-Based-Population-Registration-System-2021-45500&dil=2 Accessed February 17, 2021.

[8] Denizli Metropolitan Municipality. Marketplaces days and hours in Denizli [Denizli Pazar yerleri gün ve saatleri]. Available from: https://www.denizli.bel.tr/Default.aspx?k=content&id=13175 Accessed October 17, 2020. (Turkish)

[9] Sedgwick P, Greenwood N. Understanding the Hawthorne effect. BMJ 2015;351:h4672. doi:10.1136/bmj.h4672

[10] WHO. When and how to use masks. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public/when-and-how-to-use-masks Accessed February 12, 2021.

[11] Tam VC, Tam SY, Poon WK, Law HKW, Lee SW. A reality check on the use of face masks during the COVID-19 outbreak in Hong Kong. EClinicalMedicine 2020;22:100356. doi: 10.1016/j.eclinm.2020.100356

[12] Gunasekaran GH, Gunasekaran SSS, Gunasekaran SS, Zaimi NSIB, Halim NABA. Prevalence of facemask use among general public when visiting wet market during Covid-19 pandemic: An observational study. medRxiv [Preprint] Accessed December 23, 2020. doi: 10.1101/2020.05.17.20105023

[13] Gunasekaran GH, Gunasekaran SSS, Gunasekaran SS, Halim FHBA. Prevalence and acceptance of face mask practice among individuals visiting hospital during COVID-19 pandemic: an observational study. Preprints [Preprint] Accessed December 23, 2020. doi: 10.20944/preprints202.005.0152.v1

[14] Elachola H, Ebrahim SH, Gozzer E. COVID-19: Facemask use prevalence in international airports in Asia, Europe and the Americas, March 2020. Travel Med Infect Dis 2020;35:101637. doi: 10.1016/j.tmaid.2020.101637

[15] Elachola H, Gozzer E, Rahman NMM, et al. Photo-epidemiology to estimate face covering use in select areas in Asia versus the Americas and Africa during the COVID-19 pandemic. J Travel Med 2020;27:taaa121. doi: 10.1093/jtm/taaa121

[16] Rahimi Z, Shirali GA, Araban M, Mohammadi MJ, Cheraghian B. Mask use among pedestrians during the Covid-19 pandemic in Southwest Iran: an observational study on 10,440 people. BMC Public Health 2021;21:133. doi: 10.1186/s12889.020.10152-2

[17] Haischer MH, Beilfuss R, Hart MR, et al. Who is wearing a mask? Gender-, age-, and location-related differences during the COVID-19 pandemic. PLoS One 2020;15:e0240785. doi: 10.1371/journal.pone.0240785.

[18] Beckage B, Buckley TE, Beckage ME. Prevalence of face mask wearing in northern Vermont in response to the COVID-19 pandemic. Public Health Rep 2021;333.549.211009496. doi: 10.1177/003.335.49211009496.

[19] Newman MG. An observational study of mask guideline compliance ın an outpatient ob/gyn clinic population. Eur J Obstet Gynecol Reprod Biol 2020;255:268-9. doi: 10.1016/j.ejogrb.2020.10.048.

[20] Ho HS. Use of face masks in a primary care outpatient setting in Hong Kong: Knowledge, attitudes and practices. Public Health 2012;126:1001-6. doi: 10.1016/j.puhe.2012.09.010.

[21] Harris CR, Jenkins M, Glaser D. Gender differences in risk assessment : why do women take fewer risks than men ? Judgm Decis Mak 2006;1:48–63.

[22] Lee LYK, Lam EPW, Chan CK, et al. Practice and technique of using face mask amongst adults in the community: a cross-sectional descriptive study. BMC Public Health. 2020;20:948. doi: 10.1186/s12889.020.09087-5.

[23] Machida M, Nakamura I, Saito R, et al. Incorrect use of face masks during the current COVID-19 pandemic among the general public in Japan. Int J Environ Res Public Health 2020;17:6484. doi: 10.3390/ijerph17186484.

[24] Badillo-Goicoechea E, Chang T-H, Kim E, et al. Global trends and predictors of face mask usage during the COVID-19 pandemic. arXiv [Preprint]. Accessed December 22, 2020. Available from: http://arxiv.org/abs/2012.11678

[25] Duan T, Jiang H, Deng X, Zhang Q, Wang F. Government intervention, risk perception, and the adoption of protective action recommendations: evidence from the COVID-19 prevention and control experience of China. Int J Environ Res Public Health 2020;17:3387. doi: 10.3390/ijerph17103387.

[26] Liang XH, Tang X, Luo YT, Zhang M, Feng ZP. Effects of policies and containment measures on control of COVID-19 epidemic in Chongqing. World J Clin Cases 2020;8:2959-76. doi: 10.12998/wjcc.v8.i14.2959.

[27] Kao K, Lust E, Dulani B, Ferree KE, Harris AS, Metheney E. The ABCs of Covid-19 prevention in Malawi: authority, benefits, and costs of compliance. World Dev 2021;137:105167. doi: 10.1016/j.worlddev.2020.105167.

[28] Plohl N, Musil B. Modeling compliance with COVID-19 prevention guidelines: the critical role of trust in science. Psychol Health Med 2021;26:1-12. doi: 10.1080/13548.506.2020.1772988.

[29] Romano A, Sotis C, Dominioni G, Guidi S. The scale of COVID-19 graphs affects understanding, attitudes, and policy preferences. Health Econ 2020;29:1482-94. doi: 10.1002/hec.4143.

[30] Zhou W, Wang A, Xia F, Xiao Y, Tang S. Effects of media reporting on mitigating spread of COVID-19 in the early phase of the outbreak. Math Biosci Eng 2020;17:2693-07. doi: 10.3934/mbe.2020147.

[31] Howard J, Huang A, Li Z, et al. An evidence review of face masks against COVID-19. Proc Natl Acad Sci USA. 2021;118:e201.456.4118. doi: 10.1073/pnas.201.456.4118.

[32] Lyu W, Wehby GL. Community use of face masks and COVID-19: evidence from a natural experiment of state mandates in the US. Health Aff (Millwood). 2020;39:1419–25. doi: 10.1377/hlthaff.2020.00818.

[33] Christie A, Mbaeyi SA, Walensky RP. CDC interim recommendations for fully vaccinated people: an important first step. JAMA 2021;325:1501-2. doi: 10.1001/jama.2021.4367.

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Change in orthopedics and traumatology admissions during the COVID-19 pandemic at a university hospital in Turkey

Nusret OK[1], Raziye SAVKIN[2], Nihal BUKER[2], Mücahit OZTOP[3], Yucel YUCE[1]

[1] Department of Orthopedics and Traumatology, Faculty of Medicine, Pamukkale University, Denizli, Turkey.
[2] Department of Physiotherapy and Rehabilitation, Faculty of Physiotherapy and Rehabilitation, Pamukkale University, Denizli, Turkey.
[3] Division of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Yozgat Bozok University, Yozgat, Turkey.

Corresponding Author: Raziye SAVKIN
E-mail: raziyesavkin@hotmail.com

Submitted: 18.02.2022 **Accepted:** 06.05.2022

ABSTRACT

Objective: The rapidly increasing cases of coronavirus disease 2019 (COVID-19) in Turkey required the reorganization of the health system. The first response to the pandemic in our university hospital was to stop elective surgery on March 30, 2020, minimize outpatient consultations and reduce the number of patients in the wards. To determine the change in the number of patients admitted to our hospital, we examined the data during the pandemic process and compared them with the data of the previous year.

Methods: The number of patients admitted to Pamukkale University Orthopedics and Traumatology Outpatient Clinic and Emergency Service for orthopedic and trauma injuries between March 1, 2020 and October 31, 2020 was documented and compared with the data of the same period in 2019.

Results: During the pandemic period, the number of emergency and outpatient orthopedics and traumatology admissions decreased significantly by 15% and 42.93% respectively ($p<0.001$). The highest decrease in outpatient admissions was in April 2020 with 79.29%, and the highest decrease in emergency admissions was in March 2020 with 31.35%.

Conclusion: In conclusion, due to fear of getting infected, curfews, and decreased human mobility, there was a dramatic decrease in the number of hospital admissions between March 1, 2020, and October 31, 2020. The COVID-19 pandemic has impacted healthcare in many ways, putting some healthcare systems under great pressure and pushing others beyond their capacity. New strategies can be determined to ensure the continuity of health services and to maximize resources.

Keywords: COVID-19, Trauma, Orthopaedics, Pandemic, Outpatient, Emergency

1. INTRODUCTION

A novel coronavirus, coronavirus disease 2019 (COVID-19), which emerged in Wuhan, China, spread rapidly around the world and was declared a pandemic by the World Health Organization on March 11, 2020 [1]. On the same date, the first COVID-19 case in Turkey was detected and the first death due to the virus was reported on March 17, 2020 [2].

The world was faced with unprecedented global health challenges, with increased cases of COVID-19 causing disruption to healthcare. All over the world, there has been a significant decrease in admissions to health institutions compared to the previous year due to reasons such as lockdown, restriction of mobility due to fear of contamination, and changes in health service delivery [3,4]. The rapidly increasing cases of COVID-19 in Turkey required the reorganization of the health system. The first response to the pandemic in our university hospital was to cessation of elective surgery, minimize outpatient consultations, and reduce the number of patients in the wards on March 30, 2020. In addition, some wards were converted to COVID-19 care units. On June 1, 2020, the Turkish Ministry of Health published a "gradual" normalization plan [5], and the outpatient clinic, wards and operating rooms in our hospital started to work with 50% capacity based on the patient flow before the pandemic. As of October 2020, our hospital started to accept patients only by online appointment.

How to cite this article: Ok N, Savkin R, Buker N, Oztop M, Yuce Y. Change in orthopedics and traumatology admissions during the COVID-19 pandemic at a university hospital in Turkey. Marmara Med J 2022; 35 (3): 350-354, doi: 10.5472/marumj.1192292

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1192292
Marmara Med J 2022;35(3): 350-354

In our university hospital, which is a tertiary healthcare institution, major changes in patient flow were evident during this period. In this study, we reviewed the number of patients admitted to local university hospital orthopedics and traumatology emergency and outpatient clinics in Turkey between 1 March 2020 and 31 October 2020 and aimed to compare them with the data obtained in the same time frame in the previous year.

2. MATERIALS and METHODS

This single-center, retrospective cohort study was performed at Pamukkale University Hospital, Department of Orthopedics and Traumatology in Turkey. The ethical approval of the study was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee. Data were collected through the university hospital registry system. All consecutive patients admitted to the Orthopedics and Traumatology Outpatient Clinic and Emergency Service at Pamukkale University Hospital between March 1, 2020, and October 31, 2020 were recorded and compared to the patient admissions from the same time period in 2019. In recurrent cases, only the first admission was considered. Patients who were consulted by the emergency physician and referred to Orthopedics and Traumatology were included in the emergency service admissions.

Data Collection

Demographic data (age and gender), the admitted department (emergency/outpatient) and the date of admission were recorded. The monthly number of patients admitted to the Emergency Service and Orthopedics and Traumatology Outpatient Clinic between March and October of 2019 and the number of patients admitted during the same time period in 2020 were examined. In addition, Emergency Service and Outpatient Clinic admissions only for March 2019 and March 2020 were examined in detail in two parts: (1) The number of admissions was compared based on the date of 11 March 2020, when the first case was seen in Turkey: the first 10 days of March (1st March to 10th March), and 11th March to 31st March, (2) Weekly comparison was performed for March.

Statistical Analyses

The data was analyzed using SPSS 24.0 (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) package program. Continuous variables were given as mean ± standard deviation, median (minimum and maximum) and categorical variable values are presented as absolute numbers (n) and percentages (%). Categorical data were compared with use of the Chi-square ($\chi 2$) test. The conformity of continuous variables with normal distribution was evaluated using the Kolmogorov-Smirnov test. Independent Samples t-test was used for comparison of the groups by age. A p value of < 0.05 was considered significant.

3. RESULTS

The number of outpatient orthopedics and traumatology admissions decreased significantly by 42.93% during the pandemic period (p<0.001). A total of 7850 patients (mean age 39.85 years; 4420 males and 3430 females) were admitted to the outpatient department during the pandemic, and 13755 patients (mean age 40.08 years; 6790 males and 6965 females) were admitted between the same time period in the previous year.

Table I. *Descriptive variables of patients*

	2019 (March-October)		2020 (March-October)		
	Min-Max	X±SS	Min-Max	X±SS	p
Age (year) Policlinic	0-100	40.08±20.51	0-98	39.85±19.78	0.421
Age (year) Emergency	1-97	40.90±24.23	1-99	42.09±24.96	0.205
	n	**%**	**n**	**%**	
Gender Policlinic					
Female	6965	50.6	3430	43.7	<0.001
Male	6790	49.4	4420	56.3	
Gender Emergency					
Female	487	32.6	407	32.1	<0.001
Male	1006	67.4	862	67.9	

The number of orthopedics and traumatology emergency admissions decreased significantly by 15% during the pandemic period (p<0.001). A total of 1269 patients (mean age 42.09 years; 862 males and 407 females) were admitted to the emergency service during the pandemic, and 1493 patients (mean age 40.90 years; 1006 males and 487 females) were admitted during the same period a year earlier.

The monthly change in the number of patients in the emergency service and outpatient clinic were presented in Figure 1. During the pandemic period, there was a significant decrease in outpatient admissions in all months. The highest decrease was in April with 79.29% (1840 vs. 381), and the lowest decrease was in June with 11.21% (1356 vs. 1204). Except for May and July, there was also a decrease in emergency admissions during the pandemic period. The highest decrease was in March with 31.35% (185 vs. 127). However, an increase of 8.82% was detected in July (204 vs. 222).

Comparisons before and after March 11, 2020 were presented in Figure 2. Before March 11, both outpatient (573 vs. 591) and emergency admission (43 vs. 50) were similar in both years. However, during the pandemic period, the number of outpatient admissions decreased by 55.39% (1123 vs. 501) and number of emergency admission decreased by 45.77% (142 vs. 77) after March 11.

Weekly comparison of March is presented in Figure 3. In both years, there was a decrease in the number of outpatient admissions from the first week to the last week of March. While

http://doi.org/10.5472/marumj.1192292
Marmara Med J 2022;35(3): 350-354

this decrease was 18.63% (467 vs. 380) in March 2019, it was 80.30% (533 vs. 105) in March 2020. However, as of the second week of March, emergency applications decreased by 35.71% (42 vs. 27), 42.22% (45 vs. 26) and 56.36% (55 vs. 24), respectively, when compared to the same period in 2019.

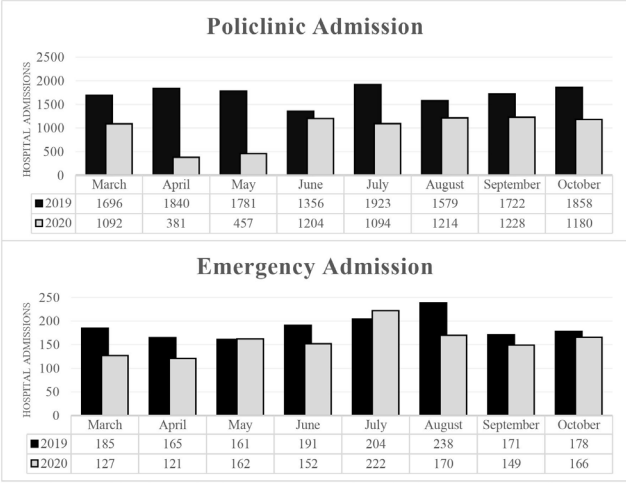

Figure 1. *The monthly change in the number of patients in the emergency and outpatient departments*

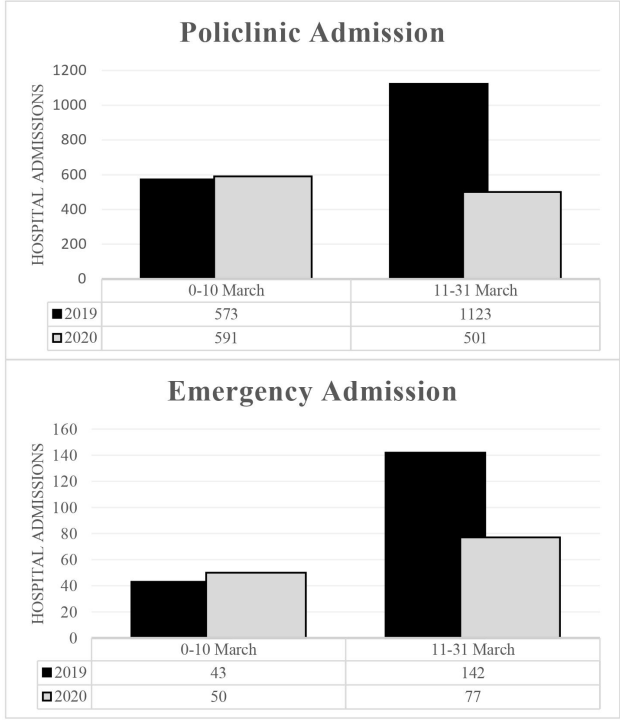

Figure 2. *The change in the number of patients in emergency and outpatients clinics before and after 11 March.*

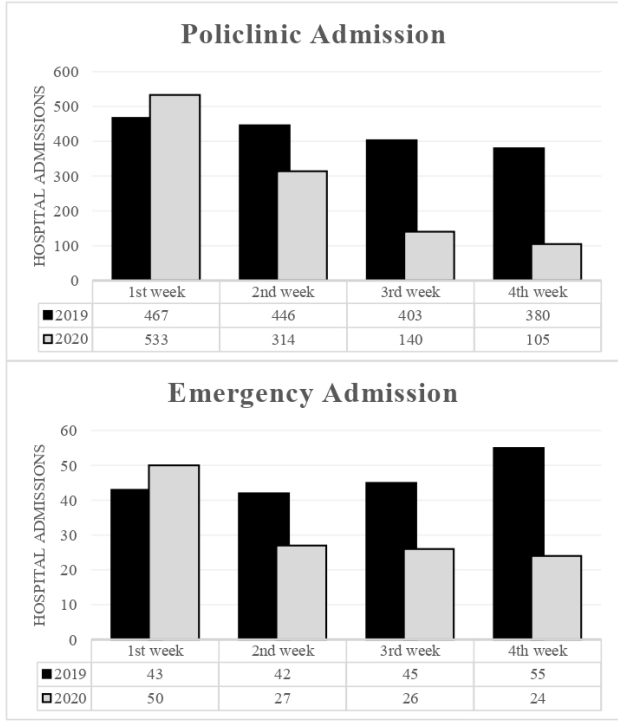

Figure 3. *Weekly comparison of March.*

4. DISCUSSION

The Turkish Ministry of Health implemented additional measures to limit the spread of COVID-19, and these measures had significant effects on hospital admissions. In this study, the change in orthopedics emergency and outpatient clinic admissions at local University hospital during the pandemic period in Turkey was examined, and it was determined that there was a 42.93% decrease in the outpatient and a 15% decrease in the emergency admissions between March 1, 2020 and October 31, 2020 compared to the previous year admissions.

In addition to the measures taken and curfews, the mobility and social life of people had impacted due to the fear-related issues caused by a pandemic that was spreading rapidly all over the world [6]. Both in Turkey and in other countries, the number of patients who admitted to the hospital after March 2020 was lower than the corresponding periods of the previous year. In studies conducted in tertiary hospitals in Turkey, Kalem et al., found that trauma cases admitted to the orthopedics department of a hospital in Ankara decreased by 50% [7], while Turgut et al., showed that the number of fracture-related applications in Izmir decreased from 1634 to 645 [8]. Significant reductions in trauma cases have been reported during the COVID-19 pandemic in Italy [9], the Republic of Ireland [10], and India [11]. An international study of 63 orthopedics and trauma surgeons from 28 countries reported that 91% of hospitals have reduced workload compared with pre-COVID-19, of which only 17% are performing elective surgeries [12]. In our study,

http://doi.org/10.5472/marumj.1192292
Marmara Med J 2022;35(3): 350-354

it was determined that there was a 42.93% and 15% decrease in orthopedics and traumatology outpatient and emergency admissions, respectively, compared to the same period in 2019. The reason for this decrease might be the measures taken in hospitals to minimize transmission, the decrease in high-energy trauma cases such as traffic accidents due to reduced human mobility during the curfew [6]. In addition, COVID-19 cases were the primary focus of the healthcare system in our hospital between March and June 2020, while other patients remained in the background and elective surgeries were postponed. Postponing or canceling hospital appointments due to COVID-19 concerns might also have caused the decline [13].

Due to the cessation of elective surgery, minimizing outpatient consultations, and reducing the number of patients in the wards on March 30, 2020, there was a significant decrease in outpatient admissions in April and May 2020. Although, there was an increase in the number of patients within the framework of the "gradual" normalization plan after June 1, 2020, it was seen that there were still fewer admissions compared to 2019. Although, there was an 8.82% increase in July compared to 2019, there were also decreases in overall emergency admissions. Similarly, when the first COVID-19 case was confirmed in the Netherlands, the rates of utilization of the emergency services in three hospitals between February 15 and May 15, 2020, had gradually decreased [6]. Hospital admissions in the US dropped dramatically in March and April 2020 with the spread of COVID-19, and there was a rebound in the late June – early July [4]. Although, in our study, the orthopedics and traumatology admissions, which decreased rapidly after the first COVID-19 case, increased with the normalization process in June 2020, yet it was still lower than in 2019. We think that this increase might be due to both the normalization process and the increased human mobility in the summer season.

The first case in Turkey was detected on March 11, 2020. The number of outpatient and emergency admissions was similar between March 1-10 in 2019 and March 1-10, 2020. However, after March 11, 2020, admissions to the emergency department and outpatient clinic dramatically decreased. The reason for this may be that with the detection of the first case, people avoid being in crowded enclosed environments such as hospitals due to the panic and fear of contamination. This decrease became more evident as of the 3rd week of March 2020. The curfew covering the age of 65 and over as of March 21, 2020 might have contributed to this [14].

This study has some limitations. The study population is limited to a tertiary hospital in a single province. Multicenter studies can be performed with larger cohorts. However, with gradual normalization, it may not be possible to generalize normalization due to new restrictions or relaxations based on the number of cases in each province in Turkey. Fear of getting infected and restrictions such as postponement of hospital appointments and elective surgeries, social isolation and closures probably kept non-urgent patients out of hospitals. The measures taken since the beginning of the pandemic to prevent and reduce the spread of the virus and maintain the health system capacity had seriously disrupted health services. However, COVID-19 is expected to stay with us for a while, and uninterrupted healthcare is now inevitable. However, limited data and changing situations with gradual normalization do not allow us to make more specific recommendations beyond sharing our experiences.

In conclusion, there is a significant decrease in orthopedics and traumatology emergency and outpatient clinic admissions between March 1, 2020, and October 31, 2020. As a result, fear of getting infected, curfews, and decreased human mobility, there was a dramatical decrease in the number of hospital admissions. The COVID-19 pandemic has impacted healthcare in many ways, putting some healthcare systems under great pressure and pushing others beyond their capacity. New strategies can be determined to ensure the continuity of health services and to maximize resources.

Compliance with Ethical Standards

Ethical Approval: Local ethical board permission was taken from Pamukkale University, Non-interventional Clinical Research Ethics Committee (Approval number: 60116787-020/73821).

Financial support: This research received no specific grant from any funding agency in the public, commercial, or non-profit sectors.

Conflict of Interest Statement: There is no conflict of interest.

Authors' Contributions: All authors contributed to the study conception and design. MO and YY: Data collection, RS and NB: Analysis and interpretation of data, RS, MO and YY: Drafting the article, NO and NB: Revising the article critically for intellectual content. All authors read and approved the final version of the article.

REFERENCES

[1] WHO Director-General's opening remarks at the mission briefing on COVID-19 – 12 March 2020. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-mission-briefing-on-covid-19—-12-march-20200. (Accessed on 02.09.2021)

[2] Republic of Turkey Ministry of Health-COVID-19 Information Page. https://covid19.saglik.gov.tr/. (Accessed on 02.09.2021)

[3] Moynihan R, Sanders S, Michaleff ZA, et al. Impact of COVID-19 pandemic on utilisation of healthcare services: a systematic review. BMJ Open 2021; 11:16:e045343. doi: 10.1136/bmjopen-2020-045343.

[4] Birkmeyer JD, Barnato A, Birkmeyer N, Bessler R, Skinner J. The Impact of the COVID-19 pandemic on hospital admissions in the United States. Health Aff 2020; 39:2010-7. doi: 10.1377/hlthaff.2020.00980.

[5] Turkey's effective fight against coronavirus. https://www.iletisim.gov.tr/images/uploads/dosyalar/Kovid_Kitap_EN.pdf. (Accessed on 02.09.2021)

[6] Barten DG, Latten GHP, van Osch FHM. Reduced emergency department utilization during the early phase of the COVID-19

pandemic: viral fear or lockdown effect? Disaster Med Public Health Prep 2020; 16:33-9. doi: 10.1017/dmp.2020.303.

[7] Kalem M, Kocaoğlu H, Merter A, Karaca MO, Özbek EA, Kınık HH. Effects of Covid-19 pandemic curfew on orthopedic trauma in a tertiary care hospital in Turkey. Acta Orthop Traumatol Turc 2021; 55:191-5. doi: 10.5152/j.aott.2021.20263.

[8] Turgut A, Arlı H, Altundağ Ü, Hancıoğlu S, Egeli E, Kalenderer Ö. Effect of COVID-19 pandemic on the fracture demographics: Data from a tertiary care hospital in Turkey. Acta Orthop Traumatol Turc 2020; 54:355-63. doi: 10.5152/j.aott.2020.20209.

[9] Luceri F, Morelli I, Accetta R, Mangiavini L, Maffulli N, Peretti GM. Italy and COVID-19: the changing patient flow in an orthopedic trauma center emergency department. J Orthop Surg Res 2020; 14:323-4. doi: 10.1186/S13018.020.01816-1.

[10] Elbardesy H. Impact of the COVID-19 pandemic on trauma and orthopaedic service in the Republic of Ireland. EMJ Microbiol Infect Dis 2020:doi: 10.33590/emjmicrobiolinfectdis/20-00116.

[11] Dhillon MS, Kumar D, Saini UC, Bhayana H, Gopinathan NR, Aggarwal S. Changing pattern of orthopaedic trauma admissions during COVID-19 pandemic: experience at a tertiary trauma centre in India. Indian J Orthop 2020; 54:374. doi: 10.1007/S43465.020.00241-0.

[12] Lezak BA, Cole PA Jr, Schroder LK, Cole PA. Global experience of orthopaedic trauma surgeons facing COVID-19: a survey highlighting the global orthopaedic response. Int Ortho 2020; 44:1519-29. doi: 10.1007/S00264.020.04644-4.

[13] Czeisler MÉ, Marynak K, Clarke KE, et al. Delay or avoidance of medical care because of COVID-19–related concerns – United States, June 2020. MMWR Morb Mortal Wkly Rep 2020; 69:1250-57. doi: 10.15585/mmwr.mm6936a4.

[14] 65 yaş ve üstü ile kronik rahatsızlığı olanlara sokağa çıkma yasağı genelgesi. https://www.icisleri.gov.tr/65-yas-ve-ustu-ile-kronik-rahatsizligi-olanlara-sokaga-cikma-yasagi-genelgesi. (Accessed on 02.09.2020)

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Association of the changes in pulmonary artery diameters with clinical outcomes in hospitalized patients with COVID-19 infection: A cross-sectional study

Aybuke SELCUK[1], Can ILGIN[2], Sait KARAKURT[3]

[1] School of Medicine, Marmara University, Istanbul, Turkey
[2] Department of Public Health, School of Health, Marmara University, Istanbul, Turkey
[3] Department of Pulmonary Medicine, School of Medicine, Marmara University, Istanbul, Turkey

Corresponding Author: Aybuke SELCUK
E-mail: aybukeselcukk@gmail.com

Submitted: 16.06.2022 **Accepted:** 27.08.2022

ABSTRACT

Objective: Enlarged pulmonary artery diameter (PAD) can be associated with mortality risk in coronavirus disease 2019 (COVID-19) patients. Our aim is to find the factors that cause changes in PAD and the relationship between radiological findings and clinical outcomes in COVID-19 patients.

Patients and Methods: In this descriptive, retrospective, and single centered study, among the hospitalized 3264 patients, 209 patients with previous chest computed tomography (CT) were included. Findings of current chest CTs of patients obtained during COVID-19 were compared with that of previous chest CTs. Pulmonary involvements, World Health Organization (WHO) Clinical Progression Scale scores and laboratory variables were recorded. Intensive Care Unit (ICU) admission, intubation and mortality were clinical outcomes that were evaluated by using uni – and multivariate analyses.

Results: Patients with high D-dimer had significantly increased risk for enlarged PAD and increase in PAD compared to previous chest CT (ΔPAD) (OR=1.18, p<0.05, OR=1.2 p<0.05). Both high D-dimer and an increase over 2 mm in PAD (ΔPAD 2mm) had significant risks for ICU admission, intubation, and mortality (OR= 1.18 p<0.01, OR=1.22 p<0.01, OR=2.62 p<0.05, OR=2.12 p<0.01, OR=2.32 p<0.01, OR=2.09 p<0.001 respectively). It was found that with enlarged PAD, risk of ICU admission and mortality increased. (OR=3.03 p<0.001, OR=2.52 p<0.01). Combined with age and lymphocyte counts, PAD predicted mortality with a 50% sensitivity, 88% specificity (AUC=0.83, p<0.001).

Conclusion: PPatients with an increase over 2 mm (ΔPAD 2mm) in PAD had significantly increased clinical severity, ICU admission, intubation, and mortality. High levels of D-dimer and CRP in patients suggest that increased inflammation and thrombosis may be effective in pathogenesis.

Keywords: Pulmonary artery diameter, COVID-19, Thromboembolism, CoRad, WHO score

1. INTRODUCTION

It is well known that increased pulmonary thromboembolic events are responsible for increased morbidity and mortality in patients with coronavirus disease 2019 (COVID-19) infection [1-5]. The detection of diffuse thromboembolism in the pulmonary arteries (PA) and dilatation of the right ventricle according to autopsy studies and clinical studies suggest that severe pulmonary hypertension (PH) secondary to pulmonary thromboembolism that develops in a large proportion of patients [6-8].

It has been stated that the enlarged pulmonary artery diameter (PAD) and PAD/Aorta ratio measurement in non-contrast chest computed tomography (CT) is a useful non-invasive method in determining pulmonary hypertension. Reference values of PAD for healthy persons have been previously established and revealed good sensitivity for excluding PH [9].

Some studies reported a relationship between enlarged PAD and severity [10,11] and mortality of COVID-19 12-14]. In a study with a small patient population comparing the change in PAD in chest CT scans between COVID-19 and pre COVID-19 periods, it was shown that increased PA diameter (ΔPAD), PAD/Aorta ratio were significantly associated with the degree of lung involvement, but no significant relationship was found with clinical severity and mortality [14].

How to cite this article: Selcuk A, Ilgin C, Karakurt S. Association of the changes in pulmonary artery diameters with clinical outcomes in hospitalized patients with COVID-19 infection: A cross-sectional study. Marmara Med J 2022;35(3): 355-361, doi: 10.5472/marumj.1195539

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1195539
Marmara Med J 2022;35(3): 355-361

The aim of this study is to investigate factors that influence the changes in PAD and the relationship between the change in PAD and clinical severity, radiological involvement, and mortality in hospitalized COVID-19 patients.

2. PATIENTS and METHODS

This study is a descriptive study. A total 3264 patients aged 18 and above, who were tested positive for SARS-CoV2 PCR and diagnosed with COVID-19 and hospitalized in tertiary university hospitals between March 2020 and June 2021 were scanned from the hospital information system and analyzed. Among them, 209 patients who had chest CT scans before the COVID-19 pandemic and whose PAD could be measurable were included in the study. Sample size was based on previous studies. Patients with missing data were excluded from the study. Chest CT scans and laboratory values of the patients obtained during the administration, as well as previous chest CT scans obtained with different indications within the last 5 years were analyzed. PAD was measured perpendicular to the vessel surfaces where the vessel walls were parallel to each other, within 3 cm of the bifurcation point, in both pre – and post-COVID-19 chest CT scans of 209 patients.

The diameter of the ascending aorta (Ao), detected in the same cross-section as the maximum PAD, was measured horizontally and vertically and averaged. PAD was evaluated by Framingham-Based on gender-specific references. Females with a diameter over 27 mm and males with a diameter 29 mm were considered having enlarged PAD, and patients were categorized into two groups as normal PAD and enlarged PAD. The normal PA/Ao ratio was considered as ≤ 0.9 [9, 15].

In addition, patients also were divided into two groups: those with an increased PAD (ΔPAD) and those without an increase in PAD, regardless of reference values, but according to the degree of change in PAD between previous chest CT scans and chest CT scans done during the COVID-19 infection.

Considering the highest Likelihood Ratio (LR) in the ROC analysis, the patients were divided into two groups as those with an increased PAD over 2 mm (ΔPAD_{2mm}) and those with PAD less than 2mm (ΔPAD_{2mm}.). In radiological evaluation, parenchymal involvement was classified in increasing percentage intervals with 0%, 5%, 25%, 50% ,75% to 100% for each lung lobe. In sum, total scores between 0 and 25 were calculated by adding scores for each lobe, such as 0, 1, 2, 3, 4, and 5 respectively [16, 17].

Two pulmonologists evaluated the chest CT scans independently, and a consensus was reached between the different results; at the point without agreement, the decision of a more senior pulmonologist was consulted. All chest CT examinations were performed on multidetector scanners with 256 rows (Philips ICT). The WHO Clinical Progression Scale was used for clinical severity [18]. Data of the patients was taken from the hospital information system. Laboratory variables including C-reactive protein (CRP), D-dimer, ferritin, fibrinogen, LDH, MPV, neutrophil, lymphocyte neutrophil/lymphocyte, lymphocyte/CRP ratios obtained in the first day of admission, and the worst values during hospitalization were used [19-22].

Human rights

The study was approved by the Marmara University, School of Medicine Clinical Research Ethic Committee (protocol number: 09.2020.1316, date: 04-12-2020). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standard.

Statistical Analysis

The statistical analysis was performed using the STATA SE 17 software (Stata Corp 2021. *Stata Statistical Software: Release 17*. College Station, TX: StataCorp 2021 lLC). Data were expressed median and interquartile range (IQR) according to normal distribution for continuous ones; counts and percentage for categorical ones. The normality assumption was tested with Shapiro Wilk W test. Chi-square is applied between categorical variables; Mann-Whitney U Test was used for two independent groups in numerical variables. Spearman's correlation test was applied to evaluate variables like radiologic involvement and pulmonary artery. Logistic regression analysis was used to examine the factors affecting the dependent outcomes. Survival analysis and multivariate analyzes in PAD and ΔPA groups were performed using the Cox proportional hazards model. Receiver Operating Characteristics (ROC) analysis was applied to see the mortality estimation of PAD and the Area Under Curve was calculated. Confidence intervals (CIs) were computed at a 95% level. If the P value was less than 0.05, it was considered statistically significant.

3. RESULTS

The Patient Characteristics

This study enrolled 209 patients (132 male, 63.16%) with previous chest CT scans. The median age of the patients was 64 years (IQR=55-75). The number of patients with oxygen requirement was 168 (80.3%), 72 patients (34.4%) were admitted to ICU. The median duration of hospitalization was recorded to be 7 days (IQR, 5-12) for patients admitted to the medical ward, and 7 days (IQR, 4-12) for patients admitted to ICU.

The previous CT scans used as baseline examinations had been performed with a median of 1.8 years (IQR, 1.04-2.65) before COVID-19 for another reason. The median PAD value of the patients before COVID-19 (PAD_{pre}) was 25.72 mm (IQR,23.54-26.63), while PAD was 27.29 mm (IQR,24.84-30.62) during COVID-19 (p<0.001).

Assessment of Lung Parenchyma

Radiological score distribution of patients were as follows: 0-5 points: 48 patients (23%), 6-10 points: 85 patients (41%) 11-15 points: 42 patients (19%), 16-20 points: 26 patients (13^%) 21-25 points: 8 patients (4%).

Radiological score showed a weak positive correlation with WHO score (R=0.28, p<0.001), and the risk of ICU admission

http://doi.org/10.5472/marumj.1195539
Marmara Med J 2022;35(3): 355-361

(OR=1.10, LR=13.34, p<0.001, 95% CI) and intubation (OR=1.05, LR=4.22, p=0.03, 95% CI) (Table II).

The enlarged PAD incidence was found to be higher in patients with a radiological score of 5 and above (OR=7.01, p<0.01, 95% CI). The increased ICU admission was found in those with a radiological score of 10 and above (p=0.008), and this relationship was clearer in those with a radiological score of 20 and above (OR = 9.64, p<0.001, 95% CI). A tendency to increase in the need for intubation was found in those with a radiological score of 15 and above (p=0.086). However, there was no significant relationship between the radiological score and mortality.

The enlarged PAD was found to be higher in patients with a radiological score of 5 and above (OR= 7.01; p<0.01). A radiological score of 5 or higher was found in 85 of 88 patients (96.6%) with enlarged PAD.

Patients with enlarged PAD

Relationship between the enlarged PAD and different variables are shown in the Table I. Risk factors affecting enlarged PAD and effect of PAD to clinical outcomes are given in Table II and Table III.

The PAD solely was a significant predictor of mortality (61% sensitivity, 65% specificity, AUC = 0.69, p<0.001). When the PAD was evaluated with age and lymphocyte, it predicted mortality better (50% sensitivity, 88% specificity, AUC = 0.83, p<0.001) (Table III). (Figure 1).

The optimal cutoff value of PAD for mortality was detected as 27.61 mm (sensitivity 67.7%, specificity 63.9%), and the cutoff for maximum effectiveness was determined as 33.54 mm (17.7% sensitivity, 97.7% specificity) (Figure 1).

Table I. *The comparisons of patients' characteristics and outcomes between normal vs enlarged PAD and without increase vs increased ΔPAD_{2mm}*

Variables	Normal PAD (n=121)	Enlarged PAD (n=88)	Without increase ΔPAD_{2mm} (n=121)	Increased ΔPAD_{2mm} (n=88)	Total (n=209)
Age	61 (54-72)	68 (59-76.5) **	65(55-75)	64 (54-73)	64 (55-75)
Female	30 (38.96%)	47 (61.04%) ***	44(%57%)	33 (%43%)	77 (%37%)
CRP	71 (121-124)	79 (37-141)	126 (62-207)	149 (84.5-214.5)	74 (27-127)
D-dimer	0.7 (0.4-1.22)	1.02 (0.6-.2.4) ***	0.71 (0.43-1.15)	1.14 (0.54-2.24) ***	0.86 (0.46-1.57)
LDH	314 (249-452)	351 (284-449)	317 (253-425)	339 (274-494)	336 (259-452)
Lymphocyte	0.8 (0.6-1.2)	0.8 (0.5-1.1)	0.9 (0.5-1.2)	0.7 (0.5-1) *	0.8 (0.5-1.1)
N/L Ratio	5.75 (3-9.6)	6.79 (3.4-11)	5.6 (2.6-10)	7.5 (4.4-11) **	5.8 (3-10.4)
L/CRP Ratio	0.01(0.004-0.05)	0.01 (0.003-0.2)	0.1 (0.005-0.05)	0.008 (0.004-0.02) **	0.1 (0.005-0.05)
WHO Score	5 (5-6)	6 (5-10) ***	5 (5-7)	5 (5-10) *	5 (5-10)
ICU Admission	29 (%40.28%)	43 (%59.72%) ***	33 (%45.83%)	39 (%54.17%) **	72 (%34%)
LOS in ICU	0(0-1)	0 (0-6.5) ***	5(2-11)	8 (5-12) **	7 (4-12)
Intubation	29(%41.43^%)	41 (%58.57%) ***	31(%44.3%)	39 (%55.7%) **	70 (%33%)
Radiological Score	97 (80.2%)	85(96.6%)	6(6-12)	8 (6-14)	9 (6-13)
Mortality	26 (41.94%)	36 (58.06%) **	28(%45.2%)	34 (%54.8%) **	62 (%29%)

p<0.05, p<0.01**, p<0.001*** CRP: C-reactive protein, LDH: Lactate dehydrogenase, N/L Ratio: Neutrophil/Lymphocyte ratio, L/CRP: Lymphocyte/ CRP ratio LOS: Length of stay, ICU: Intensive care unit.*
Data are reported as median (Interquartile Range, IQR) or number(percentage).

Table II. *The factors effecting outcomes according to Multivariable Logistic Regressions Analysis*

Variables	ICU (OR)	Intubation (OR)	Mortality (OR)
Age	1.02*	1.05***	1.07***
Covid PAD	1.16***	1.15**	1.17***
CRP	1.007***	1.007***	1.005**
D-Dimer	1.18**	1.22***	2.62*
Lymphocyte	0.3**	0.1***	0.17***
Radiological Score	1.10***	1.05*	1.03
PAD_{pre} (U)	1.21***	1.21***	1.24***
Enlarged PAD(U)	3.03***	2.76**	2.52**
ΔPAD (U)	1.09	1.14	1.02
ΔPAD_{2mm} (U)	2.12**	2.32**	2.09***

U: Univariate Regression, OR: Odds Ratio, p<0.05, p<0.01**, p<0.001****

http://doi.org/10.5472/marumj.1195539
Marmara Med J 2022;35(3): 355-361

Table III. *The group of independent non-categoric variables that affect PAD and change in PAD and outcomes according to Multivariable Logistic Regression Analysis*

Dependent variables	Independent non-categoric initial variables	Accuracy	Sensitivity	Specificity	AUC
Enlarged PAD	DD, L/CRP, Ferritin, PAD_{pre}	0.803	0.807	0.680	0.893
Δ PAD	DD, L	0.569	0.547	0.585	0.635
ΔPAD2mm	DD, L, Age	0.713	0.740	0.674	0.787
ICU admission	DD, L, Age, PAD, CRP	0.732	0.708	0.745	0.801
Intubation	DD, L Age, PAD	0.78	0.71	0.81	0.85
Mortality	L, Age, PAD	0.727	0.742	0.721	0.832

DD:D-Dimer, L/CRP: Lymphocyte/ CRP ratio, PADpre, :PAD before COVID-19
L: Lymphocyte, AUC: Area Under Curve

Table IV. *The variables effecting PAD and change in PAD according to Multiple Logistic RegressionAnalysis*

Variables	Enlarged PAD	ΔPAD	ΔPAD2mm
Age	1.02**	0.9	0.9
CRP	1.001*	1.005*	1.002
D-Dimer	1.12*	1.18*	1.18***
Ferritin	1.0004*	1.0004	0.9
Lymphocyte	0.8	0.6	0.4**

The values are Odds Ratio, p<0.05= p<0.01=** p<0.001*

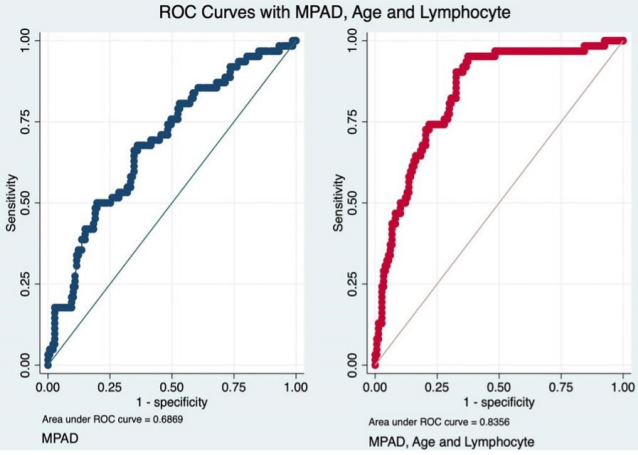

Figure 1. *Receiver Operating Characteristic Curve of PAD for predict death. The optimal cutoff value of PAD for mortality was detected as 27.61 mm (sensitivity 67.7%, specificity 63.9%), and the cutoff for maximum effectiveness was determined as 33.54 mm (17.7% sensitivity, 97.7% specificity).*

Patients with ΔPAD

It was found that the median PAD of the patients before COVID-19 (PAD_{pre}) was 25.72 mm, while PAD was 27.29 mm during COVID-19 (p<0.001). The median value changed by 1.5 mm (IQR, 0.35-3.04).

Patients were divided into two groups as increased PAD (ΔPAD) and without an increase in PAD according to the change in PAD relative to their previous chest CTs. ΔPAD increased in 144 out of 209 patients (68.9%). There was no difference in terms of demographic characteristics (age and gender). CRP, D-dimer, LDH, neutrophil, neutrophil/lymphocyte levels were significantly higher in patients with ΔPAD (p=0.005, p<0.001, p=0.04, p=0.03, p=0.007). The ratio of lymphocytes and lymphocytes/CRP were found to be significantly lower in patients with ΔPAD (p<0.001, p=0.002). While ICU admission increased significantly in patients with ΔPAD (p=0.04), there was no significant difference in WHO and radiological scores, medical ward length of stay (LOS) and ICU admission, and mortality (p=0.2, p=0.5, p=0.9, p=0.05, p=0.4). According to Cox regression analysis, it was also found that the risk of mortality significantly increased with the increase in PAD (HR=1.09, p=0.01, 95% CI).

Patients with ΔPAD_{2mm}

Considering the highest LR in the ROC analysis, the patients were divided into two groups: those with an increase over 2 mm (ΔPAD_{2mm}) and those with PAD less than 2 mm (ΔPAD_{2mm}).

Relationship between the ΔPAD_{2mm} and different variables are shown in the Table I. Effect of ΔPAD_{2mm} to clinical outcomes are given in the Table II.

When the PAD/Aorta ratio and various parameters were examined, no significant relationship could be found.

It was found that age, CRP, D-dimer, ferritin, and lymphocyte count are responsible for the change in PAD according to multiple logistic regression analysis (Table IV).

4. DISCUSSION

Pulmonary artery diameter in the chest CT of patients with COVID-19 was found to be significantly increased when compared to previous chest CT scans. In patients with enlarged PAD or ΔPAD_{2mm} ICU admission, intubation rate, and mortality increased significantly. The radiological score was found to be significantly higher in patients with enlarged PAD. In patients with ΔPAD, ICU admission was increased. It was determined that the WHO scores, ICU admission, and mortality were higher in the patients with enlarged PAD_{pre}. While, CRP levels increased significantly in patients with enlarged PAD and ΔPAD, they tended to increase in patients with ΔPAD_{2mm}. D-dimer levels were also found to be significantly higher in all patients with changes in PAD.

In this study, enlarged PAD was higher in older age, but ΔPAD was the same in both genders and among the age groups. It was reported that there was no difference between genders regarding change in PAD in a previous study [13]. It has been known that there is a positive but weak correlation between age and PAD in a healthy person [9]. So, the increase in PAD with age could be secondary to COVID-19 infection, however, it remains unknown whether this finding can be related to COVID-19

http://doi.org/10.5472/marumj.1195539
Marmara Med J 2022;35(3): 355-361

infection or age. Age can be a confounder for the relationship between COVID-19 and PAD.

High D-dimer, low L/CRP, high ferritin and enlarged PAD_{pre} were significantly increased parameters in patients with enlarged PAD. It was determined that high D-dimer and low lymphocyte count were factors that were seen in patients with significant increase in ΔPAD and ΔPAD_{2mm}. It has been reported that there is a correlation between PAD and CRP, N/L, body temperature, oxygen requirement, LDH, D-dimer, and lymphocyte during hospitalization and an increase in PAD indicates a poor prognosis [7,10,23]. Our findings are consistent with the literature. The fact that D-dimer and CRP values in the clinical course are higher in patients with enlarged PAD than in those with normal PAD, could suggest that inflammation and coagulopathy were more serious in these patients.

In our study, it was found that CRP, ferritin, lymphocyte, D-dimer had significant effects on PAD changes. It is also a crucial point that only high D-dimer levels were significantly associated with a change in PAD (Table IV). Ferritin, CRP, L/CRP are markers that show inflammation [24,25]. D-dimer is a fibrin degraded product that shows the presence of thrombi in circulation. With these findings, it can be thought that inflammation and thrombosis may be responsible for the changes in PAD. Therefore, changes in PAD were associated with poor prognosis [10].

In our study, PAD_{pre} was found to be larger than normal in 60 of 209 (28.7%) patients. In 55 of them (91%), PAD was also found to be large during COVID-19 infection. In 33 (22.14%) of 149 patients with normal PAD before COVID-19, PAD was found to be enlarged during COVID-19. As a result, PAD was found to be enlarged in 88 (42.10%) of 209 patients. ΔPAD was not found to be significantly different between patients with a previously normal or enlarged PAD. These findings suggest that patients with an enlarged PAD before COVID-19 have an increased requirement of hospitalization and increase in all the clinical outcomes.

Although, 20 of the 121 patients did not have PAD enlargement, it was noteworthy that ΔPAD2mm was significantly associated with mortality in these patients. Furthermore, ΔPAD_{2mm} was found to be more significantly associated with mortality than enlarged PAD ($p<0.01$ vs $p<0.001$). At this point, it may be important to examine patients according to previous chest CT, both to determine the risk of patients with enlarged PAD_{pre} as we mentioned above and to predict the mortality increased by ΔPAD_{2mm} in patients without enlarged PAD_{pre} or PAD.

In our study, although a median of 1.5 mm increase in PAD was detected ($p=0.001$), there was no significant relationship between ΔPAD and clinical outcomes. It has been reported that PAD increased by 3 mm ($p=0.001$) in chest CT in COVID-19 patients when compared with previous chest CT [14].. Due to the large number of patients with enlarged PAD before COVID-19 (28.7 %) in our study, a smaller increase in PAD was observed after COVID-19 when compared to the ones reported in the literature and we think that the correlation between the clinical endpoints and the ΔPAD could not reach a significant level. The increase in the size of the pulmonary artery during COVID-19 infection and the amount of this increase significantly affect the prognosis of the patients. In our study, the admission for ICU, intubation, and mortality are significantly increased in patients with enlarged PAD and/or an increase in PAD over 2 mm.

A significant correlation was found between radiological involvement and clinical severity. It was determined that the need for ICU increased significantly in patients with a radiological score of 20 and above, more specifically in patients with a radiological score of 10 and above. A significant correlation was determined between a radiological score of 15 and above and the need for intubation. A radiological score of 5 and above is significantly associated with enlarged PAD. It has been reported that there is a significant correlation between the amount of parenchymal involvement and PAD increase ($p=0.032$) and PAD/Aorta increase ($p<0.001$) [14]. Again, in parallel with the severity of pulmonary involvement, it was stated that PAD was 26.11± 3.72 mm in those without lung involvement, 26.65± 2.95 mm in those with mild pneumonia, and 28.59± 3.63 mm in those with severe pneumonia were reported ($p=0.027$) [10]. Our study helped us to make a meaningful prediction for important clinical endpoints by using different threshold values of the radiological score.

ΔPAD_{2mm} was found to be more significantly associated with mortality than enlarged PAD ($p<0.01$ vs $p<0.001$). There was no significant difference between ΔPAD_{2mm} and enlarged PAD in terms of intensive care and intubation requirements. The D-dimer level was found to be much higher in patients with ΔPAD_{2mm} compared to patients with an incase in enlarged PAD. So, it can be considered that coagulopathy is a more important feature for ΔPAD2mm than enlarged PAD.

In our study, mortality increased when PAD was 27.61 mm and above according to the ROC analysis (Sensitivity= 67.7%, Specificity =63.9%); If we selected the threshold as 33.54 mm we observed that the sensitivity decreased but the specificity increased significantly (17.7% Sensitivity, 97.7% specificity). In addition, we also found that the risk of mortality significantly increased with every 1 mm increase in PAD (HR=1.09, $p=0.01$, 95% CI) (Figure 1).

It has been reported that there was a significant increase in mortality with an increase in PAD; it was determined as an independent risk factor for mortality, and an increase in mortality in COVID-19 patients with PAD≥ 31 mm (HR=1.592[1.154–2.196], $p=0.005$, 95%CI) [13]. Likewise, it was found that mortality increased significantly with a PA over 29.15 (75% sensitivity 84% specificity) [26].

There are some studies that found both significance and insignificance between the PA/Ao ratio and mortality [26,27]. PA/Ao ratio did not reach the level of significance in our study, although, this rate tended to increase in COVID-19.

While, no significant relationship was found between ward LOS and changes in PAD, there was a significant correlation between enlarged PAD, ΔPAD and ΔPAD_{2mm} and LOS of ICU (respectively $p=0.001$, $p<0.05$ and $p=0.01$). The enlarged PAD and ΔPAD_{2mm} were significantly associated with ICU admission.

http://doi.org/10.5472/marumj.1195539
Marmara Med J 2022;35(3): 355-361

In the literature, severity of the pneumonia was significantly associated with ΔPAD and enlarged PAD [10,23,38. It was mentioned that there was no significant relationship between parenchymal abnormalities and pulmonary embolism [7,29]. These suggest that severity of parenchymal involvement could not be associated with severity of vascular involvement. In our study, the fact that the radiological score increased the risk of ICU admission, intubation, and correlated with the WHO score, but not showing its effect on mortality; could suggest that vascular involvement rather than parenchymal involvement has a greater effect on mortality. Therefore, radiological involvement increases the clinical severity, but vascular pathologies are at the forefront at the point of the issue is mortality.

This study has the following limitations. Firstly, the lack of data on etiologies that could cause enlarged PAD and PAD_{pre} like pulmonary embolism. Secondly, lack of data related to pressure pulmonary artery pressure estimated by right heart catheterization or echocardiography. Thirdly, single-center design of the study cause bias.

Conclusion

Our study indicated that PAD increased significantly in COVID-19 patients compared to previous chest CT. For the first time in the literature, this was associated with the risk of ICU admission. It was established that the clinical severity, ICU admission, the risk of intubation and mortality increased significantly in patients whose PAD increased over 2 mm. The high D-dimer level and inflammation markers in these patients suggest that increased inflammation and thrombosis may be effective in the pathogenesis.

Compliance with the Ethical Standards

Ethical Approval: This study was approved by the Marmara University, School of Medicine Clinical Research Ethics Committee (approval number: (2020/164).

Financial Support: The authors have no relevant financial information to close.

Conflict of Interest: The authors have no potential conflicts of interest to disclose.

Authors' Contributions: CI.: Medical practice, SK: Conceptualization, AS and SK: Data collection and processing, AS and CI.: Formal analysis and interpretation, AS and SK.: Writing the draft and editing. All authors read and approved the final version of the article.

REFERENCES

[1] Suh YJ, Hong H, Ohana M, et al. Pulmonary embolism and deep vein thrombosis in COVID-19: A Systematic review and meta-analysis. Radiology 2021;298: 70-80. doi: 10.1148/RADIOL.202.020.3557

[2] Fox SE, Akmatbekov A, Harbert JL, Li G, Brown JQ, Heide RSV. Pulmonary and cardiac pathology in African American patients with COVID19: an autopsy series from New Orleans. The Lancet Respir Med 2020; 8, 681-9. doi: 10.1016/S2213-2600(20)30243-5

[3] Jiménez D, García-Sanchez A, Rali P, et al. Incidence of VTE and bleeding among hospitalized patients with coronavirus disease 2019: A systematic review and meta-analysis. Chest 2021;159, 1182-96. doi: 10.1016/j.chest.2020.11.005

[4] Helms J, Tacquard C, Severac F, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: A multicenter prospective cohort study. Intensive Care Med 2020; 46, 1089-98. doi: 10.1007/s00134.020.06062-x

[5] Jevnikar M, Sanchez O, Chocron R, et al. Prevalence of pulmonary embolism in patients with COVID-19 at the time of hospital admission. Eur Respir J 2021;58:2100116. doi: 10.1183/13993.003.00116-2021

[6] D'Andrea, A, Scarafile R, Riegler L, et al. Right ventricular function and pulmonary pressures as independent predictors of survival in patients with COVID-19 Pneumonia. JACC: Cardiovasc Imaging 2020;13:2467-8. doi: 10.1016/j.jcmg.2020.06.004

[7] Jalde FC, Beckman MO, Svensson AM, et al. Widespread parenchymal abnormalities and pulmonary embolism on contrast-enhanced CT predict disease severity and mortality in hospitalized COVID-19 patients. Front Med 2021;8:666723. doi: 10.3389/fmed.2021.666723

[8] Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. NEJM 2020; 38: 120-8. doi: 10.1056/nejmoa2015432

[9] Truong Q. A., Bhatia HS, Szymonifka J, et al. A four-tier classification system of pulmonary artery metrics on computed tomography for the diagnosis and prognosis of pulmonary hypertension. J Cardiovasc Comput Tomogr 2018; 12:60-6. doi: 10.1016/j.jcct.2017.12.001

[10] Yildiz M, Yadigar S, Yildiz BŞ, et al. Evaluation of the relationship between COVID-19 pneumonia severity and pulmonary artery diameter measurement. Herz 2021; 46: 56-62. doi: 10.1007/s00059.020.05014-x

[11] Hayama H, Ishikane M, Sato R, et al. Association of plain computed tomography-determined pulmonary arteryto-aorta ratio with clinical severity of coronavirus disease 2019. Pulm Circ 2020;10: 204.589.4020969492. doi: 10.1177/204.589.4020969492

[12] Schiaffino S, Codari M, Cozzi A, et al. Machine learning to predict in-hospital mortality in covid-19 patients using computed tomography-derived pulmonary and vascular features. J Pers Med 2021;11:501. doi: 10.3390/jpm11060501

[13] Esposito A. Palmisano A, Toselli M, et al. Chest CT-derived pulmonary artery enlargement at the admission predicts overall survival in COVID-19 patients: insight from 1461 consecutive patients in Italy. Eur Radiol 2020; 31:4031-41. doi:10.1007/s00330.020.07622-x/Published.

[14] Spagnolo P, Cozzi A, Foà RA, et al. CT-derived pulmonary vascular metrics and clinical outcome in COVID19 patients. Quant Imaging Med Surg 2020;10:1325-33. doi: 10.21037/QIMS-20-546

[15] van Dam L F, Kroft LJM, van der Wal LI, et al. Clinical and computed tomography characteristics of COVID-19 associated acute pulmonary embolism: A different phenotype of thrombotic disease? Thromb Res 2020;193:86-9. doi: 10.1016/j.thromres.2020.06.010

[16] Bernheim, A. Mei X, Huang M, et al. Chest CT findings in coronavirus disease 2019 (COVID-19): Relationship to duration of infection. Radiology 2020;295,685-91. doi: 10.1148/radiol.202.020.0463

[17] Chamorro M E, Tascón D A, Sanz LI, Velez SO, Nacenta S B. Radiologic diagnosis of patients with COVID-19. Radiología (English Edition) 2021;63:56-73. doi: 10.1016/j.rxeng.2020.11.001

[18] Marshall JC, Murthy S, Diaz J, et al. A minimal common outcome measure set for COVID-19 clinical research. 20, Lancet Infect Dis 2020; p. e192-7. doi: 10.1016/S1473-3099(20)30483-7

[19] Simadibrata DM, Calvin J, Wijaya AD, Ibrahim NAA. Neutrophil-to-lymphocyte ratio on admission to predict the severity and mortality of COVID-19 patients: A metaanalysis. Am J Emerg Med 2021; 42:60-9. doi: 10.1016/j.ajem.2021.01.006

[20] Lagunas-Rangel F A. Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. J Med Virol 2020; 92:1733-4. doi: 10.1002/jmv.25819

[21] Rapkiewicz A v, Mai X, Carsons SE, et al. Megakaryocytes and platelet-fibrin thrombi characterize multi-organ thrombosis at autopsy in COVID-19: A case series. EClinicalMedicine 2020; 24:100434. doi: 10.1016/j.eclinm.2020.100434

[22] Tang N, Li D, Wang X, Sun z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020;18: 844-7. doi: 10.1111/jth.14768

[23] Zhu QQ, Gong T, Huang GQ, et al. Pulmonary artery trunk enlargement on admission as a predictor of mortality in in-hospital patients with COVID-19. Jpn J Radiol 2021;39:589-97. doi: 10.1007/s11604.021.01094-9

[24] Meng LB, Yu ZM, Guo P, et al. Neutrophils and neutrophil-lymphocyte ratio: Inflammatory markers associated with intimal-media thickness of atherosclerosis. Thromb Res 2018;170:45-52. doi: 10.1016/j.thromres.2018.08.002

[25] Okugawa Y, Toiyama Y, Yamamoto A, et al. Lymphocyte-C-reactive protein ratio as promising new marker for predicting surgical and oncological outcomes in colorectal cancer. Ann Surg 2020;272:342-51. doi: 10.1097/SLA.000.000.0000003239

[26] Erdoğan M, Öztürk S, Erdöl MA, et al. Prognostic utility of pulmonary artery and ascending aorta diameters derived from computed tomography in COVID-19 patients. Echocardiography 2021;38:1543-51. doi: 10.1111/echo.15170

[27] Eslami V, Abrishami A, Zarei E, Khalili N, Baharvand Z, Sanei-Taheri S. The association of CT-measured cardiac indices with lung involvement and clinical outcome in patients with COVID-19. Acad Radiol 2021;28: 8-17. doi: 10.1016/j.acra.2020.09.012

[28] Aoki R, Iwasawa T, Hagiwara E, Komatsu S, Utsunomiya D, Ogura T. Pulmonary vascular enlargement and lesion extent on computed tomography are correlated with COVID-19 disease severity. Jpn J Radiol 2021;39:451-8. doi: 10.1007/s11604.020.01085-2

[29] Ippolito D, Giandola T, Maino C, et al. Acute pulmonary embolism in hospitalized patients with SARS-CoV-2related pneumonia: multicentric experience from Italian endemic area. Radiol Med 2021;126: 669-78. doi: 10.1007/s11547.020.01328-2

MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

Relationship between disease severity, perceived stress, and depression in patients with seborrheic dermatitis

Esra SARAC, Emek KOCATURK GONCU

Department of Dermatology, School of Medicine, Koc University, Istanbul, Turkey

Corresponding Author: Esra SARAC
E-mail: sarac.esra@gmail.com

Submitted: 30.05.2022 **Accepted:** 27.07.2022

ABSTRACT

Objective: A significant proportion of patients with seborrheic dermatitis state that disease attacks are triggered by psychological stress and disease severity increases during stressful times. This study aimed to determine the relationship between disease severity and perceived stress and depression levels in patients with seborrheic dermatitis.

Patients and Methods: The patients' demographic characteristics, medical history, and Seborrheic Dermatitis Area Severity Index (SDASI), Perceived Stress Scale (PSS), and Beck Depression Inventory (BDI) scores were recorded, and the relationship between disease severity and the scale scores were statistically analyzed by comparing disease severity groups and using correlation analyses.

Results: The study included 120 patients with seborrheic dermatitis. The mean age of the patients was 29.5±8.2 years, and the female/male ratio was 49/71. According to disease severity, 66 (55%) patients were classified as mild, 30(25%) moderate, and 24 (20%) as severe seborrheic dermatitis. When the scale scores were evaluated, a significant positive correlation was found between SDASI and PSS (r=0.767, p<0.001), between SDASI and BDI (r=0.663, p<0.001), and between PSS and BDI (r=0.687, p<0.001).

Conclusion: A significant relationship was observed between disease severity, stress, and depression in patients with seborrheic dermatitis. There is a need for a multidisciplinary approach involving a dermatologist and psychiatrist in the follow-up and treatment of these patients.

Keywords: Depression, Disease severity, Seborrheic dermatitis, Stress

1. INTRODUCTION

Seborrheic dermatitis is a chronic recurrent skin disease that is commonly seen across the world and affects all age groups. It is considered that approximately 1-3% of the immunocompetent population is affected by this disease [1,2]. Although, the exact cause of the disease is not known, some risk factors, such as genetic predisposition, male gender, light skin color, generalized skin dryness, winter season, and proliferation of Malassezia species have been implicated in the etiology of the disease or triggering of attacks [3-6].

The effects of stress on emotional and physical health have been an interesting topic since ancient times [7,8]. Stress can be basically defined as the response to mental or physical pressure. Although, it is known that stress triggers many diseases, the presence of a dermatological disease itself can also aggravate psychological stress and cause wide range psychiatric conditions, such as depression, anxiety, and somatoform disorders [9-11].

A significant proportion of patients with seborrheic dermatitis states that disease attacks are triggered by psychological stress and disease severity increases during stressful times [12]. The involvement of visible skin areas, such as the scalp and face may also cause psychiatric comorbidities in these patients. The aim of this study was to determine the relationship between disease severity and perceived stress and depression levels in patients with seborrheic dermatitis.

How to cite this article: Sarac E , Kocaturk Goncu E. Relationship between disease severity, perceived stress, and depression in patients with seborrheic dermatitis. Marmara Med J 2022;34 (3): 35(3): 362-366. doi: 10.5472/marumj.1195298

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1195298
Marmara Med J 2022;35(3): 362-366

2. PATIENTS and METHODS

Patients

The study included patients older than 18 years without any other dermatological or chronic disease, who were diagnosed with seborrheic dermatitis. The study was approved by Koc University Ethics Committee (approval number: 2019.415. IRB1.064) and written informed consent was obtained from all the participants. The patients' demographic characteristics (age, gender, and body mass index), disease duration, family history, factors that aggravated the disease, smoking status, and alcohol use were recorded.

Disease Severity

Disease severity was measured using the Seborrheic Dermatitis Area and Severity Index (SDASI) [13]. Nine anatomical regions were scored between 0 (absent) and 3 (severe) according to the severity of desquamation, erythema, and pruritus. The score of each area was multiplied by its specific constant (forehead, 0.1; scalp, 0.4; nasolabial, 0.1; eyebrow, 0.1; postauricular, 0.1; auricular, 0.1; intermammary, 0.2; back, 0.2; and cheek or chin, 0.1), and the sum of the scores was determined as the SDASI score. The patients were classified as having a mild disease if their SDASI score was 0-4.2, moderate disease if 4.3-8.4, and severe disease if 8.5-12.6.

The patients' stress levels were evaluated using the Turkish version of the five-point Likert-type Perceived Stress Scale (PSS), which has 14 items scored between 0 (never) and 4 (usually) [14]. A lower total score in PSS indicates low level of stress perception. The reliability and validity analyses of the Turkish version of PSS were previously undertaken by Eskin et al. [15].

The validated Turkish version of the Beck Depression Inventory (BDI) was administered to evaluate and determine the risk of depression in the patients [16,17]. BDI consists of 21 items, each scored from 0 to 3 depending on the patient's response, and a higher total score indicates greater depressive symptoms. The total score that can be obtained from this scale varies between a minimum of 0 and a maximum of 63. In the current study, the patients' depressive symptoms and risk of depression were categorized as follows: 0-9 points, normal state; 10-18 points, mild depression, 19-29 points, moderate depression, and 30-63 points, severe depression.

Statistical Analyses

The statistical analyses were performed using the Statistical Package for the Social Sciences version 26.0 (IBM SPSS Inc., Chicago, IL, USA). All the numerical variables were reported as mean ± standard deviation, frequency, and percentages. As categorical variables, patient characteristics were compared using chi-squared test. Student's t-test and one-way analysis of variance were used for the comparison of quantitative variables. For non-normally distributed samples, the Mann-Whitney U or Kruskal-Wallis test was performed to compare the scores of the groups. The correlations between the scales were evaluated with the Pearson correlation analysis. Throughout the analyses, a p value of <0.05 was considered as statistically significant.

3. RESULTS

A total of 120 patients with seborrheic dermatitis were included in the study. The mean age of the patients was 29.5 ± 8.2 years, and 59% were male. According to disease severity, 66 (55%) patients were classified as having mild, 30 (25%) as moderate, and 24 (20%) as severe seborrheic dermatitis. BDI revealed normal scores in 85 (70.8%) patients, mild depression in 22 (18.4%), moderate depression in 9 (8.3%), and severe depression in 4 (3.8%). The family history of seborrheic dermatitis was positive in 34% of the patients. Patient characteristics are summarized in Table I. When evaluated in terms of disease localization, 65% of the patients had scalp and face involvement, 23% had only scalp involvement, and 12% had scalp, face, and trunk involvement. The factors that were reported to trigger attacks or increase disease severity were stress, certain food, lack of sleep, seasonal changes, alcohol consumption, nonmedical shampoos, and smoking (Table II). When the scale scores were evaluated, a significant positive correlation was found between SDASI and PSS ($r = 0.767$, $p < 0.001$), between SDASI and BDI ($r = 0.663$, $p < 0.001$), and between PSS and BDI ($r = 0.687$, $p < 0.001$) (Table III). However, there was no statistically significant correlation between the patients' scale scores and their age, disease duration, and body mass index (Table IV). Similarly, when the disease severity and PSS and BDI scores were compared between the genders, no statistically significant difference was observed ($p = 0.367$, $p = 0.667$, and $p = 0.838$, respectively).

Table I. *Patient characteristics*

Parameter		Value
Age, mean ± SD		29.5 ± 8.2
Gender, n (%)		
	Female	49 (40.8%)
	Male	71 (59.2%)
BMI, kg/m^2, mean ± SD		23.4 ± 3.7
Disease duration, months, mean ± SD		64.7 ± 65.9
SDASI, mean ± SD		4.6 ±2.8
Disease severity, n (%)		
	Mild	66 (55%)
	Moderate	30 (25%)
	Severe	24 (20%)
PSS, mean ± SD		28.7 ± 10.5
BDI, mean ± SD		9.4 ± 6.3

SD: Standard Deviation, BMI: Body Mass Index, SDASI: Seborrheic Dermatitis Area and Severity Index, PSS: Perceived Stress Scale, BDI: Beck Depression Inventory

Table II. *Disease aggravating factors*

Factor	n (%)
Stress	102 (85%)
Lack of sleep	38 (31.7%)
Certain food	56 (46.7%)
Alcohol consumption	22 (18.3%)
Smoking	15 (12.5)
Nonmedical shampoos	18 (15%)
Seasonal changes	34 (28.3%)

http://doi.org/10.5472/marumj.1195298
Marmara Med J 2022;35(3): 362-366

Table III. *Correlation between scales*

	r*	p
SDASI and PSS	0.767	<0.001
SDASI and BDI	0.663	<0.001
PSS and BDI	0.687	<0.001

**Pearson correlation coefficient, SDASI: Seborrheic Dermatitis Area and Severity Index, PSS: Perceived Stress Scale, BDI: Beck Depression Inventory*

Table IV. *Evaluation of the correlation between variables and scales*

	SDASI		PSS		BDI	
	r	p	r	p	r	p
Age	0.009	0.925	-0.015	0.870	0.024	0.793
Disease duration	0.005	0.974	0.274	0.066	0.002	0.989
BMI	0.025	0.854	0.076	0.408	0.022	0.814

SDASI: Seborrheic Dermatitis Area and Severity Index, PSS: Perceived Stress Scale, BDI: Beck Depression Inventory, BMI: Body Mass Index

4. DISCUSSION

The aim of this study was to define the relationship between disease severity, stress and depression in patients with seborrheic dermatitis. At the end of the study, it was found that there were significant correlations between disease severity and stress, between disease severity and depression and between stress and depression in patients with seborrheic dermatitis.

Since, the skin is the primary organ in the perception of beauty, diseases that involve the skin can be defined as the most important and disturbing health problem from the perspective of patients. In addition to being an external barrier against the environment, the skin can also be a reflector for internal alterations, both physical and psychological. It is known that psychological stress affects many skin diseases, including psoriasis, atopic dermatitis, alopecia areata, pruritus, chronic urticaria, hair loss, vitiligo, and acne [18-22]. The hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS) play a role in individuals' general physiological response to stress. Recent studies suggest that the skin has peripheral equivalents of these two main pathways in responding to psychological stress (19). In addition to the HPA and SNS axes, hormones and mediators, such as corticotropin-releasing hormone, adrenocorticotropic hormone, catecholamines, cortisol, prolactin, and substance P can be secreted from keratinocytes, melanocytes, fibroblasts, mast cells, immune cells, sebaceous glands, and nerve endings when the skin is under stress. Bidirectional connections between the skin and brain have an important function in regulating the release of these hormones and neurotransmitters [18,23,24]. In addition, neuroendocrine axes influence the healing process and symptoms of skin diseases by stimulating a series of cascades, including cytokine production, cell migration, and inflammation [19].

In addition to stress triggering many diseases, the presence of the disease itself can also cause psychological stress or certain psychiatric diseases. Especially, in skin diseases involving visible anatomical regions, as well as symptoms like loss of self-confidence and social phobia, psychiatric comorbidities such as anxiety, depression and somatoform disorders may accompany. Koo and Lee, who classified psychodermatological diseases, included seborrheic dermatitis in the category of psychophysiological disorders referring to the precipitation or exacerbation of a skin disease by psychological stress [25]. Many studies have shown the negative effect of stress on the severity of seborrheic dermatitis and frequency of disease attacks [12,26-29]. It is known that the quality of life is significantly affected in this chronic disease with recurrent attacks (30-33). In addition, there are studies showing an increase in anxiety and depression levels in direct proportion to the deterioration in the quality of life of patients with seborrheic dermatitis [30-32].

On completion of the current study, it was determined that as the severity of seborrheic dermatitis increased, the patients' perceived psychological stress levels and depression symptoms also increased. In a study by Aksoy et al., in which 50 patients with seborrheic dermatitis and 50 healthy controls were evaluated, the mean BDI scores of the patients were found to be 13.3, and a statistically significant difference was found compared to the control group [30]. In another study comparing 150 preoperative surgery patients and 150 patients with psychiatric disorders, it was reported that depressive patients had a high prevalence of seborrheic dermatitis [34]. Oztas et al., found an increased predisposition to depression in 30 patients with seborrheic dermatitis when compared with 30 healthy controls, although, the difference was not statistically significant [31]. In another study comparing a seborrheic dermatitis group with a healthy control group, it was shown that there was no significant difference in the risk of depression between the groups [35]. It is expected that skin diseases involving exposed body parts that are not covered by clothing during daily life psychologically affect patients to a greater extent. Misery et al., reported that patients with seborrheic dermatitis with facial involvement had higher depression scores, but there was no significant relationship between stress and depression [12]. In the current study, no significant relationship was observed between the anatomical localization of the affected seborrheic dermatitis areas and the depression levels of the patients.

It remains unclear whether stress aggravates seborrheic dermatitis or whether seborrheic dermatitis causes stress. It can be considered that stress not only triggers seborrheic dermatitis activation but is also involved in the disease cascade as a result of the chronic nature of the disease. Similarly, psychiatric comorbidities that may arise due to the psychological burden of the disease may vary depending on personal predisposition, genetics, and individual habits and experiences. In order to illuminate the complex relationship between the brain and skin, the patients' personal information, such as recent life events, social life, family relations, and sociocultural and economical status should be evaluated together with biomarkers and questionnaires.

According to the review of the literature, this is the first study to examine the severity of seborrheic dermatitis together with patients' perceived stress and depression findings and explore the relationship between these parameters. However, this study has certain limitations. First, stress and depression were

http://doi.org/10.5472/marumj.1195298
Marmara Med J 2022;35(3): 362-366

evaluated only with questionnaire-based scales. Receiving consultation from the psychiatry department and undertaking the follow-up of the patients in collaboration with a psychiatrist or psychologist would have optimized the assessment by confirming the diagnosis of psychiatric comorbidities. In addition, the inclusion of a control group and the measurement of inflammatory marker and hormone levels to evaluate the effects of stress on biochemical markers and HPA activity would have increased the power of the study.

Conclusion

The body and mind are entities that complement each other. Dermatological diseases can profoundly affect psychological well-being beyond the calculated disease severity scores and clinical findings. Therefore, the importance of mental well-being should be kept in mind in the management of seborrheic dermatitis and similar chronic skin diseases. Diagnostic questionnaires that can be applied in outpatient settings can help clinicians identify coexisting psychiatric disorders in patients with seborrheic dermatitis accompanied by stress and depressive symptoms. There is a need for a multidisciplinary approach involving a dermatologist, psychiatrist, and psychologist to create a successful treatment plan for these patients. To elucidate the skin-brain relationship in seborrheic dermatitis, further studies should be conducted with a large patient population, include a psychiatric examination and the measurement of biochemical markers, and perform comparisons between healthy controls and patients with other chronic dermatological diseases.

Compliance with the Ethical Standards

Ethical Approval: This study was approved by the Koc University Ethics Committee (Approval number: 2019.415.IRB1.064)

Financial Support: The authors have no relevant financial information to disclose.

Conflict of Interest: ES declares no conflicts of interest. EKG declares that she is on the Advisory Board of Novartis.

Author contribution: ES and EKG: Study design, ES: data collection, data analysis, writing the article.

REFERENCES

[1] Gupta AK, Madzia SE, Batra R. Etiology and management of seborrheic dermatitis. Dermatology 2004;208:89-93. doi: 10.1159/000076478.

[2] Sampaio AL, Mameri AC, Vargas TJ, Ramos-e-Silva M, Nunes AP, Carneiro SC. Seborrheic dermatitis. An Bras Dermatol 2011;86:1061-71; quiz 1072-4. doi: 10.1590/s0365.059.6201100.060.0002.

[3] Sanders MGH, Pardo LM, Franco OH, Ginger RS, Nijsten T. Prevalence and determinants of seborrhoeic dermatitis in a middle-aged and elderly population: the Rotterdam Study. Br J Dermatol 2018;178:148-53. doi: 10.1111/bjd.15908.

[4] Borda LJ, Wikramanayake TC. Seborrheic dermatitis and dandruff: a comprehensive review. J Clin Investig Dermatol 2015;3:10.13188/2373-1044.100.0019. doi: 10.13188/2373-1044.100.0019.

[5] Paulino LC. New perspectives on dandruff and seborrheic dermatitis: lessons we learned from bacterial and fungal skin microbiota. Eur J Dermatol 2017;27(S1):4-7. doi: 10.1684/ejd.2017.3038.

[6] Adalsteinsson JA, Kaushik S, Muzumdar S, Guttman-Yassky E, Ungar J. An update on the microbiology, immunology and genetics of seborrheic dermatitis. Exp Dermatol 2020;29:481-9. doi: 10.1111/exd.14091.

[7] Fink G. Stress: Concepts, Definition, and History George. In: Neuroscience and Biobehavioral Psychology. Amsterdam, The Netherlands: Elsevier, 2017.

[8] Jafferany M, Franca K. Psychodermatology: Basics Concepts. Acta Derm Venereol 2016;96:35-7. doi: 10.2340/00015.555.2378.

[9] Colavincenzo ML, Granstein RD. Stress and the skin: a meeting report of the Weill Cornell Symposium on the Science of Dermatology. J Invest Dermatol 2006;126:2560-1. doi: 10.1038/sj.jid.5700565.

[10] Baker N, Billick SB. Psychiatric consequences of skin conditions: Multiple case study analysis with literature review. Psychiatr Q 2022;93:841-7. doi: 10.1007/s11126.022.09991-6.

[11] Magin PJ, Pond CD, Smith WT, Watson AB, Goode SM. A cross-sectional study of psychological morbidity in patients with acne, psoriasis and atopic dermatitis in specialist dermatology and general practices. J Eur Acad Dermatol Venereol 2008;22:1435-44. doi: 10.1111/j.1468-3083.2008.02890.x.

[12] Misery L, Touboul S, Vinçot C, et al. Stress et dermatite séborrhéique. Ann Dermatol Venereol 2007;134:833-7. doi: 10.1016/s0151-9638(07)92826-4.

[13] Cömert A, Bekiroglu N, Gürbüz O, Ergun T. Efficacy of oral fluconazole in the treatment of seborrheic dermatitis: a placebo-controlled study. Am J Clin Dermatol 2007;8:235-8. doi: 10.2165/00128.071.200708040-00005.

[14] Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav 1983;24:385-96.

[15] Eskin M, Harlak H, Demirkıran F, Dereboy C. Algılanan stres ölçeğinin Türkçe'ye uyarlanması: güvenirlik ve geçerlik analizi. Yeni Symposium Journal 2013;51:132-40.

[16] Hisli N. Beck Depresyon Envanteri'nin geçerliği üzerine bir çalışma. Türk Psikoloji Dergisi 1988;7:3-13.

[17] Beck AT, Rush AJ, Shaw BF, Emery G. Cognitive Therapy of Depression. New York: Guilford Press, 1979.

[18] Alexopoulos A, Chrousos GP. Stress-related skin disorders. Rev Endocr Metab Disord 2016;17:295-304. doi: 10.1007/s11154.016.9367-y.

[19] Pondeljak N, Lugović-Mihić L. Stress-induced interaction of skin immune cells, hormones, and neurotransmitters. Clin Ther 2020;42:757-70. doi: 10.1016/j.clinthera.2020.03.008.

[20] Lugović-Mihić L, Ljubesić L, Mihić J, Vuković-Cvetković V, Troskot N, Situm M. Psychoneuroimmunologic aspects of skin diseases. Acta Clin Croat 2013;52:337-45.

[21] Stewart TJ, Schut C, Whitfeld M, Yosipovitch G. Cross-sectional study of psychological stress and skin symptoms in Australian university students. Australas J Dermatol 2018;59:e82ee84.

[22] Bin Saif GA, Alotaibi HM, Alzolibani AA, et al. Association of psychological stress with skin symptoms among medical students. Saudi Med J 2018;39:59e66.

[23] Chen Y, Lyga J. Brain-skin connection: stress, inflammation and skin aging. Inflamm Allergy Drug Targets 2014;13:177-90. doi:10.2174/187.152.8113666.140.522104422.

[24] Slominski AT, Zmijewski MA, Skobowiat C, Zbytek B, Slominski RM, Steketee JD. Sensing the environment: regulation of local and global homeostasis by the skin's neuroendocrine system. Adv Anat Embryol Cell Biol 2012;212:v,vii,1-115. doi: 10.1007/978-3-642-19683-6_1.

[25] Koo JYM, Lee CS. General approach to evaluating psychodermatological disorders. In: Koo JYM, Lee CS, eds. Psychocutaneous medicine. New York: Marcel Dekker, 2003: 17-28.

[26] Sanders MGH, Pardo LM, Ginger RS, Kiefte-de Jong JC, Nijsten T. Association between diet and seborrheic dermatitis: a cross-sectional study. J Invest Dermatol 2019;139:108-14. doi:10.1016/j.jid.2018.07.027.

[27] Bae JM, Ha B, Lee H, Park CK, Kim HJ, Park YM. Prevalence of common skin diseases and their associated factors among military personal in Korea. A cross-sectional study. J Korean Med Assoc 2012;27:1248-54.

[28] Lancar R, Missy P, Dupuy A, et al. Risk factors for seborrhoeic dermatitis flares: case-control and case-crossover study. Acta Derm Venereol 2020;100:adv00292. doi: 10.2340/00015.555.3661.

[29] Park SY, Kwon HH, Min S, Yoon JY, Suh DH. Clinical manifestation and associated factors of seborrheic dermatitis in Korea. Eur J Dermatol 2016;26:173-6. doi: 10.1684/ejd.2015.2706.

[30] Aksoy M, Ozkorumak E, Bahadır S, Yayli S, Aksu Arica D. Quality of life, anxiety and depression levels in patients with seborrheic dermatitis. Turkderm 2012;46:39–43.

[31] Oztas P, Calıkoglu E, Cetin I. Psychiatric tests in seborrhoeic dermatitis. Acta Derm Venereol 2004;85:68–9.

[32] Doğramacı AÇ, Havlucu DY, Savaş N. Seboreik dermatitli hastalarda yaşam kalite değerlendirmesi. Türk Dermatoloji Dergisi 2008;2:99-102.

[33] Peyrí J, Lleonart M; Grupo español del Estudio SEBDERM: Clinical and therapeutic profile and quality of life patients with seborrheic dermatitis. Actas Dermosifiliogr 2007;98:476-82.

[34] Maietta G, Fornaro P, Rongioletti F, Rebora A. Patients with mood depression have a high prevalence of seborrhoeic dermatitis. Acta Derm Venereol 1990;70:432-4.

[35] Cömert A, Akbaş B, Kılıç EZ, et al. Psychiatric comorbidities and alexithymia in patients with seborrheic dermatitis: a questionnaire study in Turkey. Am J Clin Dermatol 2013;14:335-42. doi:10.1007/s40257.013.0019-7.

MARMARA
MEDICAL JOURNAL

Case Report

https://dergipark.org.tr/tr/pub/marumj

A Viper bite in an urban area : A case report

Hasan DEMIR[1], Aykut OZKAN[2], Melis EFEOGLU SACAK[1], Nurseli BAYRAM[1], Boran ISIN UNAL[1], Cansu TIRIS[1], Murat GOL[1], Arzu DENIZBASI[2]

[1] Department of Emergency Medicine, Marmara University Research and Training Hospital,Pendik, Istanbul, Turkey.
[2] Department of Emergency Medicine, School of Medicine, Mamara University, Pendik, Istanbul, Turkey.

Corresponding Author: Melis EFEOGLU SACAK
E-mail: melisefeoglu@gmail.com

Submitted: 19.04.2022 **Accepted:** 04.06.2022

ABSTRACT

Envenomous viper snakes are not natural inhabitants of Istanbul, Turkey. The city is crowded and lacks an appropriate area for wild life. Viper snakes are mostly found in southern and middle rural Anatolia in Turkey. To our knowledge, viper bites have not been reported in our urban area previously. Here, we report a envenomous snakebite case, treated with polyvalent antivenom successfully. The snake was identified as *Vipera Pontica*, by the National Poisons Center.

Keywords: Viper, Snakebite, Venoms, Antivenins

1. INTRODUCTION

Only a small percentage of the 2,700 snake species in the world are potentially lethal. Among the venomous species, the family of Viperidae is found in the Old World: Africa, Europe, and Asia. In Turkey, viper snakes are primarily found in southern and middle rural Anatolia in Turkey. As a result, venomation of vipera was predominantly observed in Turkey's rural areas. [1,2].

Viper envenomation can cause different variety of complications from local swelling to systemic shock syndromes of anaphylaxis, cardiovascular collapse, hematotoxic and neurotoxic problems [3]. Antivenom treatment is the mainstay of the envenomous snakebites. Antivenom is the immunoglobulin of the equine or ovine, produced against the venom of the snakebite [4, 5]. The aim of the antivenom treatment is to neutralize the toxin and to protect the patient from toxin-induced neurotoxicity, hematotoxicity, systemic cardiovascular collapse and shock, all of which lead to death.

To our knowledge, no previous cases of viper envenomation have been reported in our city. The goal of this case report was to describe a unique vipera bite in Istanbul's metropolitan area and to underline the need of initiating snakebite treatment in the emergency room. In a city where snakebite is uncommon but potentially lethal, this case emphasizes the significance of early detection and details critical stages in envenomation management.

2. CASE REPORT

A 71-year-old male was admitted to the Emergency Department of our institution located in the urban area, nearby settled and populated living areas in the district of Pendik, Istanbul with the complaint of a snakebite. The patient reported that he was admitted to hospital right after a snake has bitten his right-hand middle finger at the garden of his home, which was situated in Pendik, Istanbul, close to our institution. He was admitted to emergency service policlinics, where he waited for consultation for about 30 minutes. When he was in the examination room, his next of kins brought the dead snake for identification. The patient had a medical history of hypertension, coronary artery disease, and diabetes mellitus and had been on drug therapy for twenty years. At presentation he was in good clinical condition and able to walk, talk, and cooperate. Vital signs at presentation were as follows: BP 133/80 mmHg, HR 98 bpm, Temperature 36.2 °C, SO_2 98% on room air, Glasgow Coma Scale (GCS) 15. Minor swelling and edema on the middle finger

How to cite this article: Demir H, Ozkan A, Efeoglu Sancak M, Bayram N, Isın Unal B, Tiris C, Gol M, Denizbasi A. A Viper bite in an urban area : A case report. Marmara Med J 2022; 35(3): 367-371, doi: 10.5472/marumj.1191161

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1191161
Marmara Med J 2022;35(3): 367-371

of his right hand with a subtle mark of the snake fangs were noted on inspection (Figure I). He was admitted to critical care area for monitoring and further evaluation immediately. His electrocardiogram (ECG) was within normal limits without any change from his previous medical records. There were no physical and neurological symptoms or signs except the edema on the hand. We reported and consulted the case to the National Poisons Center (NPC) on the phone (Telephone no: 114) for documentation and discussed online via the WhatsApp mobile application with the Toxicology Study Group of the Emergency Medicine Association of Turkey (EMAT) which consists of nationwide experts on the management of toxicology cases. In the meanwhile, we requested polyvalent snake antivenom from the poison center on the Anatolian side of Istanbul, which our institution is located. The snake was identified as "*Vipera pontica*" according to the consultations.

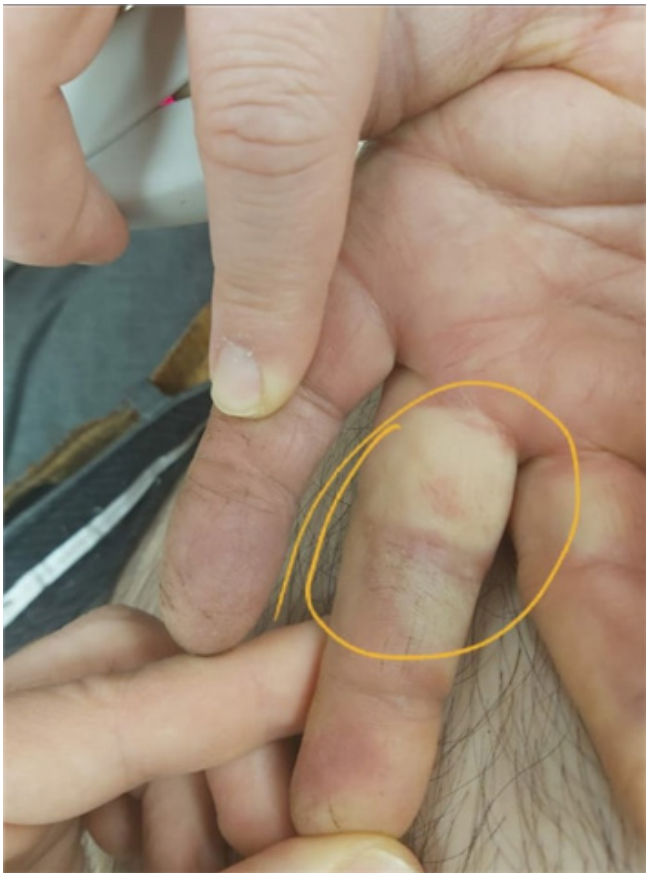

Figure I. *Right hand of the patient with snake fang mark at presentation to emergency department.*

The diagnostic blood tests including complete blood count, electrolytes, renal and hepatic function tests, blood coagulation tests and urine analysis were all within normal range. In the 30th minute of admission into the critical care area, the patient's clinical condition deteriorated. He became hypotensive and hypoxic, and the vital signs were noted as follows : BP 95/60 mmHg, HR 110 bpm, Temperature 36.2 °C, SO2 87% on room air and GCS 13 with symptoms of vomiting and swelling in the hand. His clinical stage had progressed from Grade 1 to Grade 3 of the Snakebite Severity Grading (Table I) [6]. To treat the shock state, noradrenaline infusion 1 mg/kg/hr was started in addition to fluid therapy, tetanus vaccination, and ceftriaxone 1 gr administered intravenously as well.

Table I. *Snakebite severity grading scale. 10 ml= 1 vial [6].*

Severity	Manifestations	Amount of antivenom recommended
0 (No envenomation)	Local or systemic signs or symptoms absent	-
1 (Minimal)	Local swelling, absence of systemic signs, normal labaratory findings	2 – 4 vial
2 (Moderate)	Swelling extending past bite site (15 – 30 cm), more than one systemic sign or symptom, abnormal laboratory	5 – 9 vial
3 (Severe)	Marked (30 cm) swelling, tissue loss, multiple or severe systemic symptoms, immediate systemic signs, rapid progression of symptoms	10-15 vial
4 (Very severe)	Rapid development of local reaction, ecchymoses, necrosis, sweeling severe enough to obstruct blood or lymph flow	>15 vial

A type of polivalent snake antivenom serum consisting of different venoms of viper species was readily available in NPC. 4 vials of antivenom, the whole amount that NPC stored, was obtained in 1 hour after demand and the antivenom treatment was started as soon as it arrived to the department. The required dose of the venom was calculated as 10-15 vials according to the clinical state of the patient, but the only 4 vials of antevenom were available in NPC. A slower infusion rate was used to prevent an anaphylactic reaction for the first 30 minutes and then the remaining dose was completed with the infusion rate as recommended by the NPC. Following the antivenom therapy, hypoxia and hypotension of the patient resolved dramatically. He was then transferred to the intensive care unit for hospitalization.

On admission to the intensive care unit, the vital signs were as follows : BP 120/60 mmHg, HR 95 bpm, Temperature 36.1°C, SO2 94% on room air, GCS 15. His ECG and physical and neurological examinations were normal except for the edema on the right hand. His shock state did not recur during the hospitalization period. After 24 hours of monitorization, he was handed over to ward from the intensive care unit and discharged to home afterwards. Patient's laboratory values remained within normal range during the hospital stay.

http://doi.org/10.5472/marumj.1191161
Marmara Med J 2022;35(3): 367-371

3. DISCUSSION

In Europe, the estimated yearly prevelance of snakebite is 0.4 to 1.1 per 100,000 population with four casualties each year. In a review including 5501 cases in Europe, France was the leading country in the number of reports (Figure II) [3]. In Europe, *Vipera berus*, *V. aspis*, and V. *ammodytes* are the most common snake species of the Viper genus. Other than these vipers, *Macrovipera lebetina*, *Montivipera xanthina*, and *Vipera latastei* are clinically relevant in the southern parts of Europe [3]. Most recently, a snakebite envenoming case, most probably with *Vipera latastei*, was reported from Portugal, which was potentially fatal [7]. In Istanbul which is a crowded and entirely inhabited city, snakebites are very unlikely to occur such as the other European urban areas [3, 8]. Parks and green-zone regions are not large enough to bear wildlife species such as vipers in the district of Pendik. This zone in Istanbul mainly consists of factories, manufactures, and chemical industry plants. The most probable reason for this viper case may be the transfer of the viper snake unintentionally from other regions with other commercial goods.

Figure II. *European map, with the distribution and relative number of cases according to the results of a systematic review [3].*

Although, there are many signs and symptoms following viper snakebites, the most severe complications are neurotoxicity and coagulopathy. In our patient, no coagulopathy had developed, yet his mental status deteriorated but dramatically improved following the antivenom treatment. The other severe consequences include: loss of a limb, prolonged dysfunction of the limb, prolonged length of hospitalization, admissions with later complications; particularly in children and in cases where there is delayed or inadequate treatment [9]. Acute lower extremity ischemia following a snakebite was reported recently [10]. In case of late hospital admissions, the clinician should be aware that tetanus may have complicated the clinical course [11].

Management of the snakebite starts with conventional first aid [12]. Calming the patient down, immobilization of the bitten body area, application of pressure over the bitten area, and urgent transfer of the patient to a health care center where he can receive proper antivenom treatment are the most critical initial steps. If the bitten site is on facial, abdominal or thoracic regions, the patient will not benefit from any splinting. In that case the spreading of the venom will be very fast and the patient may require early rapid sequence intubation before the transfer [4, 12]. During the transfer to the hospital, the patients should be closely monitored because these patients may deteriorate very rapidly depending on the venom type, regardless of the initial clinic.

Antivenom dosing and the indication of the clinical staging are still controversial issues although this therapy has been used for decades [5, 13, 14]. In some geographical regions, polyvalent (e.g. polyspecific) antivenoms are very important to apply. Since, these antivenoms include more than one specific antivenom, this therapy has the risk of side effects [14]. If the geographical region has only one envenomous species, monovalent antivenoms may be used; however, this is a seldom setting in clinical practice. In Turkey, polyvalent antivenoms are used in the treatment [5]. Antivenom therapy is used in patients with systemic signs and symptoms such as hypotension, hypoperfusion, cardiovascular collapse, shock, myotoxicity, neurotoxicity, bleeding and coagulation disorders, massive local swelling of the extremities with a risk for compartment syndrome, and the risk of necrosis of the extremes of the limbs. Some of the local and systemic symptoms that warrant administering antivenom are listed in Table II [9]. Laboratory tests may be an early indicator for the antivenom treatment. Leukocytosis (>20,000 WBC), severe anemia, bleeding disorders and abnormal coagulation blood tests, elevated hepatic enzymes, abnormal renal tests, myoglobinuria, hematuria, oliguria are often present [3, 9, 12]. In our patient, labaratory tests remained within normal range.

Antivenom treatment is given intravenously due to the poor absorbability via subcutaneous or intramuscular routes. While administering antivenom treatment, a physician must be alert for any signs of anaphylaxis, regardless of the rate of administration or the dilution of the antivenom, and be ready to start vasopressor therapy, as well as to discontinue the antivenom infusion; however, these cases have severely unfavorable clinical outcomes [3, 5]. The proposed dosing algorithm is the same for all age groups and the initial dosage may be repeated after about six hours.

Each patient should be monitored at the hospital even after a successful antivenom therapy at least for 48 hours because the redistribution of the venom from the snakebite site continues; furthermore, the risk for late-onset allergic reactions of the treatment is possible [4, 13].

Table II. *Site of action of venom, clinical features, testing, and management [9].*

Site of Action and/ or Systems Effected	Signs and Symptoms	Ancillary tests	Management
Local tissue	Fang marks, pain, swelling, bruising, ecchymoses, tissue necrosis (late), lymphadenopathy		Antivenom Compartment syndrome therapy
Neurological	Ptosis, diplopia, dysphagia, dyspne, paralysis, paresthesia	Neostigmine trial	Antivenom, anticholinesterase, support ventilation
Hematological	Epistaxis, bruising and ecchymoses, increase in clinical evident bleeding (melena, hematuria, etc) ecchymoses	Thrombocytopenia, anemia, prolonged aPTT and INR, decreased fibrinogen, increased D – dimer	Antivenom, blood products transfusion
Circulation	Hypotension, tachycardia, shock, altered mental state	CVP and urine monitoring	Antivenom Isotonic fluids Vasopressor therapy
Rhabdomyolysis with renal insufficiency	Red or brown urine, Oliguria	Urine dipstick, increased serum CK, potassium, BUN, creatinine	Fluid therapy Hemodialysis

aPTT activated partial thromboplastin clotting time, INR international normalized ratio, CVP central venous pressure,CK creatine kinase, BUN blood urea nitrogen

Conclusion

Viper envenomation is a rare emergency in urban European cities. Prompt recognition and medical envenomation and supportive treatment in a timely manner is crucial. Hospital preparedness for medical envenomation problems and for antivenom maintenance should be considered on a regular basis.

Compliance with Ethical Standards

Patient Consent: The patient gave his consent for images and other clinical information relating to his case to be reported in a medical publication.

Conflict of interest: The authors have no conflicts of interest to declare.

Funding: No financial support was received for this study.

Authors' Contributions: AD, NB and HD: Conceived the case report, AO, NB, BIU, CT and MG: Managed the patient, contributed materials, data, AD and MES: Drafted the manuscript and all authors contributed substantially to its revision. MES takes the responsibility for the paper as a whole.

REFERENCES

[1] Bozkurt M, Kulahci Y, Zor F, Kapi E. The management of pit viper envenomation of the hand. HAND 2008;3:324-31. doi: 10.1007/s11552.008.9114-2.

[2] Ertem K. Venomous snake bite in Turkey. First aid and treatment. Eur J Gen Med 2004;1:1-6. doi: 10.29333/ejgm/82236.

[3] Paolino G, Di Nicola MR, Pontara A, et al. Vipera snakebite in Europe: a systematic review of a neglected disease. JEADV 2020;34:2247-60. doi : 10.1111/jdv.16722.

[4] Warrell DA. Venomous bites, stings, and poisoning: an update. Infect Dis Clin N Am 2019;33:17-38. doi: 10.1016/j.idc.2018.10.001.

[5] Gülen M , Satar S , Yeşiloğlu Ö , İnce Ç , Esen Cİ , Acehan S. Comparison of two types of polyvalent snake antivenom used in treatment. Cukurova Med J 2020; 45:1230-7. doi: 10.17826/cumj.737922.

[6] Scharman EJ, Noffsinger VD. Copperhead snakebites: Clinical severity of local effects. Ann Emerg Med 2001; 38:55-61. doi : 10.1067/mem.2001.116148.

[7] Lamas A, Fragoso J, Ferreira M, Cipriano A, Alves R. Case report: Anaphylactoid shock associated with snakebite envenoming in Portugal. Am J Trop Med Hyg 2021;106: 342-44. doi: 10.4269/ajtmh.21-0671.

[8] Chippaux JP. Epidemiology of snakebites in Europe: a systematic review of the literature. Toxicon 2012; 59:86-99. doi: 10.1016/j.toxicon.2011.10.008.

[9] White J. Snakebites worldwide: Management. www.uptodate.com Literature review current through: Jan 2021. (Access date : 03.13.2021)

[10] Lu ZY, Wang XD, Yan J, Ni XL, Hu SP. Critical lower extremity ischemia after snakebite: A case report. World J Clin Cases 2021;9:7857-62. doi:10.12998/wjcc.v9.i26.7857.

[11] Habib AG. Tetanus complicating snakebite in northern Nigeria: clinical presentation and public health implications.

Acta Trop 2003 Jan;85(1):87-91. doi: 10.1016/s0001-706x(02)00234-6. PMID: 12505187.

[12] World Health Organization. Venomous snakes distribution and species risk categories. Geneva, WHO: 2016. (Accessed: 03.13.2021).

[13] Persson H. Envenoming by European vipers antivenom treatment – influence on morbidity. Prz Lek 2001;58:223-5.

[14] Lamb T, de Haro L, Lonati D, Brvar M, Eddleston M. Antivenom for European *Vipera* species envenoming. Clin Toxicol 2017; 55:557-68. doi: 10.1080/15563.650.2017.1300261.

MARMARA
MEDICAL JOURNAL

Case Report

https://dergipark.org.tr/tr/pub/marumj

Radial arterial thrombosis in COVID-19: A case report

Nurdan SENTURK DURMUS[1], Furkan Cuma CICEK[2], Cagla EYUPLER[2], Can OMUR[3], Ozge YETGINOGLU[2], Koray AK[4], Derya KOCAKAYA[3]

[1] Division of Geriatrics, Department of Internal Medicine, School of Medicine, Marmara University, Istanbul, Turkey.
[2] Department of Internal Medicine, School of Medicine, Marmara University, Istanbul, Turkey.
[3] Department of Chest Diseases and Intensive Care, School of Medicine, Marmara University, Istanbul, Turkey.
[4] Department of Cardiovascular Surgery, School of Medicine, Marmara University, Istanbul, Turkey.

Corresponding Author: Nurdan SENTURK DURMUS
E-mail: nurdan_69@hotmail.com

Submitted: 17.03.2022 **Accepted:** 25.06.2022

ABSTRACT

Thrombosis due to hypercoagulable state is an important cause of morbidity and mortality in coronavirus disease 2019 (COVID-19). . Increased D-dimer levels are an important marker of the presence and risk of thrombosis. In this report, we present that a 59-year-old male patient developed thrombosis in the distal radial arteries despite normal D-dimer level. The patient was treated with enoxaparin, iloprost infusion, and cilostazol. This case should lead us to be very careful that people diagnosed with COVID-19 with normal D-dimer levels may also have thrombosis.

Keywords: COVID-19, Radial artery, Thrombosis

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) spread all over the world in a short time after the first cases were reported from China in December 2019 [1] [1]. So far, COVID-19 has caused approximately 96 million cases and over 1.8 million deaths worldwide as of January 2021 [2]. The COVID-19 clinical spectrum ranges from asymptomatic to severe manifestations that are sepsis, acute respiratory distress syndrome (ARDS), coagulopathy, and death [1-3]. COVID-19 causes arterial and venous thrombosis with the hypercoagulable state, which is an elevation in D-dimer, prothrombin time, and fibrinogen levels [4,5].

In COVID-19, according to current literature, patients with arterial thrombosis were usually male, older, and have accompanying comorbidities [6-8]. COVID-19 related cumulative incidence of thrombotic events has been reported as 49% including 37% venous, and 3.8% of arterial thrombosis in critically ill intensive care unit (ICU) patients [3]. To date, arterial thrombosis associated with COVID-19 has occurred in many vessels such as the aorta, carotid artery, central nervous system arteries, coronary, superior mesenteric, jejunal, splenic, renal, lower, and upper extremity arteries [6-8].

In this report, we describe an acute radial arterial thrombosis in a patient receiving prophylactic anticoagulation.

2. CASE REPORT

A 59-year-old male patient with a history of hypertension and chronic obstructive pulmonary disease was admitted to our emergency department with cough and shortness of breath. On physical examination, he was hemodynamically stable and had polypnea (30 cycles per minute with 90% of blood oxygen saturation on room air). The Rox index (blood oxygen saturation/Fraction of inspired oxygen x respiratory rate) of the patient was 4.62. In initial laboratory studies, severe lymphopenia (500 x10^3/μL), elevated C reactive protein (CRP) (69.3 mg/L) and hyperfibrinogenemia (627 mg/dL) were seen. Other blood tests result such as leukocyte count, D-dimer level, prothrombin time, activated partial thromboplastin time and platelet count revealed normal limits (Table I). Nasopharyngeal swab for COVID-19 was positive. Thorax computed tomography (CT) was performed and compatible with COVID-19 pneumonia (CO-RADS – 5 [9]-scattered localized ground glass infiltration

How to cite this article: Senturk Durmus N, Cicek FC, Eyupler C, et al. Radial arterial thrombosis in COVID-19: A case report. Marmara Med J 2022; 35(3): 372-375, doi: 10.5472/marumj.1191199

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1191199
Marmara Med J 2022;35(3): 372-375

areas in both lungs and occasionally accompanying consolidated areas) (Figure 1). Based on the COVID-19 treatment policy of our hospital, the patient initiated on favipiravir, and prophylactic enoxaparin (4.000 IU once daily) on admission. As the saturation level was below 92, dexamethasone, and oxygen supportive treatment was started. The patient had severe COVID-19 pneumonia based on the COVID severity index [10]. Convalescent plasma treatment was given three times to the patient whose respiratory distress and oxygen requirement increased during the follow-up. On the 11th day of the hospital admission and the diagnosis of COVID-19, the cardiovascular surgery department was consulted when there was blue-discoloration and heat loss in the 3rd and 4th fingers of the right hand (Figure 1). In the laboratory tests performed on the day of thrombosis, the lymphocyte count increased (800 x10^3 /µL), fibrinogen level decreased (273 mg/dl), and platelet count, CRP, prothrombin time, activated partial thromboplastin time, and D-dimer levels were measured as normal. Sinus rhythm was seen on the electrocardiogram, there were no ischemic changes. The patient has no history of arterial intervention. In upper extremity CT angiography, major vascular structures were normal, digital arteries of the right 3rd and 4th fingers were found to be severely occluded of the level of proximal interphalangeal joint (Figure1). As advised by the consultant; the medical treatment was arranged as 1mg/kg twice daily enoxaparin, warming the right hand, administering intravenous iloprost infusion for 5 days, and additional cilostazol (phosphodiesterase inhibitor) twice daily. The cilostazol treatment was planned to continue for at least three months. Surgical embolectomy was not considered. After the iloprost infusion was completed, the fingers of the patient returned to normal color (Figure 1). After 1 month of hospitalization, the patient was discharged after resolution of his pulmonary symptoms and signs.

Table I. *Laboratory results of the patient on the days of admission to the hospital, thrombosis, and the discharged from the hospital*

	Admission	Thrombosis	Discharge
White blood cell (x10^3 /L)	7000	16.100	13.800
Neutrophils (#)	6000	14.600	12.300
Lymphocytes (#)	500	800	600
Neutrophils (%)	85.9	90.6	89.7
Lymphocytes (%)	6.9	4.7	4.4
Hemoglobin (g/dL)	15	14.6	13.8
Hematocrit (%)	41.8	41.7	41.7
Mean cell volume (fL)	88.8	89.9	90.3
Platelet (x10^3 /L)	230	400	234
Lactate dehydrogenase (U/L)	673	540	291
C-reactive protein (mg/L)	69.3	1.9	0.70
Fibrinogen (mg/dL)	627	273	228
D-dimer (mg/L)	0.42	0.21	0.27
Protrombin Time (s)	16.4	14.3	13.9
INR	1.24	1.09	1.05
aPTT (s)	29.9	25.2	25.9

INR: International normalized ratio, aPTT: Activated partial thromboplastin time

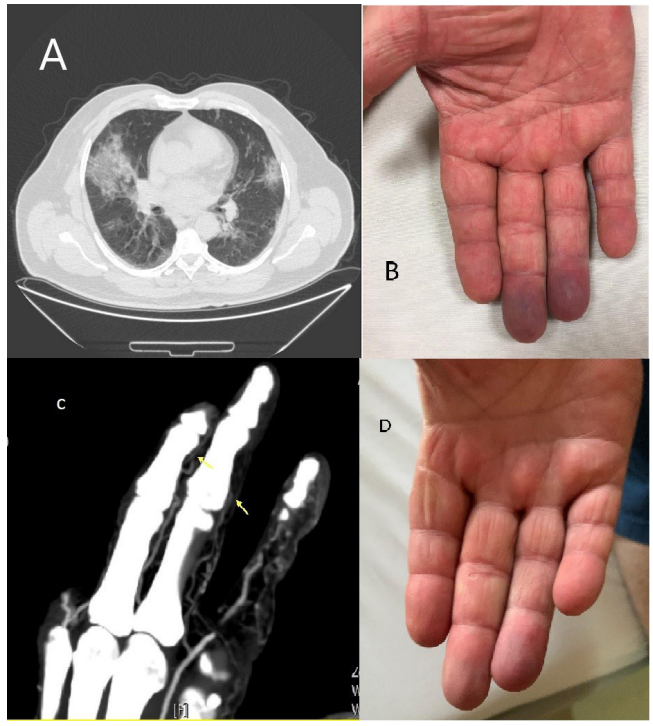

Figure 1. *Selected computed tomography (CT) of the patient. CT of the thorax (A) and upper extremity angiography (C). (B) view of the patient's right hand on the day of thrombosis, (C) an abrupt occlusion of the radial artery is evident on angiography, yellow arrow indicates the thrombosis level and (D) view of the patient's right hand on the day of discharge from the hospital.*

3. DISCUSSION

Many studies have reported a relationship between COVID-19 and hypercoagulable status [1,4,5]. Besides, elevated D-dimer levels were associated with mortality in COVID-19 patients [11]. Although, venous thrombosis is the most common, the risk of arterial thrombosis is also increased [3]. In this case, we have observed COVID-19 may have acute limb ischemia even the D-dimer level is normal. Cholesterol embolism, which will be considered in the differential diagnosis, was excluded because the major vascular vessels were normal in CT angiography.

In the case reports and series published to date, thrombosis has generally been reported in the great arteries [6-8]. In a study in the United States, thrombosis in the ulnar and radial arteries was reported only in 6.1% (3/49) patients [6]. Acute arterial occlusion is a vascular emergency, can lead to life or limb-threatening ischemia [6-8]. It may cause serious clinical findings, poor prognosis, and mortality [6,8,12,13]. In the study of Bellosta et al., only 1 patient had upper limb ischemia, and this patient died due to the development of ARDS after lower limb occlusion in the later period. [14]. Digital ischemia was observed in 2 patients in the intensive care unit, and one of these patients was discharged to the acute care rehabilitation center while the other had mortality, and concurrent venous thrombosis was also demonstrated in these two cases [15]. In another case report, a

http://doi.org/10.5472/marumj.1191199
Marmara Med J 2022;35(3): 372-375

34-year-old female patient reported stroke and brachial artery thrombosis at in same time [16]. Considering all these, it should be kept in mind that thrombosis may be seen in other organs concurrently because of coagulopathy. In COVID-19, according to published data, patients with arterial thrombosis were male, older, and have comorbidities, like our patient [6-8]. In our case, there was thrombosis in the radial artery in a 59-year-old male patient with comorbidities. Our patient was discharged home 2 weeks after thrombosis. There was no thrombosis in another organ or extremity during follow-up.

Connolly et al., reported palmar vein thrombosis in a female patient [17]. But, this patient is a carrier of factor V Leiden mutation. On the other hand, our patient did not have a known thrombophilia condition, thrombosis occurred in his digital arteries due to COVID-19 infection.

Both the COVID-19 disease itself and the thrombotic complications elevate D-dimer levels [5,6,14]. Etkin et al., reported that all 49 patients with thrombosis had high D-dimer levels [6]. In a series of 20 patients, the mean D-dimer was 2200 ng / mL [14]. In another study with 388 patients, only one patient with subsegmental pulmonary embolism had a D-dimer level in the normal range [18]. In our case, there was not any elevation in the D-dimer level that was observed both at the time of diagnosis, during the development of thrombosis, and follow-up.

Conclusion

This case should lead us to be very careful that people diagnosed with COVID-19 with normal D-dimer levels may also have thrombosis.

Compliance with Ethical Standards

This research was conducted ethically by following per under Helsinki World Medical Association Declaration.

Patient Consent: The patient gave his consent for images and other clinical information relating to his case to be reported in a medical publication.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Funding Sources: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authora' Contributions: NSD, FCC, CE, CO, OY, and DK: Collected to data, NSD, KA, and DK: Wrote the manuscript, DA: Supervised all steps of the article preparation. All authors contributed the case data. The final version was approved by all authors.

REFERENCES

[1] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395(10223):497-506. doi:10.1016/s0140-6736(20)30183-5

[2] Center JHUC-R. https://www.worldometers.info/coronavirus/countries-where-coronavirus-has-spread/. (Accessed 16 March 2021). 2021.

[3] Klok FA, Kruip M, van der Meer NJM, et al. Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: An updated analysis. Thromb Res 2020;191:148-50. doi:10.1016/j.thromres.2020.04.041

[4] Han H, Yang L, Liu R, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clin Chem Lab Med 2020;58:1116-20. doi:10.1515/cclm-2020-0188

[5] Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020;18:844-7. doi:10.1111/jth.14768

[6] Etkin Y, Conway AM, Silpe J, et al. Acute Arterial Thromboembolism in Patients with COVID-19 in the New York City Area. Ann Vasc Surg 2021;70:290-4. doi:10.1016/j.avsg.2020.08.085

[7] Cheruiyot I, Kipkorir V, Ngure B, Misiani M, Munguti J, Ogeng'o J. Arterial Thrombosis in Coronavirus Disease 2019 Patients: A Rapid Systematic Review. Ann Vasc Surg 2021;70:273-81. doi:10.1016/j.avsg.2020.08.087

[8] Putko RM, Bedrin MD, Clark DM, Piscoya AS, Dunn JC, Nesti LJ. SARS-CoV-2 and limb ischemia: A systematic review. J Clin Orthop Trauma 2020. doi:10.1016/j.jcot.2020.11.018

[9] Prokop M vEW, van Rees Vellinga T, Quarles van Ufford H. CO-RADS: A categorical CT assessment scheme for patients suspected of having COVID-19-Definition and Evaluation 2020;296:E97-e104. doi:10.1148/radiol.202.020.1473

[10] Haimovich AD, Ravindra NG, Stoytchev S, et al. Development and validation of the quick covid-19 severity index: a prognostic tool for early clinical decompensation. Ann Emerg Med 2020;76:442-53. doi:10.1016/j.annemergmed.2020.07.022

[11] Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395(10229):1054-62. doi:10.1016/s0140-6736(20)30566-3

[12] Vulliamy P, Jacob S, Davenport RA. Acute aorto-iliac and mesenteric arterial thromboses as presenting features of COVID-19. Br J Haematol 2020;189:1053-4. doi:10.1111/bjh.16760

[13] Wengerter SP, Wengerter KR, Masoudpoor H, et al. Acute aortoiliac and infrainguinal arterial thrombotic events in four patients diagnosed with the novel coronavirus 2019. J Vasc Surg Cases Innov Tech 2020;6:698-702. doi:10.1016/j.jvscit.2020.09.011

[14] Bellosta R, Luzzani L, Natalini G, et al. Acute limb ischemia in patients with COVID-19 pneumonia. J Vasc Surg 2020;72:1864-72. doi:10.1016/j.jvs.2020.04.483

[15] Schultz K, Wolf JM. Digital ischemia in COVID-19 patients: Case Report. J Hand Surg Am. 2020;45:518-22. doi:10.1016/j.jhsa.2020.04.024

[16] Shah H, Iyer A, Zaghlol R, Raparla S. Case Report: Multiple strokes and digital ischemia in a young COVID-19 patient. Am J Trop Med Hyg 2020. doi:10.4269/ajtmh.20-1101

[17] Connolly A, Walsh S, Arya R. Palmar digital vein thrombosis in a patient with COVID-19. Clin Exp Dermatol 2021;46:183-4. doi:10.1111/ced.14442

[18] Lodigiani C, Iapichino G, Carenzo L, et al. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. Thromb Res 2020;191:9-14. doi:10.1016/j.thromres.2020.04.024

MARMARA
MEDICAL JOURNAL

Case Report

https://dergipark.org.tr/tr/pub/marumj

Parvovirus-induced autoimmune hepatitis: First case in the literature

Arda YAVUZ[1], Ayse Nur TOKSOZ YILDIRIM[2], Feruze YILMAZ ENC[1], Ilyas TUNCER[1]

[1] Division of Gastroenterology, Department of Internal Medicine, School of Medicine, Istanbul Medeniyet University, Istanbul, Turkey.
[2] Department of Pathology, School of Medicine, Istanbul Medeniyet University, Istanbul, Turkey.

Corresponding Author: Feruze YILMAZ ENC
E-mail: feruzey@yahoo.com

Submitted: 12.05.2022 **Accepted:** 17.07.2022

ABSTRACT

Parvovirus B19 infection is usually self-limiting in immunocompetent individuals. Twenty-five percent of infected individuals are completely asymptomatic during infection, with 50% of them presenting flu-like symptoms and the remaining patients presenting erythema infectiosum, arthralgia, or arthritis. More rarely, transient aplastic crisis is seen in individuals with chronic haemolytic disorders. Chronic pure red cell aplasia and aplastic anaemia may also be observed in immunocompromised patients. There is sufficient evidence in the literature suggesting that B19 infections can also cause a spectrum of liver diseases, ranging from elevated transaminase levels to acute hepatitis, fulminant liver failure, and even chronic hepatitis. This case report discusses a patient with acute parvovirus infection and newly diagnosed autoimmune hepatitis (AIH) and the role of this viral infection in the disease.

Keywords: Parvovirus, autoimmune hepatitis, acute liver injury, pancytopenia

INTRODUCTION

Human parvo-virus B19 is a non-enveloped DNA virus that has tropism for erythroid progenitor cells of human beings. Patients infected with parvovirus are asymptomatic or have flu like symptoms mostly; arthralgia, arthritis and skin lesions can also be seen. Rarely red cell aplasia, bicytopenia or aplastic anemia have been reported. Parvo-virus B19 infections can also result in a spectrum of liver diseases, ranging from elevated transaminase levels to acute hepatitis, fulminant liver failure, and even chronic hepatitis [1].

CASE REPORT

A 56-year-old immunocompetent Turkish female with a history of Sjogren's and Hashimoto's disease presented to the emergency department with a 2-week history of fatigue, nausea without emesis, and weight loss. On admission, she was afebrile, and physical examination revealed jaundice but no hepatomegaly, lymphadenopathy, or rash. She had no history of herbal product use, alcohol or mushroom consumption, or substance abuse or a family history of liver disease. Laboratory data showed the following: aspartate aminotransferase 827 U/L, alanine aminotransferase 959 U/L, total bilirubin 4.95 mg/dL, direct bilirubin 3.96 mg/dL, alkaline phosphatase 206 U/L, gamma-glutamyl transferase 388 U/L, lactic dehydrogenase 332 U/L, INR 1.26, WBC 4.8 × 109/μL, Hgb 14 g/dL, and Plt 135 × 109/μL. Abdominal ultrasonography findings were unremarkable.

After admission, the patient underwent an extensive workup for acute hepatitis. Tests were negative for hepatitis A, B, C, and E; human immunodeficiency; Epstein-Barr; and cytomegalovirus. However, serological results were significantly positive for antinuclear antibodies (ANA) for 1/3200 and anti-smooth muscle antibodies for 1/100. Parvovirus IgM was also detected (first test: 44.8 IU/mL), and the patient had bicytopenia (WBC: 4×10^9/μL, Hgb: 11.3 g/dL, Plt: 86×10^9/μL). Liver biopsy was performed for suspected autoimmune hepatitis. On the third day, she developed pancytopenia, and the infection and haematology departments were consulted. Blastic cells were detected in the blood smear, and the patient's sister had a history

How to cite this article: Yavuz A, Toksoz Yildirim AN, Yilmaz Enc F, Tuncer I. Parvovirus-induced autoimmune hepatitis: First case in the literature. Marmara Med J 2022; 3; 35(3):376-378. doi: 10.5472/marumj.1195421

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1195421
Marmara Med J 2022;35(3): 376-378

of lymphoma; therefore, a bone marrow biopsy was performed. After the second positive test for parvovirus (second IgM test: 36.25 IU/mL with a positive parvovirus IgG test), the patient was treated with valacyclovir, which was administered for 7 days as suggested by the infection department. Liver histology revealed interface hepatitis lymphoplasmacytic infiltrate, lobular necroinflammatory activity, and hepatocyte rosette formation, which confirmed autoimmune hepatitis (Figures 1 and 2). After liver biopsy, 30 mg prednisolone with 50 mg azathioprine was initiated. Bone marrow histology revealed normocellular bone marrow with mild lymphoplasmacytic cell infiltration. On immunohistochemical examination, heterogeneous staining with CD3 and CD20 was observed, and mild interstitial lymphocytosis and mild plasmacytosis were detected and interpreted as reactive (Figure 3). Her hemogram parameters were normal after 4 weeks of treatment, and liver enzyme levels were normal after 6 weeks (Figure 4).

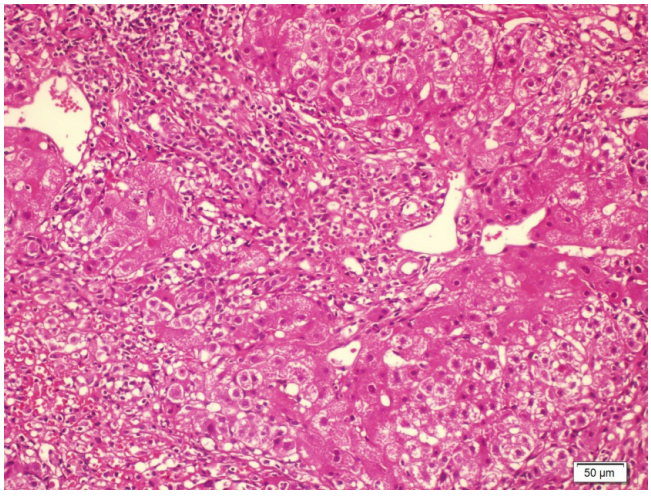

Figure 1. *Interface hepatitis lymphoplasmacytic infiltrate, lobular necroinflammatory activity (haematoxylin and eosin 20×)*

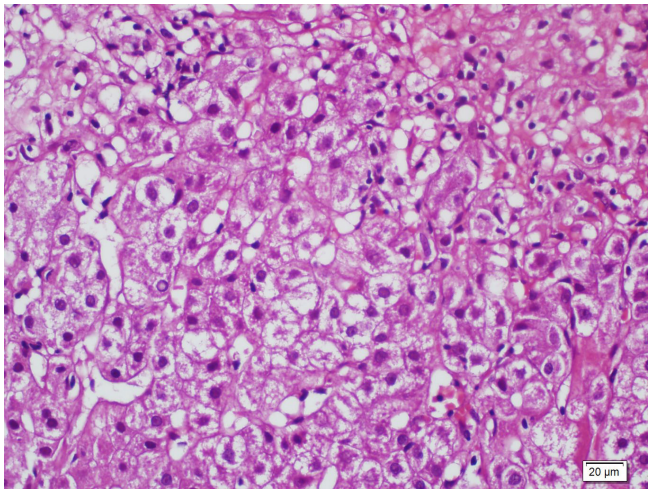

Figure 2. *Hepatocyte rosettes (haematoxylin and eosin 40×)*

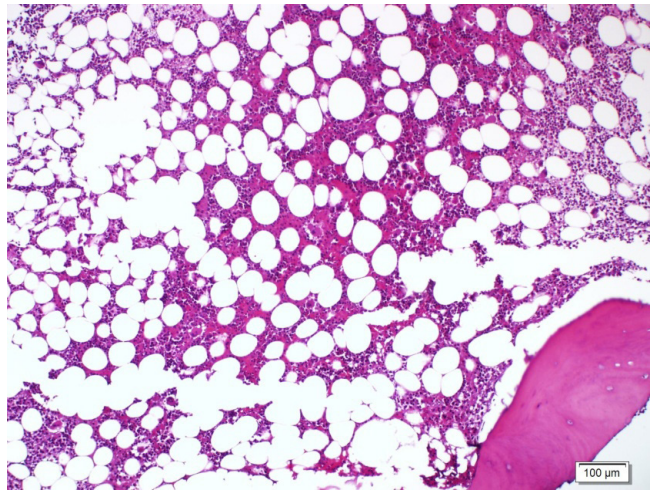

Figure 3. *Normocellular bone marrow with mild lymphoplasmacytic cell infiltration*

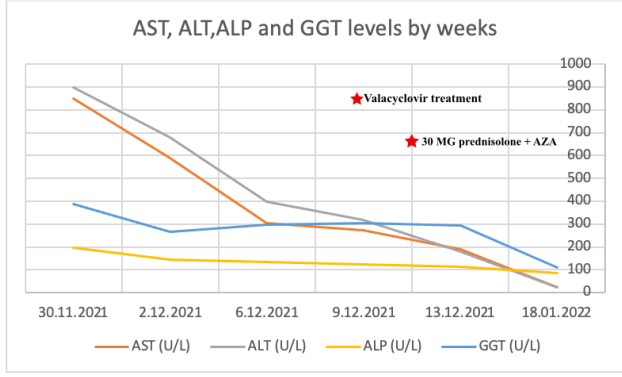

Figure 4. *AST, ALT, ALP, and GGT levels after admission*

2. DISCUSSION

Human parvovirus B19 is a non-enveloped, single-stranded DNA virus that codes for two major structural or capsid proteins, VP1 and VP2, and one non-structural protein, NS1. It exhibits tropism for erythroid progenitor cells, which possess the P-antigen. Most virus-related clinical symptoms result from cytolytic and apoptotic effects of NS1 on erythroid progenitor cells. Non-erythroid cells also express P-antigen, but to a lesser extent. VP1 also plays a role in infection and autoimmunity. Viral infections are a well-recognised prelude to autoantibody production, and many mechanisms may account for immune-mediated clinical consequences. The most probable explanation is molecular mimicry, in which specific antiviral responses cross-react with the host tissues. In addition, low-titre autoantibody production in symptomatic parvovirus B19 infections is common and may induce arthritis.

http://doi.org/10.5472/marumj.1195421
Marmara Med J 2022;35(3): 376-378

Parvoviruses may cause hepatitis, hepatitis-associated anaemia, and acute liver failure in infected patients [1]. These clinical manifestations have mainly been reported in children. Parvovirus-induced hepatitis is less common in adults than in children; however, it has a much less severe course with better patient outcomes. Most adult patients have complete and spontaneous remission, but one case required liver transplantation [2].The mechanism underlying parvovirus-induced liver injury is debatable. The cytotoxic effect of the nonstructural (NS1) protein expression may induce immune-mediated injury by interferon-gamma due to T cells or a selective defect in patients with B19 infection. This mechanism may also cause hemophagocytic syndrome via interferon-gamma and tumour necrosis factor-alpha, leading to pancytopenia and hepatic dysfunction. The second effect of the virus is the induction of other antibodies, including anti-mitochondrial antibodies. Kerr and Boyd tested 53 patients with acute parvovirus B19 infection for antibodies, and seven of them were positive for ANA-2, rheumatoid factor-1, anti-reticulin antibody-2, anti-mitochondrial antibody-2, and anti-parietal cell antibody-1 [3].

In the present case, autoimmune hepatitis developed after a symptomatic parvovirus infection. Some cases have reported autoimmune hepatitis related to Epstein-Barr virus, varicella-zoster virus, hepatitis A virus, or human herpesvirus 6. To the best of our knowledge, this is the first report of parvovirus-induced autoimmune hepatitis. We believe that viral infections trigger autoimmune diseases in patients with certain risk factors or genetic predispositions, which should always be taken into consideration.

REFERENCES

[1] Bihari C, Rastogi A, Saxena P *et al*. Parvovirus b19 associated hepatitis. Hepat Res Treat 2013;2013:472027.

[2] Krygier DS, Steinbrecher UP, Petric M *et al*. Parvovirus B19 induced hepatic failure in an adult requiring liver transplantation. World J Gastroenterol 2009; 15(32): 4067–9.

[3] Kerr JR, Boyd N. Autoantibodies following parvovirus B19 infection. J Infect 1996;32:41–7.