NTMS New Trends in Medicine Sciences

Volume 4
Issue 2
May
2023

New Trends in Medicine Sciences

Peer-Reviewed Academic Journal

ISSN: 2717- 8161
https://dergipark.org.tr/tr/pub/ntms

2023 May

New Trends in Medicine Science (NTMS) is open access, double-blind, peer-reviewed journal published triannual. It aims to contribute to scientific knowledge of medical sciences by publishing studies in basic, internal, and surgical medical sciences. The journal provides free access to the full texts of all articles immediately upon publication.

e-ISSN: *2717-8161*

Journal Abbreviation: *New Trend Med Sci/NTMS*

Web Page: *https://dergipark.org.tr/en/pub/ntms*

Correspondence Address: *ntms.editor@gmail.com*

Publication Period: *Triannual (January, May, and September)*

New Trends in Medicine Sciences *Volume 4*

Issue 2

CONTENTS

CLINICAL AND EXPERIMENTAL RESEARCHES ***RESEARCH ARTICLES***

CASE REPORT

ISSN: 2717-8161 RESEARCH ARTICLE

New Trend Med Sci 2023; 4(2): 48-51. https://dergipark.org.tr/tr/pub/ntms

Determination of Expression Levels of Interleukin 1, 6, 17, 23 and Tumor Necrosis Factor Genes in Patients with Systemic Lupus Erythematosus

Kübra Solmaz[1], Eda Balkan[1*], Meltem Alkan Melikoğlu[3]

[1]Department of Medical Biology, Faculty of Medicine, Atatürk University, Erzurum, Turkey
[2]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Atatürk University, Erzurum, Turkey

Article History
Received 16 March 2022
Accepted 06 Jan 2022
Published Online 25 May 2023

****Corresponding Author***
Eda Balkan
Department of Medical Biology
Faculty of Medicine
Atatürk University
Erzurum, Turkey.
Phone:+ +90 4423446946
E-mail: edadiyarbakir@atauni.edu.tr

Doi: 10.56766/ntms.1088689

Authors' ORCIDs
Kübra Solmaz
http://orcid.org/0000-0002-3418-1896
Eda Balkan
http://orcid.org/0000-0002-7065-8161
Meltem Alkan Melikoğlu
http://orcid.org/0000-0001-7519-9470

Abstract: Systemic lupus erythematosus (SLE) is an autoimmune, chronic, and inflammatory disease. In many studies has done till these days, in patients with Systemic Lupus Erythematosus level of IL-1, IL-6, IL-17, IL-23, and TNF-α has been shown and these cytokines have a role in the pathogenesis of SLE disease. In our study, we aimed to detect the levels of these cytokines quantitatively. This study was carried out on 30 patients with SLE who were followed up in Erzurum Atatürk University Health Research and Application Center Physical Medicine and Rehabilitation Department Policlinic. Changes in mRNA expression of IL-1, IL-6, IL-17, IL-23, and TNF-α genes were determined by quantitative Real Time. There were no statistically significant differences between IL-6 gene expression levels between patient and control groups. There was a statistically significant difference between IL-1, IL-17, IL-23, and TNF- α genes between the patient and control groups. Although there are studies supporting the role of the IL-6 gene in the pathogenesis of SLE in the literature, in our study, there was no significant difference between SLE and control group. The threefold significant difference of the IL-1 gene between the SLE group and the control group supports the important role of the IL-1 gene in the pathogenesis of SLE. IL-17, IL-23, and TNF-α genes were also found to be significantly higher than SLE patient control groups. We think that these genes will contribute to clarifying the inflammation events in the etiopathogenesis of this disease. ©2023 NTMS.

Keywords: Systemic Lupus Erythematosus; Cytokines; Pathogenesis.

1. Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune, multisystemic, inflammatory connective tissue disease characterized by a variable course and prognosis of unknown etiology [1]. Although its etiology is not fully understood, it is known that genetic predisposition and environmental triggers cause Systemic Lupus Erythematosus (SLE). Systemic Lupus Erythematosus (SLE) is one of the most common autoimmune disorders affecting women of all age groups. It mostly affects women of childbearing age and is the age group in which the incidence peaks [2]. According to the genders, the prevalence was stated to be 9/1 between female/male. In addition, the incidence of Systemic Lupus Erythematosus (SLE), which differs according to age, decreases to 2/1 in the elderly and children [3]. Systemic Lupus Erythematosus (SLE) with

Cite this article as: Solmaz K, Balkan E and Melikoğlu MA. Determination of Expression Levels of Interleukin 1-6 Tumor Necrosis Factor Interleukin 17 Interleukin 23 Genes in Patients with Systemic Lupus Erythematosus. *New Trend Med Sci*. 2023: 4(2):48-51. Doi:10.56766/ntms.1088689

a worldwide prevalence of 4.9/100.000 may show different incidence in different regions and geographical conditions[4]. Gender, ethnicity, sun exposure, age, and many genetic and environmental factors are the most influential factors in incidence and prevalence. In the United States (USA), the overall incidence is between 1.6 and 7.6 per 100,000, and the prevalence is between 14.6 and 508 per 100.000 is considered [5].

In the literature review, in animal model study of Edberg JC et al., PDCD1, known as the programmed cell death 1 gene, was reported to be associated with Systemic Lupus Erythematosus (SLE). Regarding the acute phase protein (CRP), a single nucleotide polymorphism study of the CRP gene rs3093061 was performed on patients with SLE and a correlation was found between the sensitivity of the CRP gene to Systemic Lupus Erythematosus (SLE) [6].

ITGAM (CD11b), another gene shown to be associated with Systemic Lupus Erythematosus (SLE), obtained statistically significant results in terms of SLE risk of ITGAM rs1143679 gene variants in patients with Systemic Lupus Erythematosus (SLE) of European origin.

According to data obtained from field studies, there are more than 100 gene polymorphisms that contribute to Systemic Lupus Erythematosus (SLE) susceptibility [7]. Recent research has focused on TOLL- like receptors (TLRs). Type I interferons have focused on variants of TLRs involved in immune system regulation pathways. Also, in studies on gene expression, TLR7 overexpression has been observed in patients with Systemic Lupus Erythematosus (SLE) [7].

2. Material and Methods

2.1. Materials

A patient group consisting of 30 patients (16 females-14 males) diagnosed with SLE and followed in Erzurum Atatürk University Health Research and Application Center Directorate Physical Medicine and Rehabilitation Department Polyclinic and 20 healthy individuals (10 females-10 males) without any systemic disease was included in our study. Informed Consent Form was signed by the patient and control group who agreed to participate in the study.

Blood samples collected for this study, which was approved by Erzurum Atatürk University Faculty of Medicine Ethics Committee, were used for RNA isolation (Roche) and gene expression studies (Roche Light Cycler 480 Real-Time) in Atatürk University Faculty of Medicine Laboratory of Medical Biology Department.

2.2. Methods

2.2.1. Gene expression analysis

2 ml EDTA blood samples were taken from 30 patients diagnosed with SLE and 20 control groups in Erzurum Atatürk University Health Research and Application Center Directorate.

Commercial isolation kit (High Pure) was used to synthesize cDNA from these blood samples taken from the patients of the Physical Medicine and Rehabilitation Department Polyclinic. Total RNA was obtained using RNA Isolation Kit-Roche) and stored in a deep freezer at -80 °C throughout the study. The procedure and chemicals recommended by the manufacturer were used for isolation.

The amount and quality of the obtained RNA were measured spectrophotometrically using the Nanodrop device (MaestroNano-USA). Measurement was made with 2 µl of RNA sample. Measurements made with the spectrophotometer at two wavelengths for quality; It has values between 260/280=1.5-1.9.

2.2.2. cDNA Synthesis

Commercial kit (ProtoScript® II First Strand cDNA Synthesis Kit, NEB) was used for cDNA synthesis from isolated mRNAs. In the synthesis process, the protocols determined by the manufacturer were taken as basis. The obtained cDNAs were stored at -20 ºC for use in the Real Time stage.

2.2.3. Statistical analysis

SPSS 20.0 (Statistical Packages for the Social Sciences for Windows XP Release 20.0 version) program was used to evaluate all statistical data related to the study. A Chi-square test was performed on the patient and control groups. Statistically significant differences are presented as follows: $p>0.05$ (not significant, ns) and $p<0.05$ (significant).

3. Results

A patient group consisting of 30 patients (16 females - 14 males) diagnosed with Systemic Lupus Erythematosus (SLE) and the control group consisting of 20 healthy individuals (10 females-10 males) without any systemic disease were included in this study. Expression levels of interleukin 1-6 tumor necrosis factor interleukin 17 interleukin 23 genes were examined.

Table 1: Patient and control group gene expression levels.

Gene symbol	Patient	Control	Value
IL-1	22.821	6.836	P<0.001
IL-6	11.399	10.573	P>0.05
IL-17	19.002	10.088	P<0.05
IL-23	20.062	11.392	P<0.05
TNF-α	19.554	9.914	P<0.05

When the IL-1 expression levels of the control and patient groups were evaluated, a 3- fold difference was found statistically. When the IL-6 levels of the control and patient groups were evaluated, no statistically significant difference was found. A 2-fold difference was observed in the IL-17 expression level analysis of the control and patient groups. A significant difference

was observed in the determined IL-23 expression levels of the control and patient groups.

4. Discussion

Systemic Lupus Erythematosus (SLE) is a multisystemic connective tissue disease characterized by variable course and prognosis [8]. Although complex genetic diseases and environmental factors are at the root of SLE, the exact cause of autoimmunity is unknown [9]. Studies using the MRL/lpr mouse model in the investigation of lupus and similar autoimmune diseases have reported that increased IL-1β gene expression is associated with disease severity and rapid progression of the disease. In the studies carried out to date, no investigation of the expression level of the IL-1 gene was found in Systemic Lupus Erythematosus (SLE) patients, only the serum level was examined. The study containing the most important data was conducted by Rachel M in 2018 and focused on serum levels in SLE patients [10]. In the study conducted by Hye-Young Chun in 2007, IL-2, IL-6, IL-10, IL-12 expression levels were examined, and no significant difference was observed in the IL-2 gene, while IL-10, IL-12, IL-6 genes were significantly different compared to the control group has been observed to increase [11]. Likewise, in the study of Birner P, an increase in IL-6 mRNA expression level was observed in Systemic Lupus Erythematosus (SLE) patients, and a statistically significant difference was obtained [12]. In our study, IL-6 gene expression was compared between the Systemic Lupus Erythematosus (SLE) patient and control groups, and the expression level of the said gene was not found to be statistically significant (P >0.05). In the study focused on investigating the relationship between IL-17 and T cell, conducted by Chun Kwok Wong in 2018, the expression levels were also examined, and a significant difference was found in the comparison of the expression level between the patient and control group, as in our study [13]. Tekin BG. investigated the relationship between vitamin D metabolism and interleukin 17 serum levels in SLE patients. If the study included the other, no difference could be found between the patient and the control group[14]. Likewise, in 2010, Keskin O. prepared the IL17 mRNA expression supplementary study of IL-17.IL-23 genes, and their effectiveness between the patient and control group IL-17 expression pressure no difference was found[15]. Dong G, in his study conducted in 2003, supports our study, and the difference between patient control and expression levels was found to be twofold and significant [16]. In our study, the expression levels of IL-17 genes of Systemic Lupus Erythematosus (SLE) patients were examined. The IL-17 expression level of the Systemic Lupus Erythematosus (SLE) group showed a significant 2-fold increase compared to the control group (P<0.05).

Roba M. Talaat, in his study to investigate the cytokine secretion profile in Systemic Lupus Erythematosus (SLE) patients and their possible relationship with disease activity, found a significant difference in IL-23 expression level [17].Another study parallel to our study was conducted by Xinfang Huang in 2014, and statistically significant results were obtained in this study, as in our study [18]. In addition, a statistically significant difference was found in the expression level of TNF-α gene in the study of Sabry et al.[19].

In the study, the expression levels of IL-1 genes of SLE patients were examined, IL-1 expression level of the Systemic Lupus Erythematosus (SLE) patient group increased significantly 3 times compared to the control group (P <0.001). In our study, IL-6 gene expression was compared between the Systemic Lupus Erythematosus (SLE) patient and control groups, and the expression level of the said gene was not found to be statistically significant (P>0.05). Expression levels of IL-17 genes of SLE patients were examined. Expression levels of IL-23 genes of Systemic Lupus Erythematosus (SLE) patients were examined. IL-23 expression level of the SLE patient group showed a significant increase compared to the control group (P<0.05). Expression levels of TNF-α genes of Systemic Lupus Erythematosus (SLE) patients were also examined. TNF-α expression level of the SLE patient group showed a significant increase compared to the control group (P<0.05).

5. Conclusions

As a result, according to the results of Real time PCR quantitative analysis, no statistically significant difference was observed between the patient and control groups in the expression levels of the IL-6 gene. It was observed that there was a statistical difference between the patient and control groups in IL-1, IL-17, IL-23 and TNF-α genes

Limitations of the Study

There are two major limitations in this study that can be addressed in future research. First, the sample size is larger. Second, showing that the number of interleukins involved in sle disease is higher.

Acknowledgement

None.

Conflict of Interests

The authors declare that there is no potential conflict of interest for the research, authorship, and/or publication of this article. All authors read and approved the final manuscript.

Financial Support

This study was supported by Atatürk University Scientific Research Projects Coordination Unit (Project Number: TYL-2019-7046).

Author Contributions

Design of the study: EB, Sample collection: MAM, Performed the experiments: KS, Data Collection and/or Processing: EB, KS, Writing Original Manuscript: EB, KS. EB contributed to revising the work and final approval of the final version of the manuscript..

Ethical Approval

This study was approved by the Atatürk University Faculty of Medicine Clinical Research Ethics Committee (05/01-07.06.2018).

Data sharing statement

The data that support the findings of this study are available on request from the corresponding author.

Consent to participate

Consent was obtained from the patient and control groups participating in the study.

Informed Statement

The patient and control group who agreed to participate in the study signed the informed consent form.

References

1. Bertsias G, Cervera R, Boumpas DT. Systemic Lupus Erythematosus: Pathogenesis and Clinical Features. *Eular Fpp Indd.* 2012; 1:476-505.
2. Rees F, Doherty M, Grainge MJ, Lanyon P, Zhang W. The worldwide incidence and prevalence of systemic lupus erythematosus: a systematic review of epidemiological studies. *Rheumatology*. 2017; 56(11):1945-61.
3. Villamin CA, Navarra SV. Clinical manifestations and clinical syndromes of Filipino patients with systemic lupus erythematosus. *Mod Rheumatol*. 2008; 18:161-64
4. Naleway AL, Davis ME, Greenlee RT, et al. Epidemiology of systemic lupus erythematosus in rural Wisconsin. *Lupus*. 2005;14(10):862-866.
5. Rees F, Doherty M, Grainge MJ, Lanyon P, Zhang W. The worldwide incidence and prevalence of systemic lupus erythematosus: a systematic review of epidemiological studies. *Rheumatology*. 2017; 56(11):1945-61.
6. Edberg JC, Wu J, Langefeld CD, et al. Genetic variation in the CRP promoter: association with systemic lupus erythematosus. *Hum Mol Genet*. 2008; 17(8):1147-55.
7. Nath SK, Han S, Kim-Howard X, et al. A nonsynonymous functional variant in integrin-alpha (M) (encoded by ITGAM) is associated with systemic lupus erythematosus. *Nat Genet.* 2008; 40(2):152-54.
8. Rahman A, Isenberg DA. *N Engl J Med.* 2008; 358:929-39.
9. Herrmann M, Winkler T, Gaipl U, Lorenz HM, Geiler T, Kalden JR. Etiopathogenesis of Systemic Lupus Erythematosus. *Int Arch Allergy Immunol*. 2000; 123:28-35.
10. Rachel M, et al. Analysis of Serum Interleukin (IL)-1β and IL-18 in Systemic Lupus Erythematosus. *Front Immunol.* 2018; 9:1250.
11. Chun HY, Chung JW, Kim HA, et al. Cytokine IL-6 and IL-10 as Biomarkers in Systemic Lupus Erythematosus. *J Clin Immunol.* 2007; 27:461-66.
12. Birner P, Heider S, Petzelbauer P, et al. Interleukin-6 receptor alpha blockade improves skin lesions in a murine model of systemic lupus erythematosus. *Antibodies*. 2016; 25(4):305-10.
13. KwokWonga C, WanLita LC, ShanTam K, et al. Hyperproduction of IL23 and IL-17 in patients with systemic lupus erythematosus: Implications for Th17-mediated inflammation in auto-immunity. *Clin Immunol.* 2008; 127(3): 385-93.
14. Tekin BG. Evaluation of the relationship between disease activation and vitamin D metabolism and IL-10, IL-17 and IL-23 levels in Systemic Lupus Erythematosus. Adnan Menderes Internal Medicine Department, Master Thesis. 2015.
15. Keskin O. Evaluation of clinical findings and serum IL-17 and IL-23 levels in Systemic Lupus Erythematosus patients. Ankara University İnternal Medicine Department, Master Thesis. 2010.
16. Dong G, Ye R, Shi W, et al. IL-17 induces autoantibody overproduction and peripheral blood mononuclear cell overexpression of IL-6 in lupus nephritis patients. *Chin Med J.* 2003; 116(4): 543-48.
17. Talaat RM, Mohammed SF, Bassyouni IH, Raouf AA. Th1/Th2/Th17/Treg cytokine imbalance in systemic lupus erythematosus (SLE) patients: Correlation with disease activity. *Cytokine*. 2015; 72(2):146-53.
18. Xinfang H, Hua J, Shen N, Chen S. Dysregulated expression of interleukin-23 and interleukin-12 subunits in systemic lupus erythematosus patients. *Mod Rheumatol*. 2007; 17(3):220-23.
19. Sabry A, El-Husseini A, Mahmoud K, et al. Proinflammatory cytokines (TNF-a and IL- 6) in Egyptian patients with SLE: Its correlation with disease activity. Cytokine. 2006; 35(3-4):148-53.

ISSN: 2717-8161 RESEARCH ARTICLE

New Trend Med Sci 2023; 4(2): 52-57. https://dergipark.org.tr/tr/pub/ntms

The Efficacy of Various Novel Copper-Based Antibacterial Solutions on E. Coli

Atiksh Chandra[1]* and Sahana Thayagabalu[1]

[1]Department of Biology, Cypress Bay High School, 18600 Vista Park Blvd, Weston, FL, USA.

Article History
Received 19 July 2022
Accepted 24 Aug 2022
Published Online 25 May 2023

****Corresponding Author***
Atiksh Chandra
Department of Biology
Cypress Bay High School
Weston, FL, USA
Phone: +954-743-8680
E-mail: atikshchandra@yahoo.com

Doi: 10.56766/ntms.1144829

Authors' ORCIDs
Atiksh Chandra
http://orcid.org/ 0000-0003-4509-5616
Sahana Thayagabalu
http://orcid.org/ 0000-0001-5697-5761

Abstract: The continuous rise of infection in households, businesses, and schools has accelerated the need for long-lasting sanitation solutions. Current disinfectants, like Lysol, kill bacteria and other microbes only at initial application and are ineffective under aqueous conditions. Copper (II) ions and Lactic Acid are highly regarded for their synergetic, long-lasting antibacterial properties. Although L-pyroglutamic acid holds similar properties, little research has examined its efficacy with copper metal. The purpose of this experiment is to evaluate various novel antibacterial solutions for instantaneous microbial inhibition and continued inhibition over extended periods of time in aqueous solutions. Two antibacterial solutions utilizing Copper (II) Sulfate (10 ppm) were developed with 1% Lactic Acid (Solution A) and 1% L-Pyroglutamic Acid (Solution B). The extinction rate of Escherichia coli K12 bacteria for each solution and Lysol was recorded. The concentration of E. coli was observed via spectrophotometry at 3-time intervals: Initial Introduction (28 Minutes), Short Term (2 Hours) and Long Term (72 hours). At initial introduction, there was no significant difference between solutions ($p>0.05$) ranging from 22 to 28% E. coli loss from the original sample. Significant growth inhibition ($p <0.05$) occurred in Solution A and Solution B compared to Lysol after 2 hours. Solution B sustained higher efficacy compared to Lysol after 72 hours. Overall, our Copper (II)/Lactic Acid Solution (Solution A) and Copper (II)/L-Pyroglutamic Acid Solution (Solution B) showed significant improvement when compared to the efficacy of Lysol in aqueous solutions over longer periods of time. Both solutions are cheap and long-lasting, making them pragmatic options for the future aqueous household sanitation.

Keywords: Copper; E. Coli; Lactic Acid; Pyrrolidonecarboxylic Acid.

1. Introduction

Copper metal has been highly regarded for its antimicrobial properties in the healthcare industry for the last decade [1]. Ancient civilizations utilized copper water containments and copper medicinal products long before antimicrobial mechanisms were understood [2]. In 2008, The U.S. Environmental Protection

Cite this article as: Chandra A and Thayagabalu S. The Efficacy of Various Novel Copper-based Antibacterial Solutions on E. Coli. *New Trend Med Sci.* 2023; 4(2):52-57. Doi:10.56766/ntms.1144829

program recognized copper and its alloys as the first metallic antimicrobial agent. Within 2 hours of contact, in vitro assays, copper surfaces were found to have eliminated 99.9% of microbes. In recent years, several studies have supported the utility of copper surfaces in the inhibition of antibiotic-resistant bacteria and Healthcare-associated infection in the hospital setting [3-6]. However, the application of copper sanitation to the household environment has been largely unexplored.

Furthermore, copper particles are also considered potent antibacterial agents [7]. The miniscule nature of this copper is typically considered safe for humans, although higher concentrations may lead to toxicity [8]. Usman et al. (2013) found high antibacterial activity on many bacterial strains such as, MRSA, Bacillus subtilis, Salmonella choleraesuis, and P. aeruginosa using chitosan-copper nanoparticles [9]. Antiviral properties of particle copper have also been shown against Hepatitis C Virus, Influenza A virus, and HIV-1 [10-12]. Copper is able to neutralize cellular function via numerous mechanisms including: essential ion substitution, hydrogen peroxide free radical production by membrane-bound copper, enzyme inactivation, and functional group interference [6]. While the exact mechanism for this effect is unknown, smaller particles seem to have higher antibacterial activity due to easier cell penetration [13-14].

Copper has also shown increased antimicrobial action when coupled with organic acid solutions, specifically, Lactic Acid. Used regularly in a biopreservative and food decontaminant, Lactic acid is generally regarded as safe for humans [8, 15]. Beal et al. saw a 10-fold increase in the death rate of Salm. typhimurium after lactic acid (150 mM) and copper sulfate (50 ppm) were added to a liquid pig feed.16 Several other studies exhibited similar results [10, 17]. The reasoning behind increased antibacterial efficacy with a copper/lactic acid combination is unclear. Morphological changes and increased outer membrane permeability in gram negative bacteria are a few of the proposed mechanisms examined in recent studies [8, 15]. L-Pyroglutamic acid holds similar antibacterial properties although little research has been examined on the combined effectiveness with copper.

Commercially manufactured surface disinfectants currently dominate the household sanitation market. Lysol Disinfecting Spray, a leading product, is widely recognized for its quick killing potential of nearly all microbes on various surfaces. However, disinfectants as such are known to have significantly less antibacterial efficacy in aqueous environments and over longer periods of time [18]. We hypothesized that the effective antibacterial properties, from the combination of copper (II) ions with lactic acid, would address the inadequate nature of Lysol. Thus, the purpose of this study was to (1) determine the efficacy of a copper/lactic acid solution and a copper L-Pyroglutamic Acid solution at inhibiting E. coli K12 growth at initial introduction and (2) the solutions' efficiency over longer periods of time compared to Lysol in an aqueous laboratory condition.

2. Material and Methods

Escherichia coli K12 bacterial strain obtained from Carolina Biologicals was incubated and grown with a nutrient broth as per instruction indicated in manual for 48 hours before experimentation was performed.

Solutions were developed in accordance with safety for regular household use. For this reason, specified amounts of both copper and the acids were used when creating two different solutions (A, B) with various concentrations. The Lysol Solution, the industry standard, was essentially a 2% Lysol dilution with two parts active Lysol per 100 parts distilled water. Solution A was prepared by adding equal parts 10 ppm Copper (II) and 1% L-Pyroglutamic Acid. Solution B consisted of equal parts 10 ppm Copper (II) and 1 % Lactic Acid. Both solutions were synthesized, considering the LD50 values and optimal concentrations of all the agents involved based on accepted literature.

UV spectrophotometry was used to determine changes in E. coli concentration over time. The UV spectrophotometer was used to obtain the percent transmittance of light through each cuvette at 600 nm. For precise concentration calculations, the UV spectrophotometer was utilized [19]. It was calibrated before every cuvette was tested, and the percentage transmittance was output with one decimal precision. Percentage transmittance was converted to absorbance then concentration of E. coli via the Beer Lambert Law [19]. The death rate, the percent loss of E. coli, was calculated using an initial concentration of E. coli obtained from a control sample.

2.1. Procedure

The experimentation was conducted in a constant room temperature environment (21 °C). All surfaces were thoroughly cleaned prior to and after experimentation. The cuvettes were disposed safely, using a 10% bleach solution to eliminate all bacterial growth.

For each experimental group, 7 labelled cuvettes were filled with the initial E. coli broth culture (1.35 mL) and testing solution (0.3 mL) at minute zero in a distinct order. The broth was stirred well before every cuvette was filled as for cultures to be evenly distributed. Each cuvette was filled one minute after the last cuvette. Then, transmittance was recorded via spectrophotometry one minute after the last to maintain consistent reading throughout the experiment.

Spectrophotometry readings of transmittance were taken for all seven cuvettes over the course of 72 hours in the same order, broken into three subcategories of investigation: "initial", "short term" and "long term." Initial data represents percent of E. coli loss after 28 minutes from start. Readings were taken at minute 0, 7, 14, 21, and 28. Short term data represents percent of E. coli loss after 127 minutes from start. Readings were taken at minute 120 and 127. Long term data represents

the percent of E. coli loss after 72 hours from start. Seven trials were conducted for solution A, solution B and Lysol solution. At each reading point, the average of the seven trials per solution was recorded.

2.2. Statistical Analysis

The data was analyzed by one-sided t-tests on the slopes to measure the positive statistical difference for Solutions A and B compared to Lysol in the percent loss of E. coli. A nonparametric test was not conducted due to insufficient evidence of consistent dispersion. Statistical significance was evaluated where $P<0.05$ was considered significant, and $P<0.001$ highly significant.

3. Results

The efficacy of the Copper (II)/Lactic Acid Solution (Solution A) and Copper (II)/L-Pyroglutamic Acid Solution (Solution B) were similar to that of Lysol in the initial time interval (28 minutes). In the short-term interval (2 hours) both solutions successfully exhibited higher efficacy than that of Lysol. After 72 hours, the long-term interval, Solution B continued to sustain high efficacy compared to Lysol.

Figure 1 (Initial interval) represents the average percent loss of E. coli from its initial concentration to the end of the 28-minute period. There was no significant difference found between the effectiveness of both solution A ($P=0.149$, $t=1.365$) and B ($P=0.063$, $t=2.012$) compared to Lysol. Both solutions as well as Lysol exhibited relatively equal antibacterial efficiency with respect to loss of E. coli.

Figure 2 (Short-term interval) denotes the average percent loss of E. coli from its initial concentration over 128 minutes. We found significant differences between the effectiveness of both solution A ($P<0.001$, $t=23.053$) and B ($P<0.001$, $t=58.415$) compared to Lysol. Solution A reached 46.5 % bacterial death and Solution B reached 42.3 % bacterial death by the two-hour mark highlighted in yellow. Lysol remained rather stagnant over this two-hour period holding at 27 % E. coli loss.

Figure 3 (Long-term interval) presents the average percent loss of E. coli from 128 minutes till 72 hours. Our results indicated significant differences between the effectiveness of both solution A ($P<0.001$, $t=-0.318$) and B ($P<0.001$, $t=2.650$) compared to Lysol. The slope of Solution A, the orange line, peaks after two hours and approaches a constant rate during this interval. Solution B, on the other hand, the gray line, continued eliminating E. coli for the duration of 72 hours peaking at 58% bacterial death at the 4320 minutes. Lysol also has an increase in bacterial death, peaking its percentage loss at 42%. The long-lasting high efficacy of Solution B in comparison to Solution A and Lysol is exhibited in this analysis.

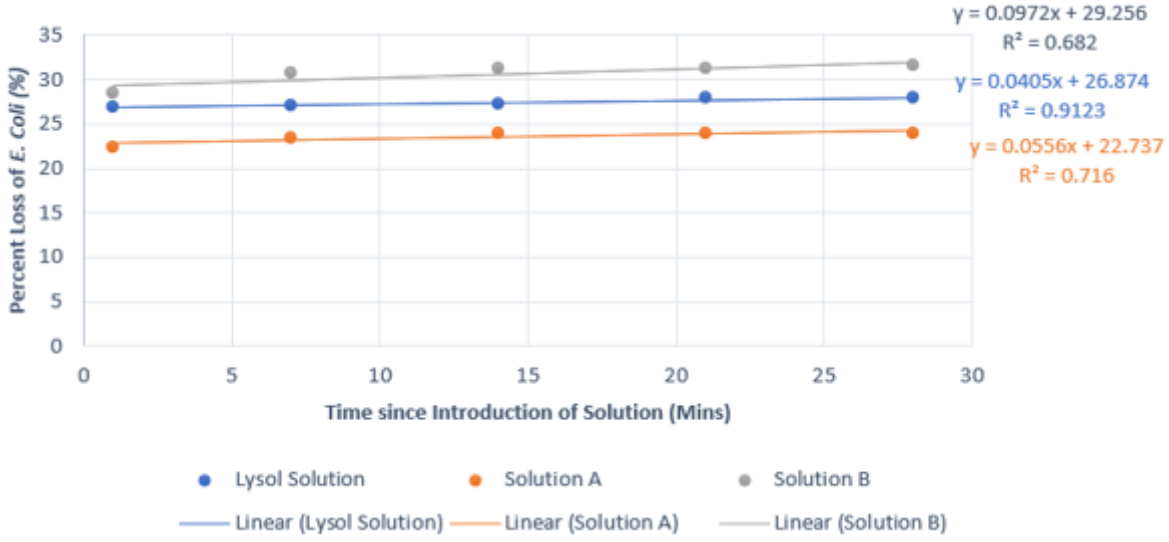


Figure 1: Average Percent Loss of E. Coli Over 28 Minutes. The scatterplot presents the immediate change in E. Coli concentration once each solution is introduced.

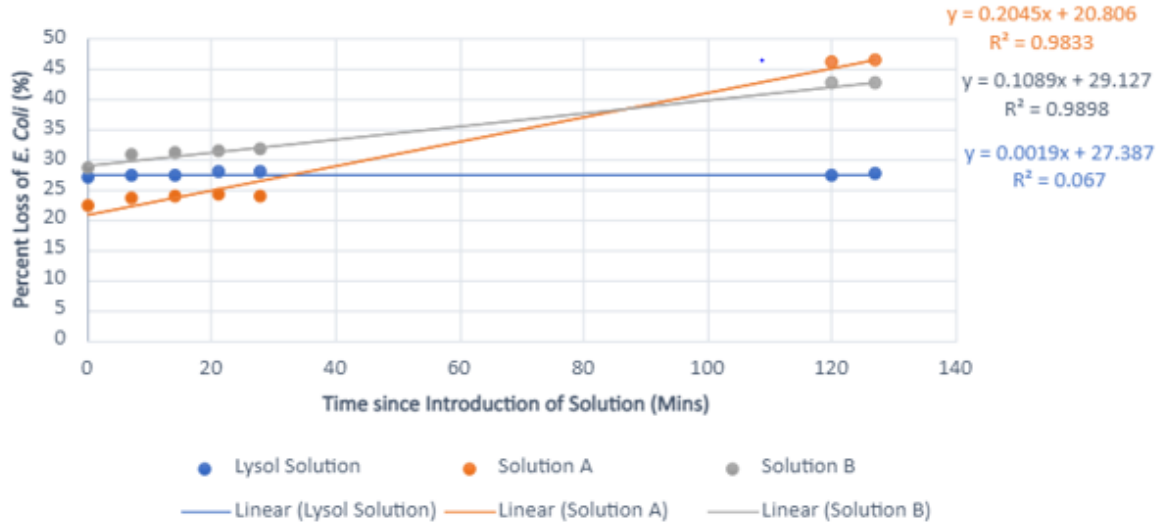


Figure 2: Average Percent Loss of E. Coli Over 128 Minutes. The scatterplot presents the "Short-term" change in E. Coli concentration once each solution is introduced.

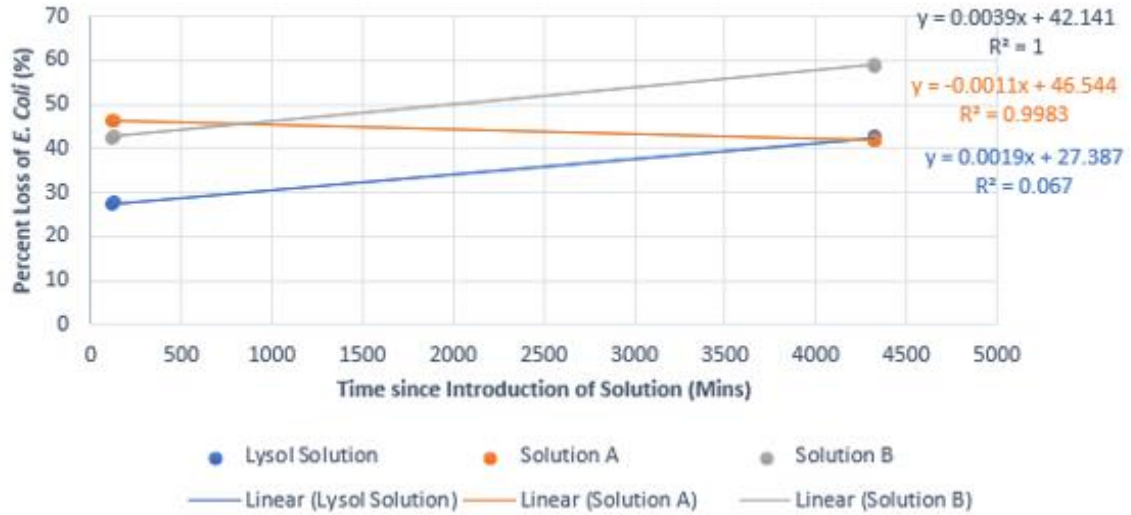


Figure 3: Average Percent Loss of E. Coli Over 72 Hours. The scatterplot presents the "Long- term" change in E. Coli concentration once each solution is introduced.

4. Discussion

Overall, our Copper (II)/Lactic Acid Solution (Solution A) and Copper (II)/L-Pyroglutamic Acid Solution (Solution B) showed significant improvement when compared to the efficacy of Lysol in aqueous solutions over longer periods of time.

Our results are fairly consistent with Gyawali et al. (2011) and Al-Holy (2010). Gyawali et al. evaluated the combination of lactic acid and Copper (II) on E. coli O157:H7. On both lettuce and tomato samples as well as laboratory medium, significant growth inhibition was observed [20]. Al-Holy studied the natural antimicrobial effect of Copper (II), lactic acid, and Monolaurin on Cronobacter. With low concentrations of these components, Al-Holy was able to achieve complete elimination of Cronobacter [21]. Diminished antibacterial properties were observed after a 24-hour time period in both studies. Our study presents similar results as our Copper (II)/Lactic Acid Solution had comparable high efficacy after 2 hours, but severely decreased efficacy after 72 hours. We believe the synergetic inhibitory activity of Solution A to attributed to Lactic Acid permeabilizing the outer membrane of Gram-negative bacteria, as suggested in the prior literature [22]. Free copper (ii) ions are then able to permeate the membrane, disabling enzymes and inactivating functional groups of proteins [22].

Current research on antibacterial properties of L-Pyroglutamic acid is rather limited. Two studies, Tan et al. [23] and Gang et al. [24], presented preliminary evidence of antibacterial and antifungal properties of L-Pyroglutamic acid. However, we were unable to find any relevant literature utilizing the combination of L-Pyroglutamic acid and Copper (II) ions as a synergetic antibacterial agent. While we did not evaluate the antibacterial action mechanisms of L-Pyroglutamic

acid, our study pioneers the involvement of L-Pyroglutamic acid in the disinfecting space. Our results from Solution B highlight the potential of L-Pyroglutamic acid over longer periods of antibacterial inhibition along with copper particles, although further research should be conducted.

The data from our experiment supported both of our hypotheses. Hypothesis 1 was supported from our initial testing phase where we saw similar percent loss across all three solutions. Hypothesis 2 was supported from the short term and long-term testing phases. After 2 hours the Copper/L pyroglutamic acid solution was most effective and after 72 hours Copper/Lactic Acid solution was most effective. Given the high availability, low cost, and low concentration of key ingredients, our cost analysis indicates cheap commercial production of both solutions. As for safety, our solutions utilize concentrations (g/L) of Copper (II) Sulfate, L-Pyroglutamic Acid, and Lactic Acid that are less than 4% of the LD50 value per average adult body mass (g). Both solutions are suitable for household use based on LD50 values comparison and all-natural ingredients. In summary, both solutions A and B are strong contenders as antibacterial alternatives to Lysol. The research presented has practical applications in regard to disinfection. Because of the low cost, our solutions can be applied in two main avenues: Aqueous Disinfecting and Household Disinfecting. Our sanitation formula, having been tested in aqueous environments, will combat microbial growth in standing waters and wastewaters continuously. Our product is most practical as a household disinfectant via wipes and aerosol spray cans. The aqueous nature of this sanitation formula makes our solution easy to implement in a variety of antibacterial products offered to the average consumer. Although we do not speculate any health concerns, the human safety of both solutions should be formally regulated before household implementation. Since this method is cheap, easy to implement, and long-lasting, it is a pragmatic option for future household sanitation.

In the future, we wish to continue testing our solutions with varied concentrations to maximize the longevity of their antibacterial potential. We would like to further research the antiviral potential of our solutions on other molecules including COVID-19. A study conducted by Govind et al. (2021) demonstrated promising results on the antiviral capabilities of copper through its production of certain toxins which combat COVID-19 viral attacks [25]. Another study by Kassaa et al. (2014) analyzed the antiviral potential of lactic acid bacteria through direct probiotic-virus interaction and other mechanisms [26]. The antifungal properties of L-pyroglutamic acid may also be explored.

5. Conclusions

We synthesized copper-based aqueous antibacterial solutions with 1% Lactic Acid (Solution A) and 1% L Pyroglutamic Acid (Solution B). The efficacy of the solutions reflected in the average percent loss of E. coli in comparison to 2% Lysol dilution over 28 minutes, 128 minutes, and 72 hours' time-intervals. Optimal efficacy was exhibited in Solution B given its long-lasting antibacterial activity. Coupled with low production cost, our results foreground the potential of L-pyroglutamic Acid/copper solutions in aqueous household disinfecting. Future research must be conducted with an array of microorganisms and health risks must be considered.

Limitations of the Study

Our research was limited by time constraints for data collection. Thus, the testing-intervals were restricted to 28 minutes, 128 minutes, and 72 hours. To increase the range of data collection, longer time-intervals are recommended.

Acknowledgement

We would like to thank Mr. Jay Rosenberg of Cypress Bay High School for his valuable input and contributions.

Conflict of Interests

Authors did not have any conflicts.

Financial Support

It was partially supported by Cypress Bay High School.

Author Contributions

Both authors had equal contributions.

Ethical Approval

No ethical approval was needed for this study.

Informed Consent

Informed consent form was submitted to the journal.

Availability of Data and Materials

Not applicable for this study.

References

1. Montero DA, Arellano C, Pardo M et al. Antimicrobial properties of a novel copper-based composite coating with potential for use in healthcare facilities. *Antimicrob Resist Infect Control.* 2019; 8(3):1-10.
2. Grass G, Rensing C, Solioz M. Metallic copper as an antimicrobial surface. *Appl Environ Microbiol.* 2011; 77(5):1541-47.
3. Espirito Santo C, Wen Lam E, Elowsky CG et al. Bacterial killing by dry metallic copper surfaces. *Appl Environ Microbiol*. 2011; 77: 794-802.
4. Espirito Santo C, Morais PV, Grass G. Isolation and characterization of bacteria resistant to metallic copper surfaces. *Appl Environ Microbiol*. 2010; 76:1341-48.
5. Espirito Santo C, Taudte N, Nies DH, Grass G. Contribution of copper ion resistance to survival of Escherichia coli on metallic copper surfaces. *Appl Environ Microbiol*. 2008; 74:977-986.
6. Faundez G, Troncoso M, Navarrete P, Figueroa G. Antimicrobial activity of copper surfaces

against suspensions of Salmonella enterica and Campylobacter jejuni. *BMC Microbiol.* 2004; 4:19.

7. Vincent M, Duval RE, Hartemann P, Engels-Deutsch M. Contact killing and antimicrobial properties of copper. *J Appl Microbiol*. 2018; 124(5):1032-46.
8. Trevors JT, Cotter CM. Copper toxicity and uptake in microorganisms, *J Industr Microbiol*. 1990; 6(2):77-84.
9. Usman MS, El Zowalaty ME, Shameli K, Zainuddin N, Salama M, Ibrahim NA. Synthesis, characterization, and antimicrobial properties of copper nanoparticles. *Int J Nanomedicine*. 2013; 8:4467-79.
10. Fujimori Y, Sato T, Hayata T, et al. Novel antiviral characteristics of nanosized copper(I) iodide particles showing inactivation activity against 2009 pandemic H1N1 influenza virus. *Appl Environ Microbiol.* 2012; 78(4):951-55.
11. Borkow G, Lara HH, Covington CY, Nyamathi A, Gabbay J. Deactivation of human immunodeficiency virus type 1 in medium by copper oxide-containing filters. *Antimicrob Agents Chemother*. 2008; 52(2):518-25.
12. Hang X, Peng H, Song H, Qi Z, Miao X, Xu W. Antiviral activity of cuprous oxide nanoparticles against Hepatitis C Virus in vitro. *J Virol Methods.* 2015; 222:50-57.
13. Applerot G, Lellouche J, Lipovsky A et al. Understanding the antibacterial mechanism of CuO nanoparticles: revealing the route of induced oxidative stress. *Small*. 2012; 8(21):3326-37.
14. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. *Int J Nanomed*. 2012; 7:6003-9.
15. Dickson JS, Anderson ME. Microbiological Decontamination of Food Animal Carcasses by Washing and Sanitizing Systems: A Review. *J Food Prot 1 February*. 1992; 55(2):133-40.
16. Beal J, Niven S, Campbell A, Brooks P. The effect of copper on the death rate of Salmonella typhimurium DT104:30 in food substrates acidified with organic acids. *Letters Applied Microbiol.* 2004; 38:8-12.
17. Ibrahim DSS, Amer MN, El-Shishtawy HM, Elmaghraby MMK, Abdellatif AAM, El-Deriny MM. Nematicidal Activity of Lactic Acid Bacteria against Root-Knot Nematodes. *J Microbiol Biotechnol*. 2022; 7(1):000216.
18. Schmidt MG, Fairey SE, Attaway HH. In situ evaluation of a persistent disinfectant provides continuous decontamination within the clinical environment. *Am J Infec Control.* 2019; 47(6):732-34.
19. Kolev S, McKelvie I. Advances in Flow Injection Analysis and Related Techniques. In: Barcelo D. Comprehensive Analytical Chemistry, 1st ed. Elsevier Science. 2008: 311-42.
20. Gyawali R, Ibrahim SA, Abu Hasfa SH, Smqadri SQ, Haik Y. Antimicrobial Activity of Copper Alone and in Combination with Lactic Acid against Escherichia coli O157:H7 in Laboratory Medium and on the Surface of Lettuce and Tomatoes. *J Pathog*. 2011; 2011:1-9.
21. Al-Holy M, Castro L, Al-Qadiri H. Inactivation of Cronobacter spp. (Enterobacter sakazakii) in infant formula using lactic acid, copper sulfate and monolaurin. *Letters Applied Microbiol.* 2010; 50:246-51.
22. Alakomi HL, Skyttä E, Saarela M, Latva-Kala K, Helander IM. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Appl Environ Microbiol.* 2000; 66(5):2001-5.
23. Tan SW, Chai CL, Moloney MG, Thompson AL. Synthesis of mimics of pramanicin from pyroglutamic acid and their antibacterial activity. *J Organic Chem.* 2015**;** 80(5):2661-75.
24. Gang F, Zhu F, Lİ X, Wei J, Wu W, Zhang J. Synthesis and bioactivities evaluation of L-pyroglutamic acid analogues from natural product lead. *Bioorg Med Chem.* 2018; 26(16):4644-49.
25. Govind V, Bharadwaj S, Sai Ganesh MR et al. Antiviral Properties of Copper and Its Alloys to Inactivate Covid-19 Virus: A Review. *BioMetals.* 2021; 34:1217–35.
26. Al Kassaa I, Hober D, Chihib N, Drider D. Antiviral Potential of Lactic Acid Bacteria and Their Bacteriocins. *Probiotics Antimicrob.* 2014; 6:177-85.

ISSN: 2717-8161 RESEARCH ARTICLE

New Trend Med Sci 2023; 4(2): 58-65. https://dergipark.org.tr/tr/pub/ntms

Effect of a TNF-Alpha Inhibitor on Anxiety and Depression-Like Behaviors in a Mouse Chemobrain Model

Mehmet Oz[1*], Nurhan Akaras[2]

[1]Department of Physiology, Faculty of Medicine, Aksaray University, Aksaray, Turkey
[2]Department of Histology and Embryology, Faculty of Medicine, Aksaray University, Aksaray, Turkey

Article History
Received 19 Jan 2023
Accepted 07 Mar 2023
Published Online 25 May 2023

[*]***Corresponding Author***
Mehmet Oz
Department of Physiology
Faculty of Medicine
Aksaray University
Aksaray, Turkey
Phone: +90 03822882900
E-mail: ozmhmt@gmail.com

Doi: 10.56766/ntms.1239435

Authors' ORCIDs
Mehmet Oz
http://orcid.org/0000-0003-4167-2623
Nurhan Akaras
http://orcid.org/ 0000-0002-8457-9448

Abstract: Although chemotherapy increases the survival rate of cancer patients, it causes significant side effects such as deterioration in cognitive functions that generate a decline in their living standards. In our study, the effect of adalimumab (TNF-Alpha inhibitor) on anxiety and depression-like behaviors in mice with cognitive impairment with methotrexate was investigated. In our study, a total of 24 mice, 6 mice in each group, were used, and the first group was considered as the control. A single dose of methotrexate (40 mg kg^{-1}) was administered intraperitoneally to the other two groups, and a chemobrain model was created. Adalimumab (10 mg kg^{-1}) was administered twice, 1 hour and 5 days before methotrexate and/or vehicle administration. Anxiety-like behaviors were measured with elevated plus maze (EPM) test and open field test (OF), depression-like behaviors were measured with tail suspension test (TST), and hippocampal tissue was examined histopathologically. Methotrexate decreased the time spent in the central zone in the open-field arena, the time spent in the open arms in the elevated plus maze test, and increased the duration of immobility in the tail suspension test in rats. Methotrexate caused a decrease in the number of neuronal cells in the CA3 region of the hippocampus, as well as neurodegenerative and atrophic changes. Adalimumab improved the time spent in open arms in the OF test and the number of open arm entries in the EPM, immobility time on TST, and histopathological changes. In this study, it was shown that methotrexate-related anxiety and depression-like behavioral disorders were prevented by adalimumab treatment, but further studies are recommended to investigate the mechanisms mediating the therapeutic effect of adalimumab. ©2023 NTMS.

Keywords: Methotrexate; Adalimumab; Anxiety; Depression; Neuroinflammation.

1. Introduction

Associated with the increase in the prevalence of malignant diseases, the use of different types of drugs at more aggressive doses for therapeutic purposes, as well as the development of a large number of new chemotherapeutic agents, has led to reduced recurrence and increased survival rate for many types of cancer. However, it is inevitable to observe different complications that lead to a decrease in living standards. One of these is cognitive dysfunction associated with the use of systemic chemotherapy, which includes mild cognitive impairments, also known as 'chemophog' or 'chemobrain'. Such

Cite this article as: Oz M and Akaras N. Effect of a TNF-Alpha Inhibitor on Anxiety and Depression-Like Behaviors in a Mouse Chemobrain Model. *New Trend Med Sci*. 2023; 4(2):58-65. Doi:10.56766/ntms.1239435

conditions are non-fatal but persistent, so long-term survivors' quality of life is significantly impaired and clinically important as they prevent a return to their pre-cancerous state [1]. Methotrexate (Mtx) is a chemotherapeutic that reduces the growth and proliferation of tumor cells and is widely used in the treatment of types of cancer, and significantly increases the survival rates of patients with acute lymphoblastic leukemia and lymphoma [2]. Mtx is a folic acid antagonist that negatively affects important cellular bioprocesses such as DNA, RNA and protein synthesis by inhibiting the dihydrofolate reductase enzyme, which acts by changing the intracellular folate distribution [3]. One of the important complications of Mtx is the pathophysiological changes that lead to neurodegenerative processes in the brain, especially in areas where cognitive functions are carried out, such as the hippocampus. Thus, in addition to improving survival, Mtx therapy is associated with persistent impairments in survivors' cognitive functions such as attention, reasoning, memory, learning, and executive function [4]. The pathophysiological changes associated with this neurotoxicity are multifaceted and are of increasing interest by researchers. Numerous mechanisms have been suggested to explain the underlying mechanism of Mtx therapy-induced cognitive impairment. Mtx causes increased hippocampal endoplasmic reticulum stress[5], decreased hippocampal antioxidant enzyme activity, such as SOD, CAT, GPx and GSH, and decreased levels of some neurotrophic factor, such as BDNF, increased lipid peroxidation [6], induced apoptosis and associated disruption of hydrogen sulfide production in the hippocampal CA1 region [5, 7], altered dendritic branching and spine morphology [8], reduced cell proliferation, survival and cell differentiation in the hippocampal DG region [9–11], increased amount of proinflammatory cytokines that cause neuroinflammation [12, 13].

The genes involved in the generation of the inflammatory response are normally suppressed, but become active when cells sense information about infection or injury, and this response must be strengthened to mount an effective immune response and initiate antimicrobial or antiviral activity. Some of the important enhancers of this immune response are proinflammatory cytokines, such as TNF-alpha, and IL-1beta [14]. TNF-alpha, a 25 kDA type II transmembrane protein, is produced by active macrophages, as well as by different types of cells such as kupffer cells of the liver, ovarian cells, beta and T cells in the immune system, and astrocytes in the brain [15]. Neuroinflammation, which plays an important role in the etiology of neurodegenerative disorders that cause deterioration in cognitive activities such as anxiety, depression and learning-memory, is closely related to the excessive increase in the expression of these proinflammatory cytokines [12, 13]. In the literature, there are studies reporting improvement in cognitive functions as a result of suppressing or reversing neurodegenerative processes by inhibiting proinflammatory cytokines, especially TNF-alpha, with plant-derived [16] or different chemicals [17]. Adalimumab (Ada), a recombinant monoclonal human antibody, is a tnf-alpha inhibitor, it can exert its effect in two ways, binds directly to tnf-alpha, preventing it from binding to its receptor or it dissolves to avert TNF-alpha from binding with cell surface receptors and inhibits the functions of TNF-alpha by binding to its receptors [18, 19]. Ada cannot cross the blood-brain barrier, but a recent study has shown that the TNF-alpha inhibitor, which cannot cross the blood-brain barrier, is as effective as its analogue that can cross the blood brain barrier [20]. The number of studies showing the positive effects of Ada use in critical pathologies where inflammation plays an important role is increasing, and Ada has also been proven to be effective in cognitive disorders due to neuroinflammation [17, 19]. Therefore, we hypothesized that Ada would be effective in the "chemobrain" model, which develops due to chemotherapeutic use and in which neuroinflammation participates. In addition, we could not find any study in the medical literature showing the effect of Ada on methotrexate-induced anxiety and depression-like behaviors in mice.

With this background, this study was planned to evaluate the effect of Ada, a TNF-alpha inhibitor, on anxiety-like and depression-like behaviors in a chemotherapeutic drug-induced "chemobrain" mouse model, as well as histopathological changes in the brain hippocampal region.

2. Material and Methods

2.1. Experimental Animals and Design

In this study, 10-week-old male Swiss Albino mice obtained from Aksaray University Experimental Animals Unit were used. Mice weighed an average of 32.29±2.03 g, groups were formed with 6 mice in each cage, and the mice were taken to the cages 10 days before the study and adapted to each other. Throughout the study, mice were kept in a quiet and noise-free environment and standard laboratory conditions (light-dark period, 55% humidity, 22-24 °C temperature), with unlimited access to water and food. The experimental procedure was carried out with care and attention, in accordance with the care and use guidelines of experimental animals, after the approval of Aksaray University Experimental Animals Local Ethics Committee (Ethics Committee Date and Number: 19.09.2022-44). The study consisted of a total of 4 groups, with 6 mice in each group; the first group is considered as the control group, no drug was administered, the second group is the Mtx group; A single intraperitoneal dose of 40 mg/kg methotrexate was administered 24 hours before the behavioral tests. The third group is Mtx plus Ada; It consisted of mice administered both methotrexate and Ada. The fourth group is Ada; Two doses of 10 mg/kg Ada were administered intraperitoneally as described above.

2.2. Drug administration

All the drugs used in our study were prepared fresh just before use and the remaining drugs were evaluated as medical waste. Mtx (Metoart Con, Kocak Farma Drug and Chemical Industry A.Ş. Istanbul, Turkey) was administered intraperitoneally as a single dose of 40 mg/kg, since it is the lowest dose of Mtx that causes depression and anxiety-like behaviors in mice [12]. While the half-life of Ada (Humira, AbbVie Tıbbi İlaçlar San. Tic. Ltd. Şti, Turkey) is about 2 weeks in humans, this period is shorter in rodents [21]. For this reason, Ada was administered at a dose of 10 mg/kg[22] in two doses, 5 days apart (1 hour and 5 days before Mtx injection). The drugs were diluted with physiological saline, mice not treated with Mtx and/or Ada were injected with the same volume of saline to avoid the placebo effect. Behavioral tests were started 24 hours after MTX administration, with a 2-hour break between each test. 2 hours after the end of behavioral tests, brain tissues were removed from all mice under end-stage anesthesia (ketamine & xylazine) and immediately placed in 10% formaldehyde solution.

2.3. Open Field Test (OF)

The OF test was achieved to evaluate the anxiety-like behaviors and locomotor activities of the mice. The OF arena consisted of an empty 40 x 40 x 40 cm box made of water and chemical resistant wood, and the albino mice were contrastingly dark. Before the study, the floor of the open field arena was marked as 16 equal squares, 4 squares in the center and 12 squares on the side were coded as perimeters. Each mouse was placed in the middle of the arena and allowed 5 minutes to explore the area. Time spent in the central zone, vertical movements (Rearing count; mice standing up on their hind legs) and horizontal movements (crossing number; at least three paws in the same square) were evaluated [23]. After each experiment, the OF arena was cleaned so that residual odor did not affect the next mouse.

2.4. Elevated Plus Maze (EPM)

In our study, the EPM paradigm was used to measure anxiety-like behaviors. The EPM arena consisted of 4 arms, 35x5 cm in length, two of which were open arms and the other two closed arms. These 4 arms were combined with a 5x5 cm square in the center, giving the arena a cross shape. The closed arms were open at the top and had 36 cm high walls. The arena was positioned 45 cm above the floor. Each mouse was placed in the square in the middle of the arena, facing the open arm, and their behavior on the EPM arena was recorded for 5 minutes. During this time, the total time spent in open arms and the number of entries in each arm were measured [24].

2.5. Tail Suspension Test (TST)

The TST was achieved to assess the depression-like behavior of the mice. For this purpose, in the compartment made of black material resistant to water and chemicals, there was a hook for hanging each mouse by its tail with the help of an adhesive tape. Four mice were able to do this test at the same time, but the mice were prevented from seeing each other with a wall placed between them. The mice suspended by their tails were video-recorded for 6 minutes, during which time they remained motionless was recorded. Moments when the mice remained passive, made no effort to escape, and remained completely motionless were considered as immobility.

2.6. Histopathological examination of brain tissues

As part of the histopathological analysis, mouse brains were stored in 10% formalin for fixation for 48 hours. After the fixed brain tissues were washed in water overnight, blocks were prepared by performing routine histological procedures. Sections of 4 μm thickness were taken from the pre-prepared blocks using microtone and Hematoxylin-Eosin (H&E) staining was applied. Histopathological analyzes were photographed with a computer-assisted microscope (Olympus Cx 43; Japan) and reviewed by the histologist.

2.7. Statistical analysis

All data from the study were presented as mean ± SD. One-way analysis of variance and post hoc test were used to determine statistical differences between groups (ANOVA and TUKEY)

The differences observed when the p was less than 0.05 were considered significant.

3. Results

3.1. Effect of Ada on locomotor activity in a mouse chemobrain model

In our study, OF was used to evaluate locomotor activity. In the statistical analysis between the groups, no significant discrepancy was found in locomotor activity evaluated by crossing number (Figure 2A, $p>0.05$). Thus, it can be said that the cognitive data obtained from all mice are independent of locomotor activity.

3.2. Effect of Ada on anxiety-like behaviors in a mouse chemobrain model

Anxiety-like behaviors were assessed using the OF and the EPM test. The time spent in the central area of the mice that received only Mtx injection was found to be lower than the control mice (Figure 2B, $p<0.001$) in the OF test. This indicates that Mtx administered at a dose of 40 mg/kg 24 hours before the behavioral test causes anxiety-like behaviors. Ada treatment improved mtx-induced reduced time spent in the central area of the OF test (Figure 2B, $p<0.001$). The findings in mice receiving only Ada treatment were similar to the control group. Mice standing on their hind legs and looking around in the open field arena is considered exploratory behavior (Rearing number) 23. In our study, no significant difference was found in terms of the number of rearing (Figure 2C, $p >0.05$).

In the EPM test, mice that received an injection of Mtx had a shorter open-arm stay compared to the control

(Figure 3A, p<0.001). The decrease in the open arm time in this test is associated with anxiety-like behaviors 24. In mice receiving Mtx, Ada treatment improved their time in the open arm (Figure 3A, p<0.001). This time was higher than for mice in the Mtx group, but not enough to reach the level of mice in the control and Ada group (Figure 3A). In the EPM test, the number of open arm entries was similar in the control and Ada groups, and it was found to be significantly higher than the Mtx and Mtx plus Ada groups. (Figure 3B, p>0.05).

3.3. Effect of Ada on depression-like behaviors in a mouse chemobrain model

The TST was used to evaluate depression-like behaviors in the mouse chemobrain model induced by Mtx. In this test, all mice are hung from their tails to a special apparatus and video recording is made for 6 minutes. Immobility time is used as a measure of depression-like behavior. In our study, it was found that mice in the Mtx group showed higher immobility time than the control group (Figure 4, p<0.001). This result suggests that Mtx injection induced depression-like behavior in mice. On the other hand, Ada treatment administered in two doses 1 hour and 5 days before the Mtx injection resulted in the improvement of depression-like behaviors (Figure 4, p<0.001). In the Ada-only group, the immobility time was the same as in the control and Mtx plus Ada groups (p>0.05), but lower than the mice in the Mtx group (Figure 4, p<0.001).

3.4. Effect of Ada on histopathological changes in brain tissue

When the H&E staining results were evaluated, it was seen that the arrangement and density of neuron cells in the hippocampus region of the mice in the control group were normal. As a result of histological examination in the dentate gyrus (DG) and corpus ammonis (CA) regions, the morphology of the neurons was pyramidal, healthy and easily distinguishable (Figure 5A). When the hippocampus images of the mice in the Mtx-treated group were examined, the pyramidal cells were sparse, irregular, and the images were unclear compared to the control. In addition, degenerative and atrophic changes were observed in neurons in this group (Figure 5B). In the groups given Mtx and Ada together, it was observed that the pathological changes related to Mtx decreased and the number of neuron cells increased. Improvements were detected in neuron and glial cells in mouse hippocampus in this group (Figure 5C). There was no significant change in any of the neurons belonging to the hippocampus parts of the groups that were given only Ada, and it was determined that they showed a characteristic arrangement close to the control.

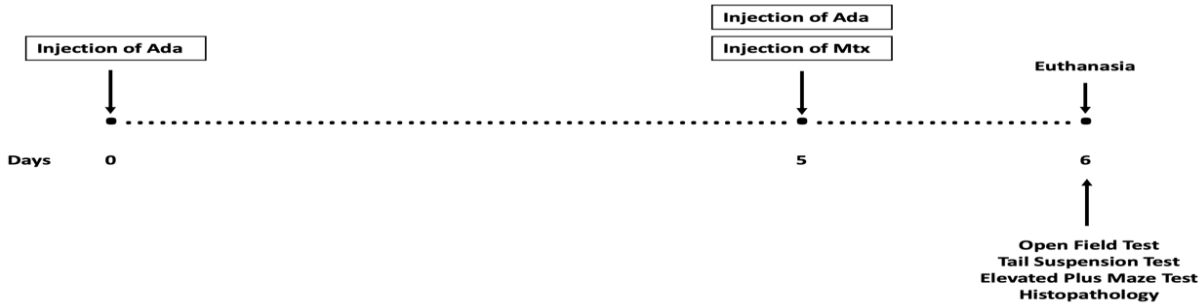
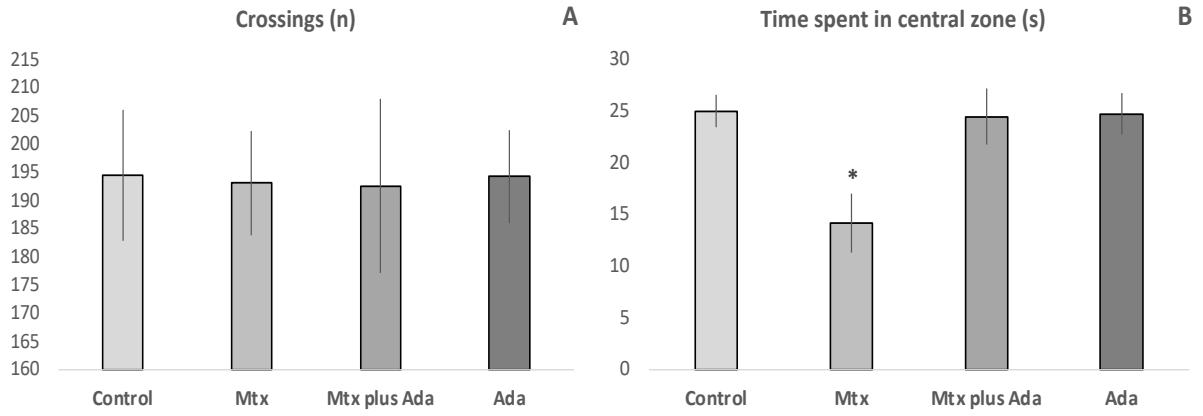


Figure 1: Schematic presentation of the protocol used for the study.



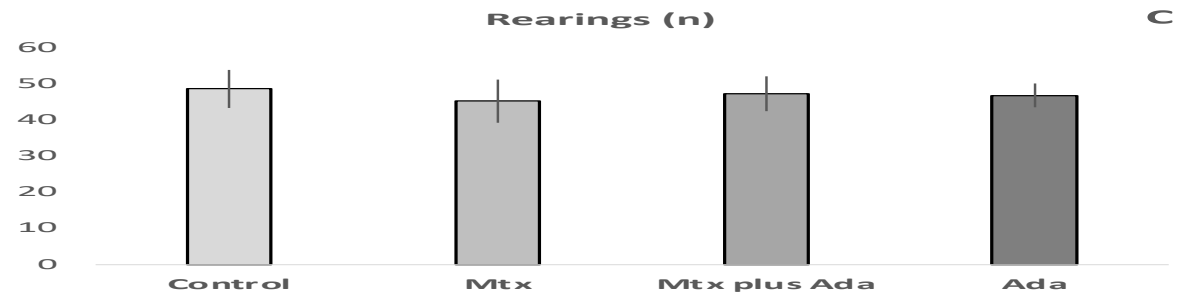


Figure 2: Effect of Ada on (A) time spent in central zone (B) number of rearing (C) number of crossing in the open field test in mice. The data are expressed as the Means±SD. Asterisk (*) indicates significance compared with other groups. p<0.001; one-way ANOVA.

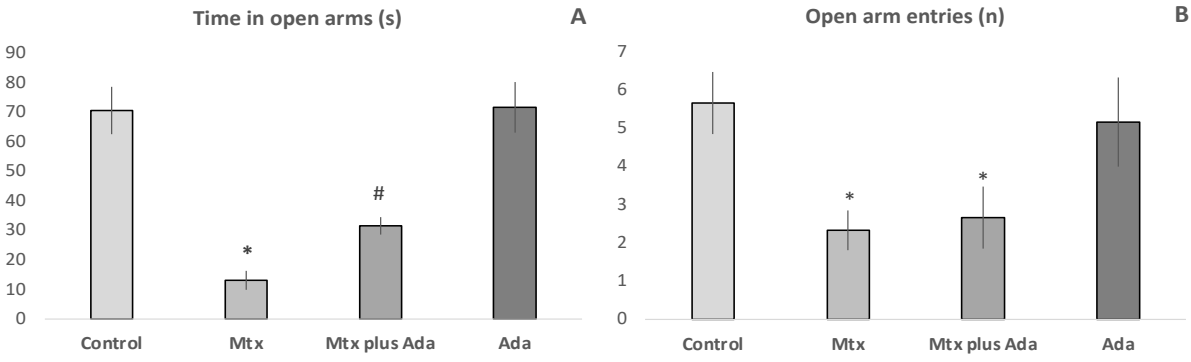


Figure 3: The effects of Ada on open arm time (A) and open arm entries (B) in the elevated plus maze test. Data presented as means ± SD. Asterisk (*) indicates significance compared with other groups. Hash (#) indicates significance compared with other groups. p<0.001; one-way ANOVA.

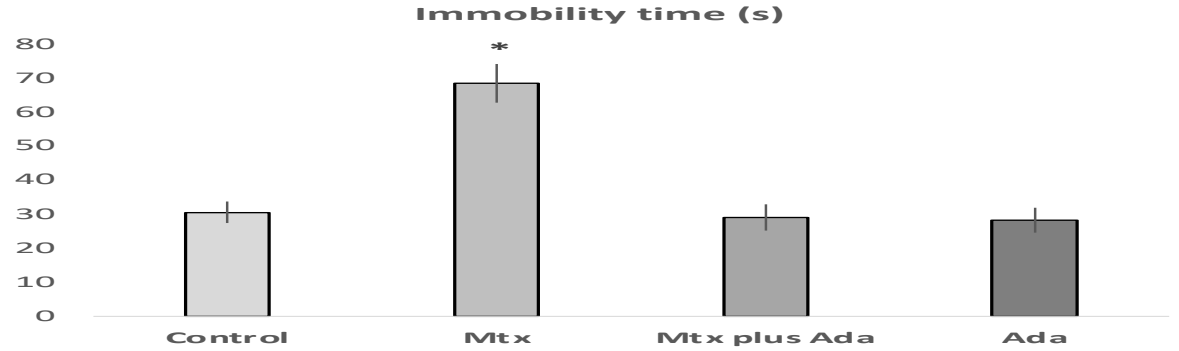


Figure 4: Changes in the immobility time in tail suspension test among each group. The data are expressed as the Means±SD. Asterisk (*) indicates significance compared with other groups. p<0.001; one-way ANOVA.

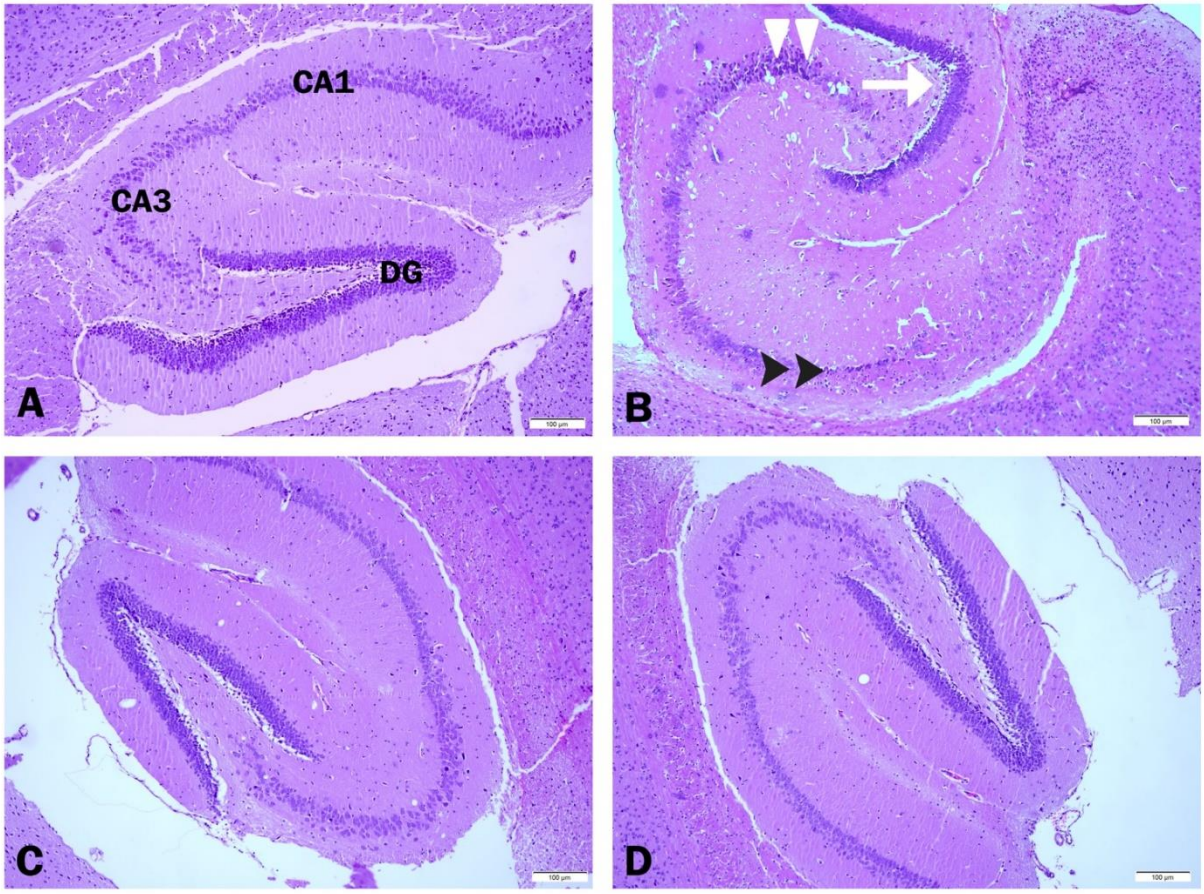


Figure 5: Light micrographs of brain hippocampus regions in mice. A) Control group; Corpus Ammonis 1 (CA1), Corpus Ammonis 3 (CA3) and Dentate Gyrus (DG) regions in normal morphology, (B) Mtx group; Neuronal degeneration in hippocampal region (white arrowhead), decrease in neuronal cells in CA3 region (black arrowhead), dentate gyrus structural disorder (arrow), (C) Mtx plus Ada group, (D) Ada group; H&E staining; scale bar=100 microns.

4. Discussion

In our study, the protective effects of Ada, a TNF-alpha inhibitor, on methotrexate-induced anxiety and depression-like behaviors were investigated. Administration of methotrexate to mice caused anxiety-like behaviors such as decreased time spent in the central zone in the OF, decreased time spent in the open arm and the number of open arm entries in the EPM, and depression-like behaviors such as increased immobility time in the TST. Ada administered in two doses before methotrexate injection increased the time spent in the central zone in the open field test, in the open arm in the EPM, and decreased immobility time in the TST. These results suggest that Ada may be a useful instrument to ameliorate mtx-dependent anxiety and depression-like pathologies.

One of the side effects of chemotherapeutic drugs that we cannot avoid in the treatment of malignant diseases is the deterioration of cognitive functions, which is called chemobrain [1]. Because cognitive impairment due to chemotherapeutic use negatively impacts post-survival life, researchers often use experimental animal models to better define the precise mechanism of this physiopathological phenomenon. Mtx, a folate inhibitor, is one of the chemotherapeutics commonly used for this purpose, causing cognitive dysfunction in both rats and mice [5, 12]. Mtx treatment causes mice to underperform in new object location and novel object recognition tests assessing spatial and recognition memory [6, 9, 11], and in the Morris water maze test, a well-established learning and memory paradigm in experimental animals [5, 7, 11], as well as the passive avoidance test, a hippocampal learning paradigm [12]. Mtx is not only acutely effective as in the well-organized studies mentioned above, but it has also been reported that cognitive deterioration still continues in the chronic period [25] even after 6-16 months [26] after the end of mtx administration. In addition to negatively affecting learning and memory performance, Mtx also affects depression-like behaviors. It has been shown that Mtx administered as a single dose of 40 mg/kg intraperitoneally leads to more immobility time in the forced swimming test, which is a test evaluating depression-like behaviors [12]. In our study, behavioral tests were performed 24 hours after injecting a single dose of 40 mg/kg Mtx into mice. Mtx decreased the time spent in the central zone in the OF test (Figure 2B), the time spent in the open arm in the EPM test (Figure 3A), while it increased the immobility time in the TST test (Figure 4). These results suggest that MTX induces anxiety and depression-like behaviors, respectively. There was no statistically significant difference

between all groups in locomotor activity evaluated with the OF test (p> 0.001, Figure 2A), so the results obtained from our study are independent of locomotor activity. The first limitation of our study is that we did not evaluate well-defined learning and memory impairment in Mtx-related chemobrain models. However, we thought that targeting the physiopathology of anxiety and depression, which may adversely affect the pathogenesis of the disease due to its comorbid relationship with the disease, may contribute to better management of complications.

Possible mechanisms of cognitive impairment due to Mtx are various, such as decreased hippocampal tissue antioxidant enzyme activity and increased lipid peroxidation [6], decreased hippocampal neurogenesis [5, 10, 12, 25], and impaired cell proliferation in the hippocampal dentate gyrus [9–11]. In addition, a growing body of evidence indicates that neuroinflammation plays an important role in cognitive impairment due to Mtx [9–11]. The increase in proinflammatory cytokines responsible for neuroinflammation due to chemotherapeutic drugs has led to an increased interest in studies that can improve cognitive dysfunctions by suppressing these cytokines. We evaluated the effect of Ada, a tnf alpha inhibitor shown to ameliorate neuroinflammation in an experimental model of chronic cerebral hypoperfusion [17], on chemotherapy-induced anxiety and depression-like behaviors associated with increased proinflammatory cytokines. According to the findings of our study, Ada administered twice intraperitoneally (1 hour and 5 days before Mtx injection) at a dose of 10 mg/kg improved Mtx-related anxiety and depression-like behaviors. Ada increased the time spent in the central zone in the OF test (Figure 2B, p<0.001), the time spent in the open arm in the EPM test (Figure 3A, p<0.001), and decreased the immobility time in the FST test (Figure 4, p <0.001). Our results are consistent with similar studies, Ada improves cognitive functions by increasing hippocampal tissue BDNF level, decreasing TNF alpha and IL-6 levels in Alzheimer mouse model [19], reducing oxidative stress and inhibiting NF-κB signaling in chronic cerebral hypoperfusion model [17]. Although we have shown that Ada inhibits impaired anxiety and depression-like behaviors in the chemotherapy-induced chemobrain model, the second limitation of our study is that a physiopathological parameter related to the mechanism of this effect was not evaluated. However, Ada ameliorated the reduction in neuronal cells, neuronal degeneration, and atrophic changes in the CA3 region of the hippocampus due to methotrexate (Figure 5). In addition, this report is the first study in the literature to show the effect of Ada on anxiety and depression-like behaviors in an Mtx-induced chemobrain model.

5. Conclusion

Based on the results of this study, adalimumab supplementation prevented impaired anxiety and depression-like behaviors in a mouse model of Mtx-induced chemobrain. It also improved hippocampal tissue degenerative and atrophic changes. This suggests that Ada may be a therapeutic agent, especially in neurodegenerative disorders accompanied by neuroinflammation causing anxiety and depression.

Limitations of the Study

The main limitations of our study are that the learning and memory activities of the mice were not evaluated after the chemobrain model was created, and that tnf-alpha levels were not detected both in the brain tissue and in the blood. In addition, the anxiety and depression-producing mechanism of MTX and the inability to study the mechanism of the therapeutic role of Ada in this effect are other limitations.

Acknowledgement

None.

Conflict of Interests

There is no conflict of interest.

Financial Support

This study received no financial support.

Author Contributions

Conseption-Oz M; Design-Oz M; Supervision-Oz M; Materials-Oz M, AKARAS N; Data Collection and/or Processing-Oz M, AKARAS N; Analysis and/or Interpretation-Oz M, AKARAS N; Literature Review-Oz M, AKARAS N; Writing-Oz M; Critical Review-Oz M, AKARAS N.

Ethical Approval

The study was approved by Aksaray University Experimental Animal Ethics Committee (Approval Date: 19.09.2022 and Approval number: 44).

Data sharing statement

None.

Consent to participate

None.

Informed Consent

None.

References

1. Taillibert S, Voillery D, Bernard-Marty C. Chemobrain: is systemic chemotherapy neurotoxic? *Curr Opin Oncol*. 2007; 19(6):623-27.
2. Schrappe M, Reiter A, Riehm H. Prophylaxis and treatment of neoplastic meningeosis in childhood acute lymphoblastic leukemia. *J Neurooncol*. 1998; 38(2-3):159-65.
3. Strober BE, Menon K. Folate supplementation during methotrexate therapy for patients with psoriasis. *J Am Acad Dermatol*. 2005; 53(4):652-59.
4. Argyriou AA, Assimakopoulos K, Iconomou G, Giannakopoulou F, Kalofonos HP. Either called "chemobrain" or "chemofog," the long-term chemotherapy-induced cognitive decline in cancer survivors is real. *J Pain Symptom Manage*. 2011; 41(1):126-39.
5. Lv S, Wu N, Wang Q, Yang LH. Endogenous hydrogen sulfide alleviates methotrexate-induced cognitive impairment by attenuating endoplasmic

reticulum stress-induced apoptosis via CHOP and caspase-12. *Fundam Clin Pharmacol*. 2020; 34(5):559-70.

6. Sritawan N, Suwannakot K, Naewla S, et al. Effect of metformin treatment on memory and hippocampal neurogenesis decline correlated with oxidative stress induced by methotrexate in rats. *Biomed Pharmacother*. 2021; 144:112280.
7. Wu LL, Lin DN, Yu LH, Yang LH. Endoplasmic reticulum stress plays an important role in methotrexate-related cognitive impairment in adult rats. *Int J Clin Exp Pathol*. 2017; 10(10):10252-60.
8. Alexander TC, Simecka CM, Kiffer F, et al. Changes in cognition and dendritic complexity following intrathecal methotrexate and cytarabine treatment in a juvenile murine model. *Behav Brain Res*. 2018; 346:21-28.
9. Sirichoat A, Anosri T, Kaewngam S, et al. Neuroprotective properties of chrysin on decreases of cell proliferation, immature neurons and neuronal cell survival in the hippocampal dentate gyrus associated with cognition induced by methotrexate. *Neurotoxicology*. 2022; 92:15-24.
10. Sritawan N, Prajit R, Chaisawang P, et al. Metformin alleviates memory and hippocampal neurogenesis decline induced by methotrexate chemotherapy in a rat model. *Biomed Pharmacother*. 2020; 131:110651.
11. Sirichoat A, Krutsri S, Suwannakot K, et al. Melatonin protects against methotrexate-induced memory deficit and hippocampal neurogenesis impairment in a rat model. *Biochem Pharmacol*. 2019; 163:225-33.
12. Yang M, Kim JS, Kim J, et al. Acute treatment with methotrexate induces hippocampal dysfunction in a mouse model of breast cancer. *Brain Res Bull*. 2012; 89(1-2):50-56.
13. Aslankoc R, Ozmen O, Yalcın A. Astaxanthin ameliorates damage to the cerebral cortex, hippocampus and cerebellar cortex caused by methotrexate. *Biotech Histochem*. 2022; 97(5):382-93.
14. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell*. 2010; 140(6):918-934.
15. Aggarwal BB, Gupta SC, Sung B. Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. *Br J Pharmacol*. 2013; 169(8):1672-92.
16. Kaur H, Patro I, Tikoo K, Sandhir R. Curcumin attenuates inflammatory response and cognitive deficits in experimental model of chronic epilepsy. *Neurochem Int*. 2015; 89:40-50.
17. Xu JJ, Guo S, Xue R, et al. Adalimumab ameliorates memory impairments and neuroinflammation in chronic cerebral hypoperfusion rats. *Aging*. 2021; 13(10):14001-14.
18. Correger E, Marcos J, Laguens G, Stringa P, Cardinal-Fernández P, Blanch L. Pretreatment with adalimumab reduces ventilator-induced lung injury in an experimental model. *Rev Bras Ter Intensiva*. 2020; 32(1).
19. Park J, Lee SY, Shon J, et al. Adalimumab improves cognitive impairment, exerts neuroprotective effects and attenuates neuroinflammation in an Aβ1-40-injected mouse model of Alzheimer's disease. *Cytotherapy*. 2019; 21(6):671-82.
20. Ou W, Yang J, Simanauskaite J, et al. Biologic TNF-α inhibitors reduce microgliosis, neuronal loss, and tau phosphorylation in a transgenic mouse model of tauopathy. *J Neuroinflammation*. 2021; 18(1):312.
21. Allez M, Karmiris K, Louis E, et al. Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: Definitions, frequency and pharmacological aspects. *J Crohns Colitis*. 2010; 4(4):355-66.
22. Durmaz S, Kurtoğlu T, Barbarus E, Eliyatkın N, Yılmaz M. TNF-alpha inhibitor adalimumab attenuates endotoxin induced cardiac damage in rats. *Acta Cir Bras*. 2020; 35(2):e202000202.
23. Wang JY, Zhang Y, Chen Y, et al. Mechanisms underlying antidepressant effect of transcutaneous auricular vagus nerve stimulation on CUMS model rats based on hippocampal α7nAchR/NF-κB signal pathway. *J Neuroinflammation*. 2021;18(1):291.
24. Fathalizade F, Baghani M, Khakpai F, Fazli-Tabaei S, Zarrindast MR. GABA-ergic agents modulated the effects of histamine on the behaviour of male mice in the elevated plus maze test. *Exp Physiol*. 2022; 107(3):233-42.
25. Wen J, Maxwell RR, Wolf AJ, Spira M, Gulinello ME, Cole PD. Methotrexate causes persistent deficits in memory and executive function in a juvenile animal model. *Neuropharmacology*. 2018; 139:76-84.
26. Berlin C, Lange K, Lekaye HC, et al. Long-term clinically relevant rodent model of methotrexate-induced cognitive impairment. *Neuro Oncol*. 2020; 22(8):1126-37.

ISSN: 2717-8161 RESEARCH ARTICLE

New Trend Med Sci 2023; 4(2): 66-72. https://dergipark.org.tr/tr/pub/ntms

Traumatic Stress and Health Anxiety in Intensive Care Workers During the Covid-19 Pandemic

Esra Demiryürek[1*], Havva Kocayigit[1]

[1]Department of Psychiatry, Sakarya Private Clinic, Sakarya, Turkey
[2]Department of Anesthesiology and Reanimation, Sakarya University Education and Research Hospital, Sakarya, Turkey

Article History
Received 25 Nov 2022
Accepted 11 Oct 2023
Published Online 25 May 2023

****Corresponding Author***
Esra Demiryurek
Department of Psychiatry
Sakarya Private Clinic
Sakarya, Turkey
Phone: +90 5448250668
E-mail: esraozdil09@gmail.com

Doi: 10.56766/ntms.1209313

Authors' ORCIDs
Esra Demiryurek
http://orcid.org/0000-0002-5708-3631
Havva Kocayigit
http://orcid.org/0000-0002-8719-7031

Abstract: Intensive care professionals are the individuals who are most exposed to the psychological effects of the COVID-19 pandemic. The aim of the present study was to evaluate and compare the, traumatic stress symptoms and health anxiety in intensive care unit (ICU) workers and non-ICU workers who have been playing an active role in caring for COVID-19 patients. The sociodemographic characteristics of all the healthcare professionals working in the ICU and other units, were recorded. In addition, their history of COVID-19 infection, living with an elderly person, intensive care hospitalization history of relatives, and death status of relatives due to COVID-19 were recorded. The participants were divided into two groups, namely ICU workers and non-ICU workers. The Health Anxiety Inventory (HAI)and the Impact of Event Scale-Revised (IES-R) scales were applied to all the participants. Of the 150 healthcare workers initially identified, 116 agreed to participate in this study. Sixty-nine (59.5 %) of them were working in the ICU, while 47 (40.5 %) were in non-ICU. The mean IES-R score of all participants was 40.19±15.73. When the IES-R scores and ICU results with non-ICUs were compared, the healthcare workers who work in ICU had significantly higher total IES-R scores (42.83±14.65, p<0,01) and sub-scores, including hyperarousal (11.75±5.24, p<0.01), avoidance (14.90±5.52, p<0.01), intrusion (16.17±6.38, p<0.01), than those who does not work in ICU. The HAI scores did not show a significant difference between the two groups (19.59±7.50 for the ICU group vs. 18.40±7.04 for the non-ICU group) (p=0.392). Present study predicts that the COVID-19 pandemic increases traumatic stress, especially in healthcare workers working in ICU. For this reason, psychological assistance is vital to protect healthcare professionals from the acute and long-term effects of trauma. ©2023 NTMS.

Keywords: Health Anxiety; Traumatic Stress; Covıd-19.

1. Introduction

The World Health Organization (WHO) declared COVID-19 to be a pandemic on March 11, 2020, after infections and deaths began to increase exponentially worldwide. The first cases of COVID-19 had been reported during December 2019 in Wuhan, China [1]. The COVID-19 outbreak represents a significant source of traumatic stress that has affected the entire global population and so negatively impacted the

Cite this article as: Demiryurek E and Kocayigit H. Traumatic Stress and Health Anxiety in Intensive Care Workers During the Covid-19 Pandemic. *New Trend Med Sci.* 2023; 4(2):66-72. Doi:10.56766/ntms.1209313

psychological health of human society. The pandemic has been reported to cause trauma to individuals due to factors such as the uncertainty of its duration and outcome, the lack of fully effective treatment, the lack of consensus as to the general nature of the virus, the number of people infected worldwide, the high mortality rate, the high transmission rate, the fear of death, the losses experienced, the associated economic losses, and the prospect of unemployment [2,3].

Undoubtedly, healthcare professionals are the individuals who are most exposed to the psychological effects of the COVID-19 pandemic. Indeed, healthcare professionals have reported struggling with difficulties such as the increased workload, the risk of themselves and their families being infected, the frequently changing treatment protocols, the lack of protective equipment, the difficult ethical decisions they have to make, and their colleagues becoming infected [4]. Such difficulties increase the psychologic stress experienced by healthcare workers and, therefore, have significant short- and long-term effects on their mental health [5]. It has been found that healthcare workers who directly care for COVID-19 patients have a higher risk of developing psychological stress and symptoms such as anxiety, depression, and post-traumatic stress disorder (PTSD) [6,7]. COVID-19, as a new disease whose etiology and pathology remains unknown, has a high mortality rate and no effective treatment, which increases the symptoms of PTSD experienced by the healthcare workers having to deal with it [8].

PTSD involves the development of specific negative symptoms following exposure to one or more traumatic events. It is a mental health condition characterized by hyperarousal, avoidance, and intrusive memories [9].

Health anxiety involves the belief that the individual has a serious illness, which stems from the misinterpretation of somatic symptoms or the fear of catching a disease. Regardless of its classification, severe health anxiety has significant negative impacts on the individual's well-being, social and occupational functioning, and healthcare resource utilization. More anxiety and increased mental dysfunction have been identified during the COVID-19 pandemic in individuals with severe health anxiety [10].

There is no study that we are aware of that compares the psychological challenges faced by medical staff caring for COVID-19 patients in the ICU and other departments. The aim of the present study was to evaluate and compare the PTSD symptoms and health anxiety in intensive care unit (ICU) workers and non-ICU workers who have been playing an active role in caring for COVID-19 patients.

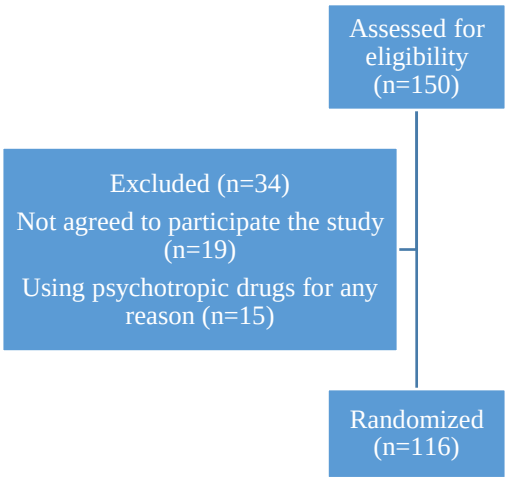
2. Material and Methods

This study was conducted in accordance with the Helsinki Declaration's ethical standards. Moreover, informed consent was obtained from all the participants.

2.1. Participants

This study was conducted in Sakarya University, which was designated a pandemic hospital by the Turkish Ministry of Health between November 2020 and December 2020. As such, the hospital is actively involved in the care of COVID-19 patients. Healthcare professionals who had been involved in the treatment of COVID-19 patients for at least three months were included in the present study.

Some 116 healthcare workers out of 150 healthcare professionals working at the hospital were included in this study. Post hoc power analysis was performed to assess the adequacy of the sample size of the study. In the power analysis, it was determined that the power was 0.99 at a significance level of 0.05 and a confidence interval of 95% (Correlation H1=0.717, lower critical r=-0.112, Upper Critical r=0.112, power 0.99). This value indicated that the sample size was adequate[11].



2.2. Data collection and study design

The sociodemographic characteristics of all the healthcare professionals working in the ICU and other units, including their age, gender, smoking and alcohol use, regular medical treatment, and chronic systemic disease history, were recorded. In addition, their history of COVID-19 infection, living with an elderly person, intensive care hospitalization history of relatives due to COVID-19, and death status of relatives due to COVID-19 were recorded.

The participants were divided into two groups, namely ICU workers and non-ICU workers. The non-ICU group comprised healthcare professionals who worked in outpatient clinics, inpatient services, and operating rooms. The Health Anxiety Inventory (HAI) scale was applied to all the participants to determine their level of health anxiety, while the Impact of Event Scale-Revised (IES-R) was used to evaluate their trauma.

The HAI was developed by Salkovskis et al. to assess health anxiety [12]. It is a self-report scale consisting of 18 items. The first 14 items of the scale consist of statements with four sequential answers that question the mental state of the patient. In terms of the remaining four questions, patients are asked to consider what their mental state might be if they had a serious illness, and

they are then questioned accordingly. The scoring of the scale is between zero and three for each item, with a high score indicating a high level of health anxiety [13]. The highest possible score for the scale is [54].

The IES-R is a 22-item self-report scale developed to measure the subjective tension and strain caused by traumatic events. Weiss and Marmar [14] rearranged the scale initially developed by Horowitz et al. [15], and their scale was adapted into Turkish by Corapcioglu et al. [16]. In the scale, the hyperarousal subscale includes six items, while both the avoidance and reliving subscales consist of eight items. Each symptom included in the scale is scored on a five-point Likert scale ranging from "none" to "very much" based on the frequency of its occurrence during the last week. The lowest and highest scores for the scale range from 0-88.

2.3. Statistical Analysis

Descriptive analysis of the variables was expressed as Mean±SD in normal distribution, and parameters with abnormal distribution were expressed as median of the 25^{th}-75^{th} percentile (interquartile range). Categorical data are expressed as proportions. The chi-square and the Student's t-test were used for categorical and continuous variables, respectively. Fisher's exact test was applied in analysing small samples. For continuous variables, differences between the two groups were evaluated using the Student's t-test when data were normally distributed and the Mann-Whitney U test when the assumption of normality was not met. Binary logistic regression analysis was performed to determine independent factors associated with mortality. A P-value less than 0.05 was considered statistically significant. Statistical analyses were performed using statistical software (SPSS 20.0, IBM Corporation, Armonk, Chicago, IL, USA).

3. Results

Sociodemographic and clinical characteristics of 116 healthcare professionals participating in the study are shown in Table 1.

Participants were divided into two groups according to their workplace as ICU workers and non-ICU workers. Sixty-nine (59.5 %) of them were working in the ICU, while 47 (40.5 %) were in non-ICU. Table 2 demonstrates the IES-R and HAI scores of both groups. The mean IES-R score of all participants was 40.19±15.73.

Table 1: Sociodemographic and clinical features of the participants.

	n=116
Age (year)	30.18±6.79
Gender, n (%)	
Female	71 (61.2)
Male	45 (38.8)
Occupation, n (%)	
Physician	31 (26.7)
Nurse	81 (69.8)
Technician	4 (3.4)
Marital Status, n (%)	
Married	64 (55.2)
Single	52 (44.8)
Smoking, n (%)	50 (43.1)
Alcohol consumption, n (%)	17 (14.7)
Presence of comorbidity, n (%)	13 (11.2)
Cohabit with elderly, n (%)	11 (9.5)
Recovered from Covid-19, n (%)	17 (14.7)
Relative died due to Covid-19, n (%)	29 (25.0)
Workplace, n (%)	
Intensive Care Unit	69 (59.5)
Other departments	47 (40.5)

When the IES-R scores and ICU results with non-ICUs were compared, the healthcare workers who work in ICU had significantly higher total IES-R scores (42.83±14.65, $p<0.01$) and sub-scores, including hyperarousal (11.75±5.24, $p<0.01$), avoidance (14.90±5.52, $p<0.01$), intrusion (16.17±6.38, $p<0.01$), than those who does not work in ICU.

Forty-eight (76.2 %) ICU workers and 19 (69.9 %) non-ICU workers received a score of 24 or higher, indicating the fulfilment of diagnostic criteria for PTSD. The HAI scores did not show a significant difference between the two groups (19.59±7.50 for the ICU group vs. 18.40±7.04 for the non-ICU group) ($p=0.392$).

Sociodemographic characteristics for PTSD were evaluated by binary logistic regression analysis as independent risk factors. Female gender [OR: 0.143 %95 CI: 0.021-0.989 $p=0.049$], working in ICU [OR: 0.019 CI: 0.002-0.154 $p<0.01$], cohabit with elderly [OR:15.599 CI:2.092-116.3 $p<0.01$], alcohol consumption [OR:0.041 CI:0.002-0.769 $p=0.033$] were identified as risk factors for PTSD. Age, occupation, marital status, being recovered from COVİD-19, having a relative died due to COVİD-19, and smoking

habit was not found predictor for PTSD (Table 3). Sociodemographic characteristics (Gender, age, workplace, cohabit with elderly, alcohol consumption, occupation, marital status, being recovered from COVID-19, having a relative died due to COVID-19, smoking) and IES-R scores for HAI were evaluated by binary logistic regression analysis as independent risk factors and none were found to be predictors of health anxiety.

Table 2: IES-R Scores in ICU Personnel and Non-ICU Personnel.

	Total n=94	ICU Personnel n=69	Non-ICU Personnel n= 47	*P**
IES-R Intrusion	14.94±6.68	16.17±6.38	11.15±6.5	<0.01
IES-R Avoidance	14.37±5.83	14.90±5.52	10.30±5.39	<0.01
IES-R Hyperarousal	10.88±5.36	11.75±5.24	7.53±4.66	<0.01
Total IES	40.19±15.73	42.83±14.65	28.94±13.62	<0.01
HAI-1	15.0±5.77	15.48±5.94	14.77±5.86	0.525
HAI-2	4.14±2.72	4.12±2.86	3.64±2.26	0.341
Total HAI	19.77±7.27	19.59±7.50	18.40±7.04	0.392

IES-R= Impact of Events Scale–Revised, HAI: Health Anxiety Inventory. *: ICU personnel vs non-ICU personnel. P<0.05 statistically significant. Mean±Standard Deviation, *Student's t-test.

Table 3: Independent factors associated with PTSD using binary logistic regression analysis.

	Exp (B) (Odds Ratio)	95% Confidence Interval	*p*
Age	1.044	0.951-1.146	0.370
Gender (female vs male)	0.143	0.021-0.989	0.049*
Occupation	13.404	0.132-1359	0.134
Work place (ICU-non-ICU)	0.019	0.002-0.154	<0.01*
Marital status (single vs married)	0.932	0.973-1.034	0.934
Cohabit with elderly (yes vs no)	15.599	2.092-116.3	<0.01*
Recovered from covid-19 (yes vs no)	0.184	0.017-2.039	0.168
Relative died due to covid-1 (yes vs no)	0.895	0.220-3.646	0.877
Smoking (yes vs no)	3.689	0.866-15.70	0.077
Alcohol consumption (yes vs no)	0.041	0.002-0.769	0.033*

*: p<0.05 was considered statistically significant. Mean±Standard Deviation. Binary logistic regression analysis.

4. Discussion

As a result of our study, the healthcare workers who work in ICU had significantly higher total IES-R scores and sub-scores, including hyperarousal, avoidance and intrusion than those who does not work in ICU. However, the HAI scores did not show a significant difference between the two groups.

Psychiatric trauma can occur if there is an imbalance between the stressful event and the capacity to cope with it psychologically [17]. It has been reported that pandemics also had traumatic effects on the society in the past, and the symptoms of acute stress disorder and PTSD increased during pandemic periods [18]. During the COVID-19 pandemic, it has been shown that the prevalence of PTSD has increased worldwide, especially for healthcare professionals [2,3]. Looking at the effects of the Covid-19 pandemic, especially at the mental health of healthcare workers, it has been shown that the most affected group is front line workers. In similar studies, healthcare workers who cared for primary COVID-19 patients and second line workers were compared, and it was shown that anxiety, depression and traumatic symptoms were higher on the front line [19-21].

A study conducted in France showed that 27% of healthcare workers who treat COVID-19 patients in ICU had PTSD symptoms [22].

There are studies reporting that PTSD is seen at higher rates in intensive care nurses than nurses working in other departments [23, 24]. We attribute the higher incidence of PTSD symptoms in nurses than doctors and technicians, to the fact that they spend more time with patients and therefore they may be more likely to establish emotional bonds with patients.

It is known that nurses working in the ICU provide care to patients who are clinically worse, therefore have witnessed deaths of many patients [25]. Participation in a resuscitation can create an increased level of psychological stress. New research suggests that PTSD in resuscitation providers at baseline is 9.6% [26]. In our study, the IES-R scores and sub-scores of the healthcare workers working in the ICU were found to be statistically higher than the non-ICU workers.

Although all healthcare workers are affected during the pandemic process, the group with front liners has a higher risk of PTSD due to witnessing all phases of COVID-19 disease. When the patients in ICU who died before the pandemic generally used to be were older in

age, now the loss of younger patients, ICU staff witnessing the long-term relationships they have made deteriorate, patients dying without making their farewells to their loved ones and ICU staff being the ones breaking the news to their families and feeling the guilt from it, high contamination risk from long periods of physical contact, long hours of work and living isolated because the risk of infecting other people are the reasons why we think ICU staff have more traumatic stress.

In this study, depression, anxiety and health anxiety levels were higher in women, showing that the psychiatric impact during the COVID-19 pandemic may be greater on women. Several previous studies have shown that female gender has been identified as a predictor of PTSD symptoms after pandemics [19, 20]. In our study, being a female was determined as a risk factor for the development of PTSD.

Different from the study of Lai et al in our study, age was not determined as a risk factor for PTSD development. However, in Turkey, a high proportion of the elderly population (22.6%) live in extended family, thus identifies as a risk factor in our study for development of PTSD among healthcare workers [27]. Those living with elderly are at higher risk of developing PTSD due to the fear of these workers to infect their family members. In our study we found a positive association between PTSD and alcohol consumption, in past studies this relationship was commonly explained by using alcohol to cope with unpleasant symptoms of PTSD [28, 29].

There are few studies in the literature examining the relationship between health anxiety and COVID-19 pandemic. In COVID-19 pandemic, the average HAI score in the general population in Germany was found to be 14.68±6.58 [29]. In another study the average HAI score in the Turkish general population during the COVID-19 pandemic was found to be 15.1±7,0 [30]. In our study, HAI scores were found to be higher than these studies (19.77±7.27).

When the relationship between knowledge about the virus and health anxiety is examined;

Blakey and Abramowitz (2017) and Lei et al. (2020) found that a higher level of knowledge about the virus is associated with increased anxiety [31, 32]. Furthermore In a study recently reported in Turkey, it was found that stress status along with anxiety in healthcare workers during COVID-19 was significantly higher and significantly impaired the quality of life[33]. In this respect, higher HAI scores in our study can be interpreted as all participants in our study were healthcare workers and had more knowledge about COVID-19 than the general population.

Although HAI scores were found to be high in our study, sociodemographic characteristics were not found to be an independent risk factor for health anxiety. Unlike our study, in the study of Ozdin et al., female gender, accompanying chronic disease and previous psychiatric history were found as risk factors for health anxiety [30].

There were several limitations in the present study. First, low number of participants in a single center enrolled in this study which can cause higher variability. Second, the cross-sectional nature of the study is not appropriate to assess the direction of causation. Third, use of self-rating scales in this study which may cause biased entries. Fourt, healthcare professionals were not evaluated in subcategories. Last limitation is that no comparison was made between the ICU workers in COVID-19 intensive care unit and ICU workers in non-COVID-19 intensive care unit.

5. Conclusion

In conclusion, present study was the first to assess health anxiety in healthcare workers during the current COVID-19 outbreak. We predict that the COVID-19 pandemic increases traumatic stress, especially in healthcare workers working in ICU. For this reason, psychological assistance is vital to protect healthcare professionals from the acute and long-term effects of trauma. Furthermore, other long-term studies are needed to evaluate the psychiatric effects of the pandemic on healthcare workers.

Limitations of the Study

The limitations of this study are that the cross-sectional nature of the study is not appropriate to assess the direction of causation and use of self-rating scales in this study which may cause biased entries.

Acknowledgement

None declared by the authors.

Conflict of Interests

The authors declared no conflict of interest.

Financial Support

No funding was received to produce this article.

Author Contributions

ED and HK designed the research. ED participated in data collection and data analysis. ED and HK wrote the manuscript, read and approved the final script.

Ethical Approval

This study was approved by the clinical research ethics committee of the Sakarya University. Date: 02.03.2021 number: E14837.

Data sharing statement

The material used in the study and without the permission of the authors.

Consent to participate

Not applicable.

Informed Consent

The authors accept their responsibilities in the study.

References

1. World Health Organization. Coronavirus Disease 2019 (COVID-19): situation report e 66. https://www.who.int/emergencies/diseases/novel-coronavirus2019/situation-reports.
2. Kira, IA, Shuwiekh HA, Rice KG et al. Measuring COVID-19 as Traumatic Stress: Initial Psychometrics and Validation. *J Loss Trauma*. 2020; 20:220-37.

3. Benfante A, Di Tella M, Romeo A, Castelli L. Traumatic Stress in Healthcare Workers During COVID-19 Pandemic: A Review of the Immediate Impact. *Front Psychol.* 2020; 11:569935.

4. Walton M, Murray E, Christian M.D. Mental health care for medical staff and affiliated healthcare workers during the COVID-19 pandemic. Eur Heart *J Acute Cardiovasc Care*. 2020; 9(3):241-47.

5. Hall H. The effect of the COVID-19 pandemic on healthcare workers' mental health. *J Am Acad Pas*. 2020; 33(7):45-48.

6. Chew NWS, Lee GKH, Tan BYQ et al. A multinational, multicentre study on the psychological outcomes and associated physical symptoms amongst healthcare workers during COVID-19 outbreak. *Brain Behav Immun*. 2020; 88:559-65.

7. Tan BYQ, Chew NWS, Lee GKH et al. Psychological Impact of the COVID-19 Pandemic on Health Care Workers in Singapore. *Ann Intern Med*. 2020; 173(4):317-20.

8. Carmassi C, Foghi C, Dell'Oste V, et al. PTSD symptoms in healthcare workers facing the three coronavirus outbreaks: What can we expect after the COVID-19 pandemic. *Psychiatry Res.* 2020; 292:113312.

9. Psychiatric Association (APA). Diagnostic and statistical manual of mental disorders. DSM-5. American Psychiatric Press, Washington DC, 2013.

10. Asmundson GJG, Taylor S. How health anxiety influences responses to viral outbreaks like COVID-19: What all decision-makers, health authorities, and health care professionals need to know. *J Anxiety Disord*. 2020; 71:102211.

11. Çapık C. Statistical Power Analysis and Its Use in Nursing Research: Basic Information. *Anatolian J Nurs Health Sci.* 2014; 17(4):268-74.

12. Jalkovskis PM, Rimes KA, Warwick HM, Clark DM. The Health Anxiety Inventory: development and validation of scales for the measurement of health anxiety and hypochondriasis. *Psychol Med.* 2002; 32:843-53.

13. Aydemir Ö, Kırpınar İ, Satı T, Uykur B, Cengisiz C. Reliability and Validity of the Turkish Version of the Health Anxiety Inventory. *Arch Neuropsychiatry*. 2013; 50:325-33.

14. Weiss DS, Marmar CR. The Impact of Event Scale-Revised: In Wilson J, Keane T (editors). Assessing psychological trauma and PTSD. New York: Guildford. 1997; 399-411.

15. Horowitz M, Wilner N, Alvarez W. Impact of event scale: A measure of subjective stres. *Psychosom Med.* 1979; 41:209-18.

16. Çorapçıoğlu A, Yargıç İ, Geyran P, Kocabaşoğlu N. The study of the realibility and validity of the Turkish version of the Impact of Event Scale-Revised (IES-R). *New Symposium.* 2006; 44:14-22.

17. Fischer G, Riedesser P. Lehrbuch der Psychotraumatologie. München: Ernst Reinhardt Verlag. 1999.

18. Chew QH, Wei KC, Vasoo S et al. Narrative synthesis of psychological and coping responses towards emerging infectious disease outbreaks in the general population: practical considerations for the COVID-19 pandemic. *Singapore Med J.* 2020; 61(7): 350-56.

19. Lai J, Ma S, Wang Y, Cai Z, Hu J, Wei N, et al. Factors Associated with Mental Health Outcomes Among Health Care Workers Exposed to Coronavirus Disease 2019. *JAMA Netw Open.* 2020; 3(3):e203976.

20. Liu, N, Zhang, F, Wei, C, Jia, Y, Shang, Z., Sun, L., Liu, W. Prevalence and predictors of PTSS during COVID-19 outbreak in China hardest-hit areas: Gender differences matter. *Psychiatry Res.* 2020; 287:112921.

21. Rossi, R, Socci, V, Pacitti, F, et al. (2020). Mental health outcomes among frontline and second line health care workers during the Coronavirus disease 2019 pandemic in Italy. *JAMA Netw Open*. 2020; 3(5):e2010185.

22. Caillet A, Coste C, Sanchez R, Allaouchiche B. Anaesth Psychological Impact of COVID-19 on ICU Caregivers. *Crit Care Pain Med.* 2020; 39(6):717-72.

23. Mealer M, Jones J, Newman J, McFann KK, Rothbaum B. & Moss M. The presence of resilience is associated with a healthier psychological profile in intensive care unit (ICU) nurses: results of a national study. *Int J Nurs Stud.* 2012; 49(3):292-99.

24. Karanikola M, Giannakopoulou M, Mpouzika M, Kaite CP, Tsiaousis GZ, Papathanassoglou EDE. Dysfunctional psychological responses among Intensive Care Unit nurses: a systematic review of the literature. *Rev Esc Enferm USP*. 2015; 49(5):847-57.

25. Straus SE, Wilson K, Rambaldini G, et al. Severe acute respiratory syndrome and its impact on professionalism: qualitative study of physicians' behaviour during an emerging healthcare crisis. *BMJ*. 2004; 329:83.

26. Spencer, SA, Nolan, JP, Osborn, M, et al. The presence of psychological trauma symptoms in resuscitation providers and an exploration of debriefing practices. *Resuscitation*. 2019; 142: 175-81.

27. Turkey Family Structure Survey (2006), the Ministry of Family and Social Policies, Ankarahttps://ailevecalisma.gov.tr/uploads/athgm/uploads/pages/indirilebilir-yayinlar/research-on-family-structure-in-turkiye-2006.pdf (accessed 16 of December 2006).

28. Khantzian EJ. The self-medication hypothesis revisited: The dually diagnosed patient. *Primary Psychiatry*. 2003; 10:47-48.

29. Saladin ME, Brady KT, Dansky BS, Kilpatrick DG. Understanding comorbidity between PTSD and substance use disorders: Two preliminary investigations. *Addictive Behaviors*. 1995; 20:643-55.

30. Ozdin S and Ozdin SB. Levels and predictors of anxiety, depression and health anxiety during COVID-19 pandemic in Turkish society: The importance of gender. *Int J Soc Psychiatry*. 2020; 66(5):504-11.

31. Blakey SM, Abramowitz JS. Psychological predictors of health anxiety in response to the Zika virus. *J Clin Psychol Med Settings*. 2017; 24:270-78.

32. Lei L, Huang X, Zhang S, Yang J, Yang L, Xu M. Comparison of prevalence and associated factors of anxiety and depression among people affected by versus people unaffected by quarantine during the COVID-19 epidemic in Southwestern China. *Med Sci Monitor*. 2020; 26: e924609.

33. Gunaydin N, Bol C, Yilmaz S. Evaluation of Anxiety in Doctors Working in New Type 2019 COVID and Non-COVID Services. *Turk J Intensive Care*. 2021; 19(Suppl 1):87-94.

NTMS New Trends in Medicine Sciences

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

ISSN: 2717-8161 RESEARCH ARTICLE

New Trend Med Sci 2023; 4(2): 73-82. https://dergipark.org.tr/tr/pub/ntms

Predictive Role of Posterior Communicating Artery Spasm on Axonal Degeneration in Oculomotor Nerve Root Following Subarachnoid Hemorrhage: Experimental Study

Mete Zeynal[1*], Mehmet Hakan Şahin[1]

[1]Department of Neurosurgery, Faculty of Medicine, Atatürk University, Erzurum, Turkey

Article History
Received 05 Apr 2023
Accepted 01 May 2023
Published Online 25 May 2023

[*]***Corresponding Author***
Mete Zeynal
Department of Neurosurgery
Faculty of Medicine
Atatürk University
Erzurum, Turkey
Phone: +90 5057545887
E-mail: dr.metezeynal@gmail.com

Doi: 10.56766/ntms.1277530

Authors' ORCIDs
Mete Zeynal
http://orcid.org/0000-0002-7398-443X
Mehmet Hakan Şahin
http://orcid.org/0000-0002-5309-4165

Abstract: The oculomotor nerve root's medial aspect in the cisternal space is closely associated with the posterior communicating artery and receives blood supply from it. This study investigates whether ischemic damage to oculomotor nerve roots results from posterior communicating artery spasm in subarachnoid hemorrhages. A total of 18 rabbits participated in this study. Baseline pupil diameters were measured using sunlight and ocular tomography. Rabbits were divided into control (GI, n=5), SHAM (GII, n=5; 0.75 cc serum physiologic injection), and subarachnoid hemorrhage-induced groups (GIII, n=8; 0.75 cc autologous blood injection). Pupil diameters were re-measured after the experiment and daily for three weeks. The animals were observed for one week before euthanasia. The posterior communicating artery vasospasm index (VSI) was determined using the wall surface/lumen surface ratio. Stereological methods were employed to examine the normal and degenerated axon densities of the oculomotor nerves. The Kruskal-Wallis and Mann-Whitney U tests were used to evaluate degenerated axon density (n/mm^2) and VSI values. A p-value of less than 0.005 was considered significant. The degenerated axon numbers in per square millimeter (n/mm2) of posterior communicating artery and average equatorial diameter of lens (mm) were 3±1/0.936±0.212 in GI; 18±4/1.578±0.235 in GII; and 212±34/2.515±0.347 in GIII. The p-values were $p<0.005$ for GI/GII, $p<0.0005$ for GII/GIII, and $p<0.001$ for GI/GIII. The posterior communicating artery vasospasm plays a significant role in oculomotor nerve root injury. ©2023 NTMS.

Keywords: Subarachnoid Hemorrhage; Oculomotor Nerve; Vasospasm; Ischemic Demage.

1. Introduction

The basilar, posterior cerebral, superior cerebellar (SCA), and posterior communicating arteries (PCom) are responsible for providing nourishment to the oculomotor nerve (OcN) network. According to Zhang et al [1], the PCom predominantly supplies blood to the nerve roots. Additionally, the cisternal segment of the OcN is often supplied by the mesencephalic perforators [2].

Clamping the PCom can lead to third nerve palsy due to inadequate blood flow to the third cranial nerve root and mechanical harm to the nerve [3]. The primary cause of third nerve palsy is PCom aneurysm [4]. Blunt head injuries can result in widespread axonal damage and OcN detachment [5]. Localized subarachnoid hemorrhage may contribute to delayed oculomotor palsy [6].

Cite this article as: Zeynal M and Şahin MH Predictive Role of Posterior Communicating Artery Spasm on Axonal Degeneration in Oculomotor Nerve Root Following Subarachnoid Hemorrhage: Experimental Study. *New Trend Med Sci.* 2023; 4(2):73-82. Doi:10.56766/ntms.1277530

Facial nerves cause vasodilation in cerebral arteries [7]. Facial ischemia can worsen PCom spasms, increasing the risk of OcN ischemia. The ciliary ganglia regulate light and accommodation reflexes by causing miosis. According to Onen et al, it is not only the degenerated neuron density of the ciliary ganglion caused by parasympathetic pupilloconstrictor palsy that leads to pupil dilation, but also the high neuron density present in the pupillodilatory superior cervical sympathetic ganglia must be taken into account as a significant contributing factor [8]. Subarachnoid hemorrhage and aneurysmal compression of PCom can result in axonal degeneration in OcN's and denervation degeneration in ciliary ganglions [9-11]. Facial and trigeminal nerves contribute to vasodilation in cerebral arteries [7]. In the presence of facial ischemia, PCom spasms can worsen, increasing the risk of OcN ischemia.

2. Material and Methods

This study was carried out with the approval of the ethics committee (E-45361945-000-2200224815) from Atatürk University, Faculty of Medicine. In this study, 18 rabbits weighing between 2.5-3 kg were divided into three groups: baseline controls (Group I, n=5), SHAM (Group II, n=5), and the experimental group (Group III, n=8). The researchers initially measured the pupil diameters of all rabbits using natural light and ocular tomography, and these measurements were used as control data for the study. The rabbits belonging to both the experimental and SHAM groups were subjected to anesthesia through subcutaneous injection drugs in proper doses. After preparing the occipito-cervical region, subarachnoid hemorrhage (SAH) was induced in the experimental group by injecting 0.75 cc of blood obtained from the auricular arteries into the cisterna magna. In contrast, the SHAM group was administered 0.75 cc of saline solution instead. Rabbits were monitored for a week with daily pupil diameter measurements and were given a standard diet and unrestricted water access. After the observation period, the rabbits were euthanized, and their OcN roots were collected and examined histologically.

To conduct light microscopic analysis, the samples were prepared as paraffin blocks. Hematoxylin & eosin and GFAP techniques were used to stain the sections. Before the analysis, all brain samples had their cranial nerves and vascular structures removed as much as possible. The Cavalieri method was implemented to evaluate the normal and degenerated axon numbers in both OcN's. Furthermore, the vasospasm index of the PCom, which supplies the OcN, was determined using a previously described calculation method. The vasospasm index is determined by the value of vessel wall surface area divided by lumen area volume and can be formulated as VSI = (R2-r2)/r2). The Cavalieri volume estimation method was utilized to determine the total number of axons in each OcN. Total axon numbers were calculated by multiplying the number of 45-degree portions of OcN's by [8]. The numerical density of axons in each OcN is depicted. The pupil diameter measurement method is summarized in Figure 11. Specific findings are detailed in figure legends, with axon counts presented as mean±SD.

Lens diameters were measured by ocular tomograthy devices. They were followed three weeks and decapitated. The normal and degenerated axon densities of oculomotor nerves were examined by Stereological methods. Lens diameters and degenerated axon density (n/mm2) of oculomotor nerves were evaluated by the Kruskal-Wallis and Mann-Whitney U test. Differences were significant at $p< 0.005$. Data were analyzed using nonparametric statistics with the Mann-Whitney U Test.

3. Results

The number of degenerated axons per square millimeter (n/mm2) and VSI values of the PCom were 3±1/0.936±0.212 for the control group, 18±4/1.578±0.235 for the SHAM group, and 212±34/2.515±0.347 for the experimental group. A negative correlation was found between the degenerated axon density in OcN's and the PCom VSI values.

Anatomical analysis of the brain samples revealed swelling, pink-purple subarachnoid spaces, adhesions, clotted degenerations, cortical injury, and periarterial adhesions. OcN sections were conducted 3 mm after the nerve's origin (Fig 1). Figure 2 shows the typical histological features of OcN's and PCom in a healthy rabbit. The brain histopathological examinations revealed the presence of subarachnoid blood accumulations, inflammations, thickening, pia-arachnoid adhesions, arterial spasms and narrowings, endothelial damage, inner elastic membrane convolutions, muscular hypertrophy, thrombosis, and intimal edema in the arteries that supply OcN roots.

Researchers conducting macroscopic brain examinations on the experimental group identified the presence of brain edema caused by subarachnoid hemorrhage, clot formation, displacements, and leakage of bloody material into the OcN roots and basal brain arteries. They also observed the occurrence of microembolism in the basilar artery and arachnoid pia adhesions. However, due to the presence of meningeal adhesions, they were unable to observe the basal cisterns and subarachnoid spaces of the OcN's. Microscopic brain examinations of the experimental group yielded similar findings. Upon histopathological evaluation of the OcN's, uneven surfaces and axons with noticeable indentations in some nerves were observed. Axonal displacement to the periphery, axonal thinning, and the formation of a peri-axonal halo resulting from axonal regression were identified as degenerated axons. Using the GFAP method, researchers were able to identify axonal degeneration in OcN's in the experimental group. All histopathological examinations are demonstrated in Figure 1-10.

This study has revealed that the ischemia of the radicular OcN and the resulting axonal degeneration

play critical roles in the development of mydriatic pupils. It is noteworthy that the high density of degenerated axons present in OcN's can be considered as a significant contributing factor in the development of mydriatic pupils. This phenomenon can occur in both normal individuals and in various neurological pathologies that affect the light reflex.

The degenerated axon numbers in per square milimeter (n/mm^2) of posterior communicating artery and average equatorial diameter of lens (mm) were 3±1/0.936±0.212 in Group I, 18±4/1.578±0.235 in Group II, and 212±34/2.515±0.347 in Group III. The p-values were $p<0.005$ for Group I/Group II, $p<0.0005$ for Group II/Group III, and $p<0.00001$ for Group I/Group III.

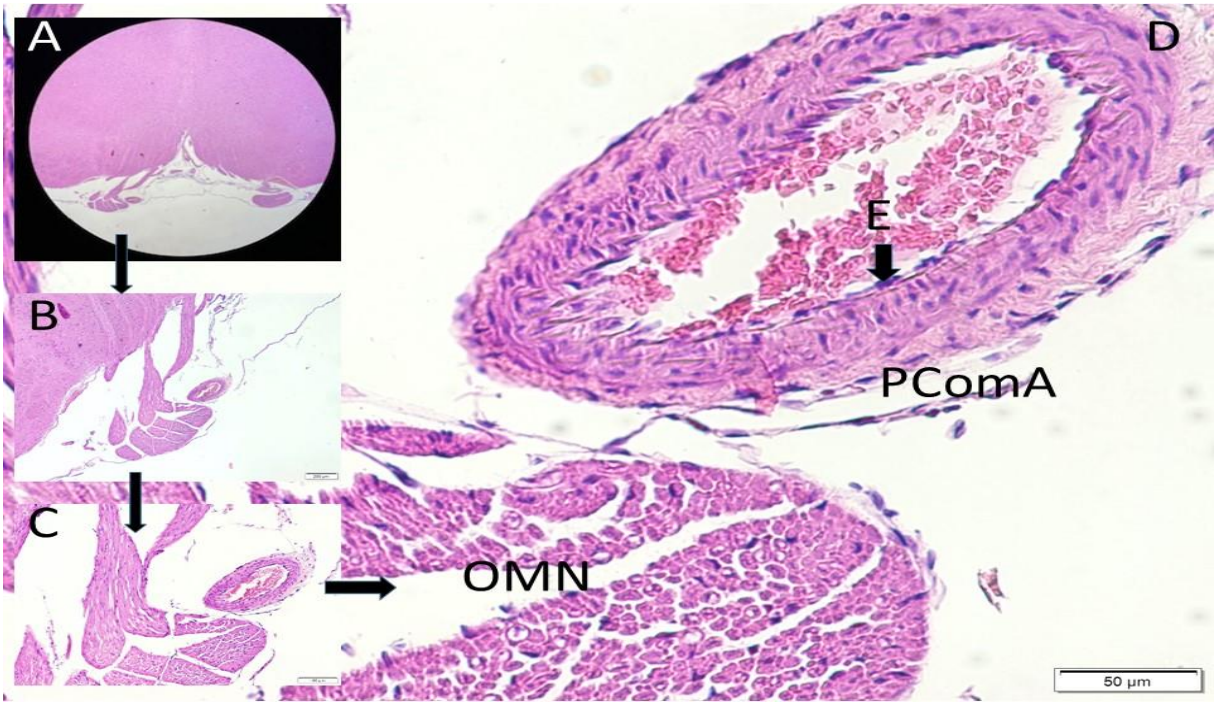


Figure 1: Posterior communicating artery (PComA) and oculomotor nerve (OMN) in normal animal (LM, H&E, x4/AB; x10/C; xx20/D).

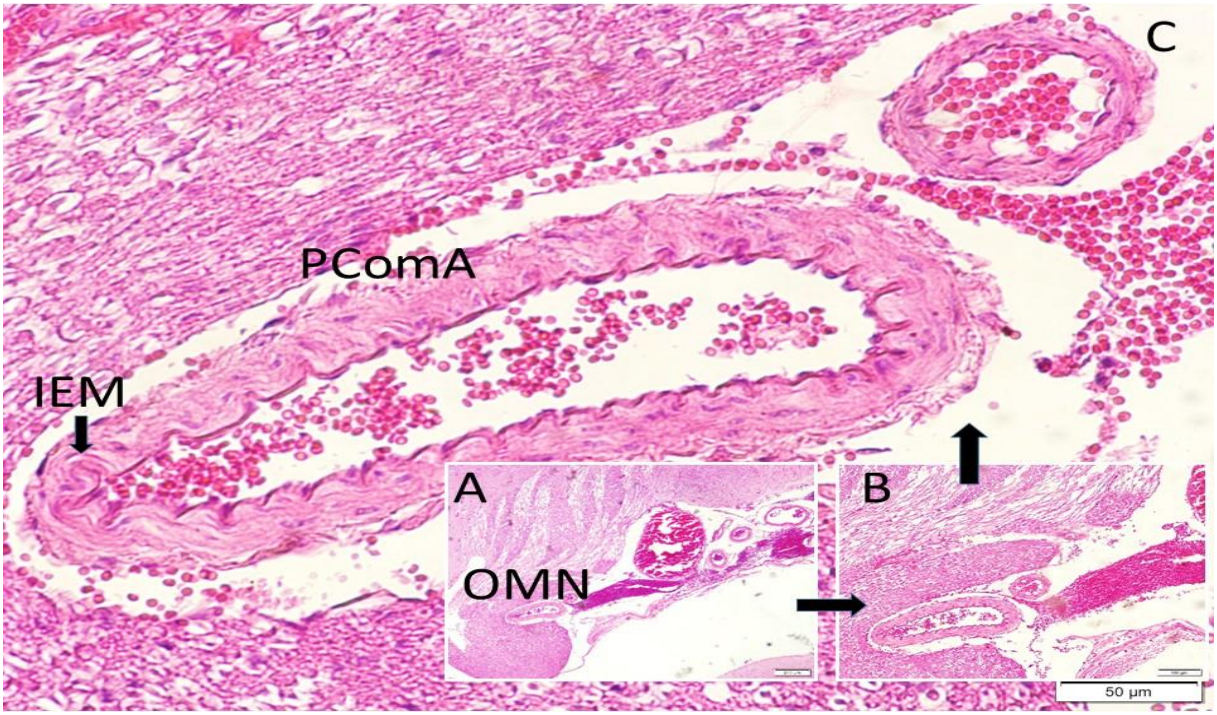


Figure 2: Posterior communicating artery (PComA), internal elastic membrane (IEM) and oculomotor nerve (OMN) is seen in bloody cisternal part of OMN is seen in SAH cerated animal (LM, H&E, x4/AB; x10/C; xx20/D).

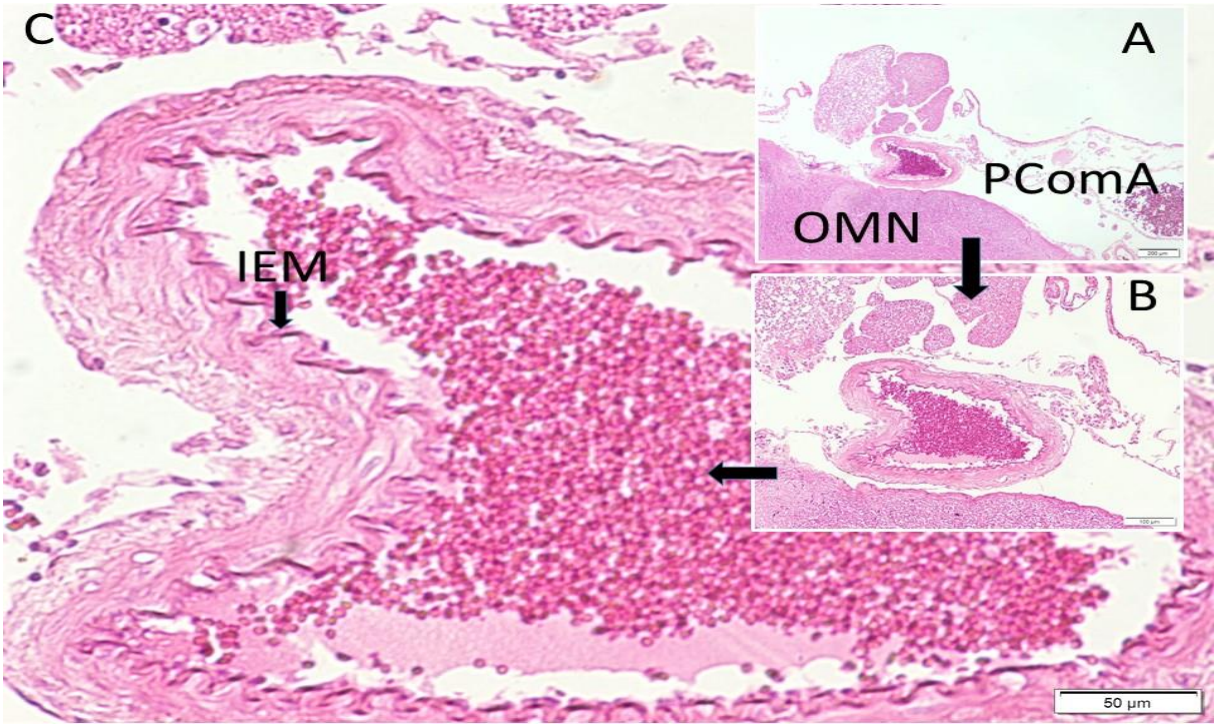


Figure 3: Posterior communicating artery (PComA), convoluted internal elastic membrane (IEM) and edematous nerve oculomotor nerve (OMN) is seen in bloody cisternal part of OMN is seen in SAH cerated animal (LM, H&E, x4/A; x10/B; x20/C).

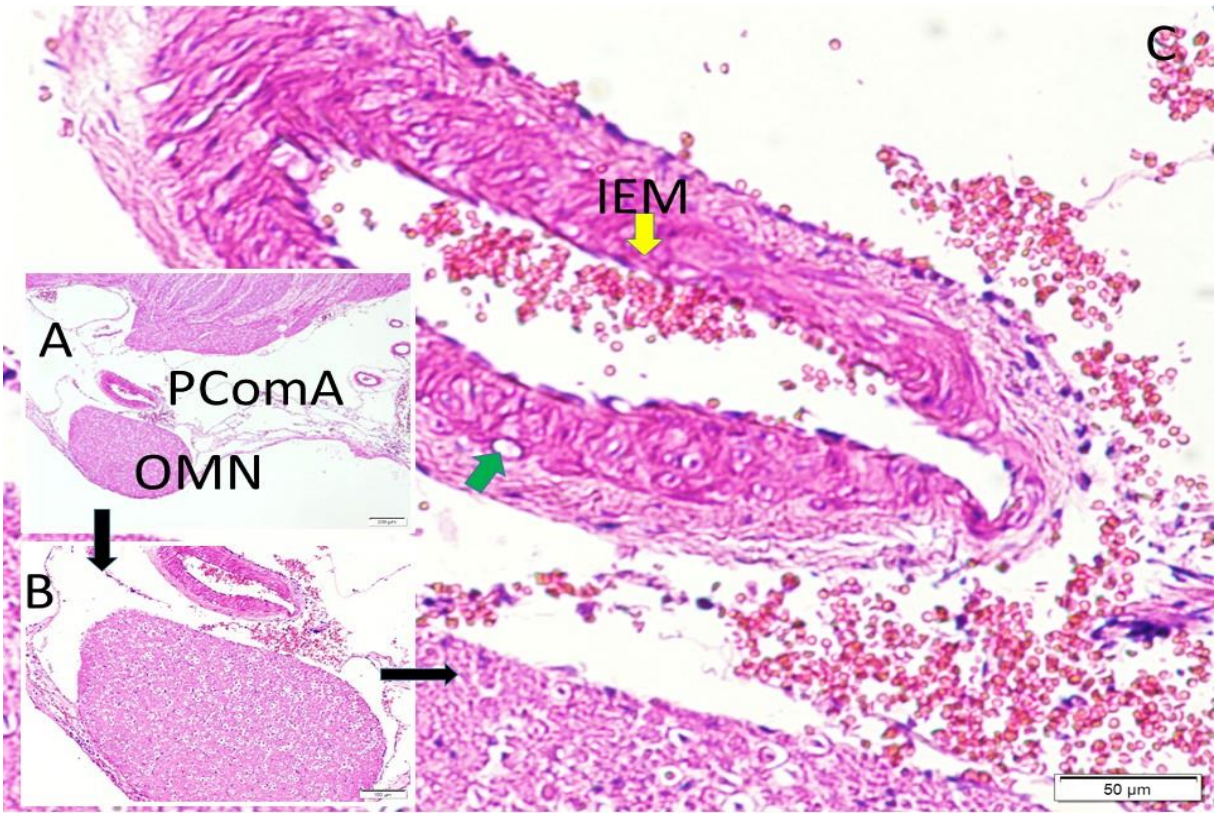


Figure 4: Posterior communicating artery (PComA), convoluted internal elastic membrane (IEM) and significsntly edematous and water collected (Green arrow) in nerve oculomotor nerve (OMN) is seen in bloody cisternal part of OMN is seen in SAH cerated animal (LM, H&E, x4/A; x10/B; x20/C).

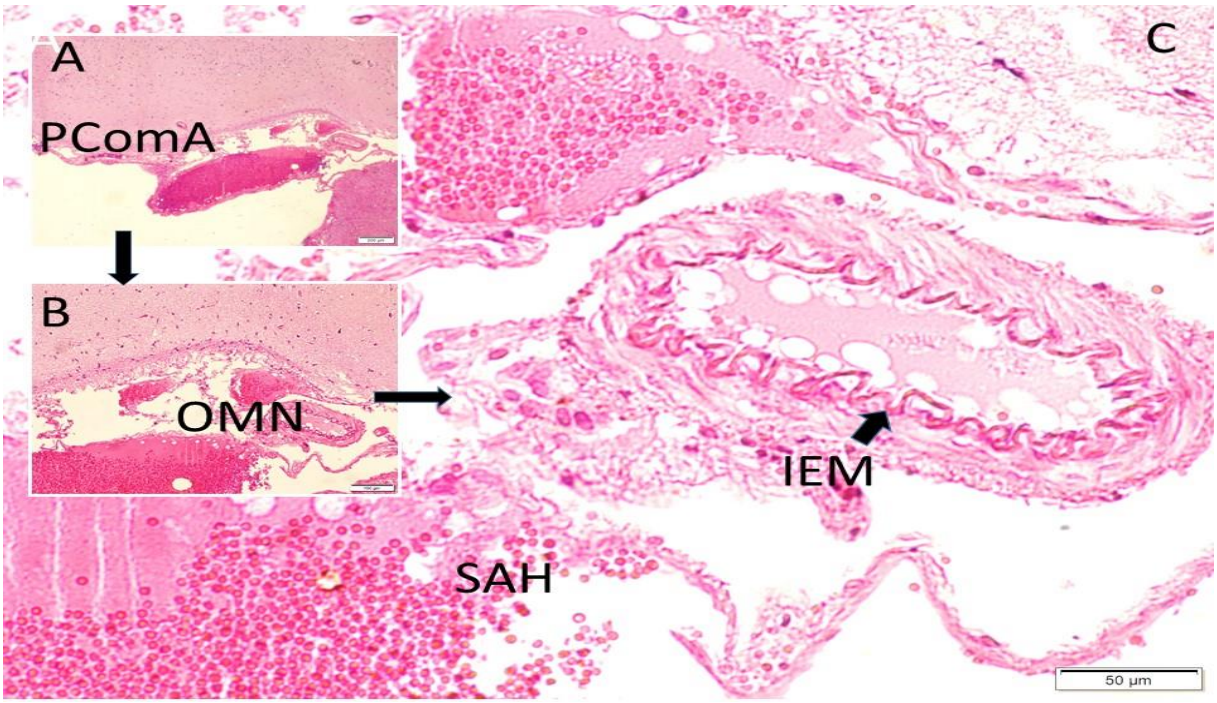


Figure 5: Posterior communicating artery (PComA), convoluted desquamated internal elastic membrane (IEM) and significantly contracted artery is seen in bloody cisternal part of OMN is seen in SAH cerated animal (LM, H&E, x4/A; x10/B; x20/C).

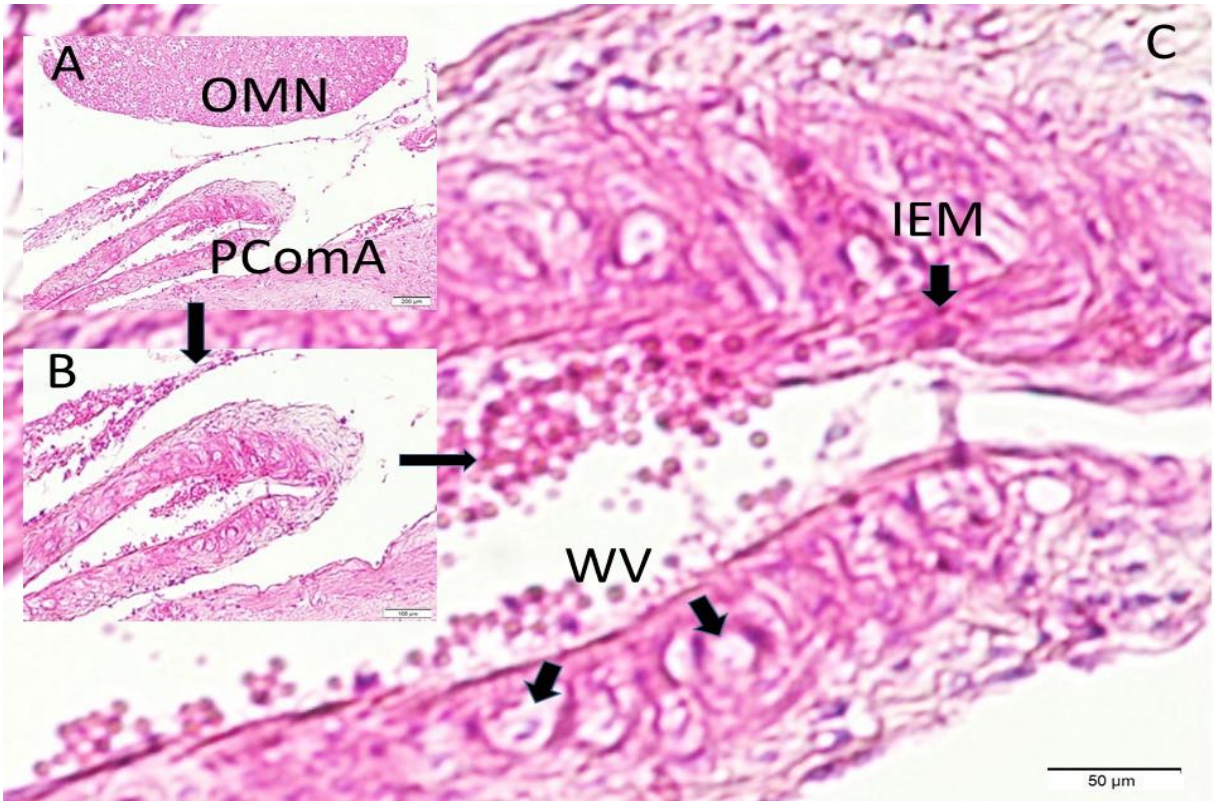


Figure 6: Posterior communicating artery (PComA), convoluted internal elastic membrane (IEM), hyperthrophied muscles and significantly edematous and water collected (WV) in nerve oculomotor nerve (OMN) is seen in bloody cisternal part of OMN is seen in SAH cerated animal (LM, H&E, x4/A; x10/B; x20/C).

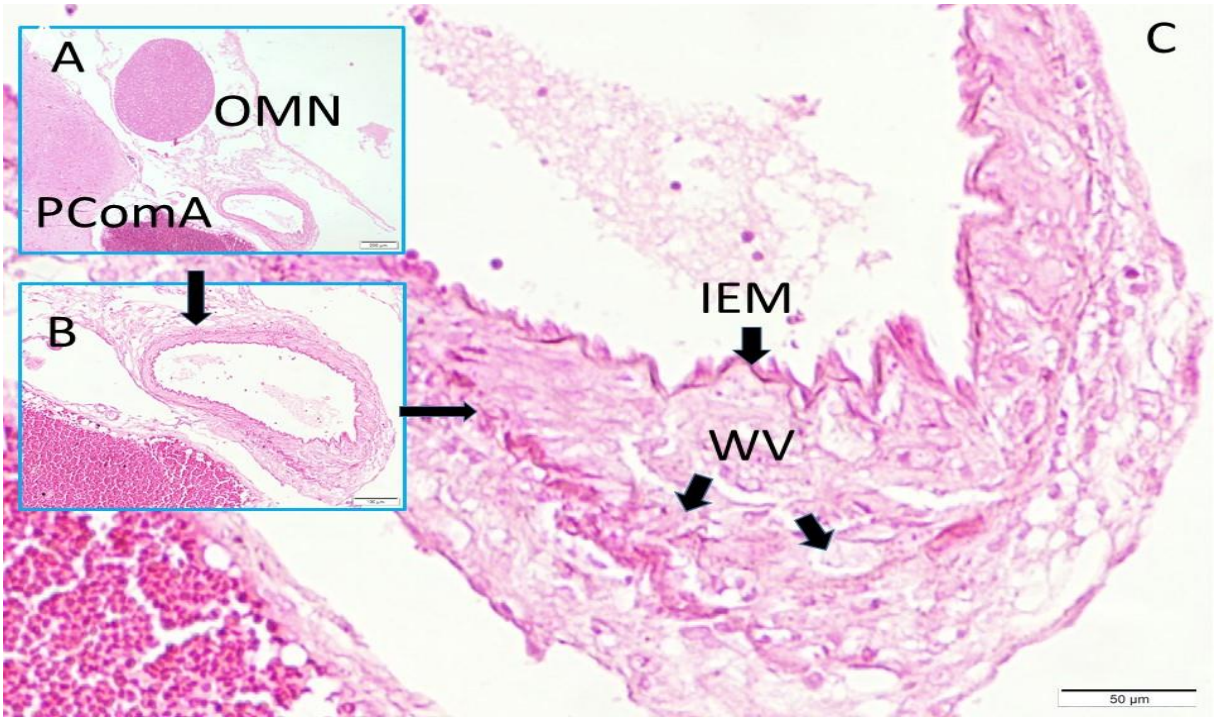


Figure 7: Hyperthrophised posterior communicating artery (PComA), convoluted internal elastic membrane (IEM) and significantly edematous and water collections among ruptured muscles in arterial wall (WV) in PComA is seen in bloody cisternal part is seen in SAH cerated animal (LM, H&E, x4/A; x10/B; x20/C).

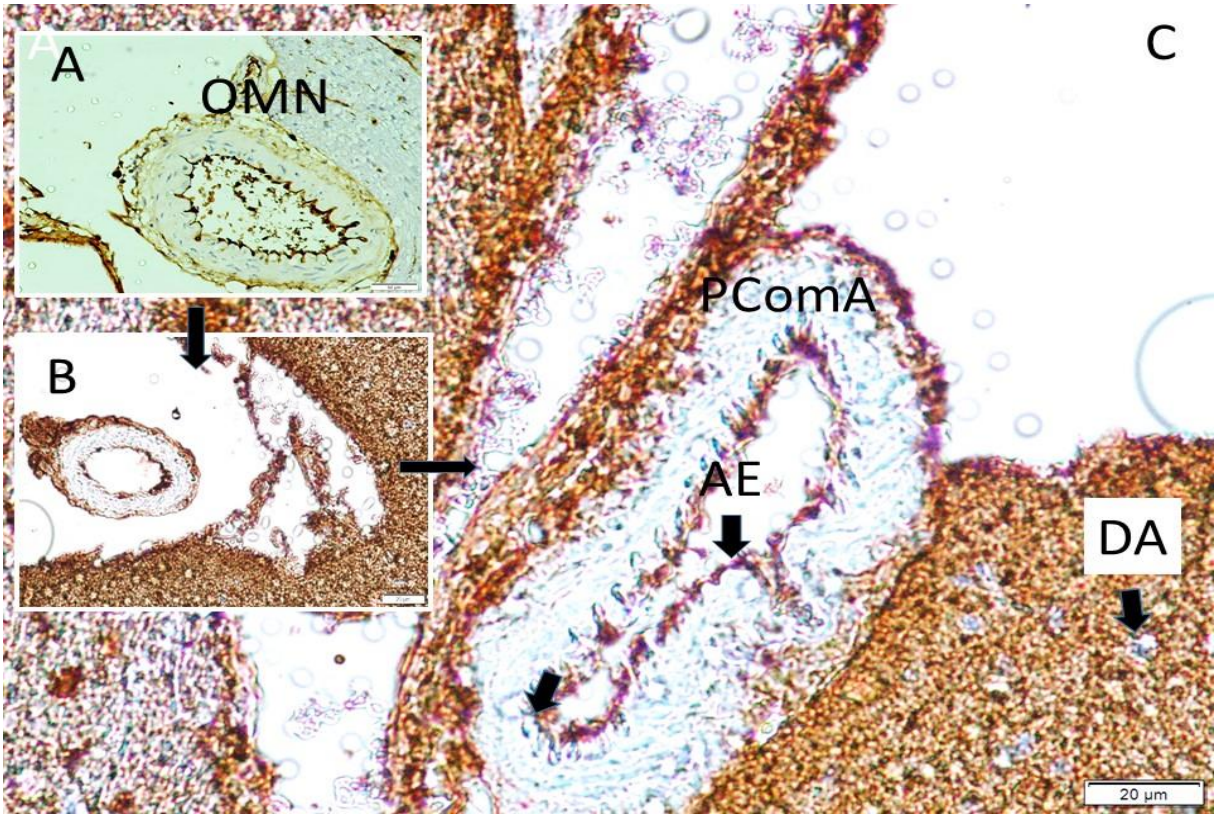


Figure 8: Posterior communicating artery (PComA), convoluted internal elastic membrane (IEM) and significantly edematous and water collected PComA and OMN, degenerated OMN axons (DA) is seen in bloody cisternal part of OMN is seen in SAH cerated animal (LM, Tunel, x20).

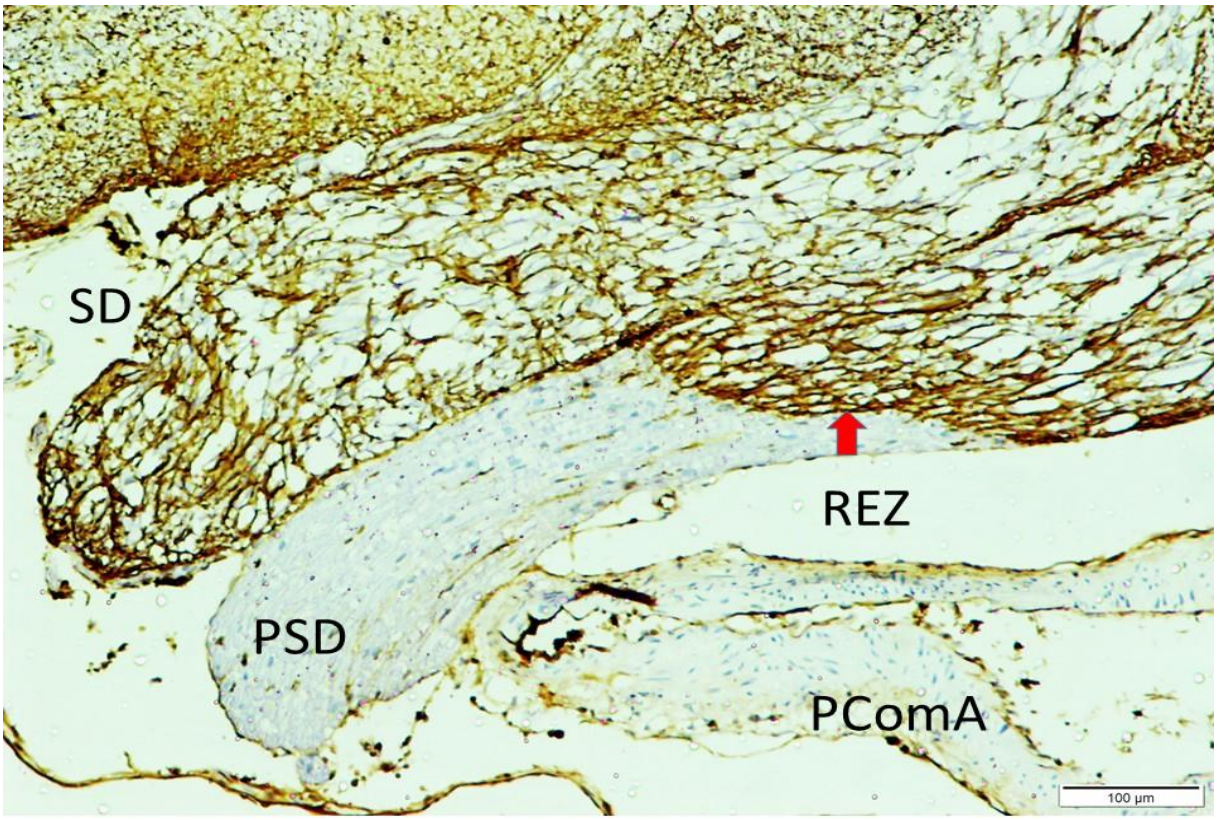


Figure 9: Oculomotor nerve (OMN) with their parasympathetic division (PSD), somatomotor division (SD), root entry zone (REZ) and PComA is seen in cisternal part of OMN is seen in a normal animal (LM, NSE, x4/A; x10/B; x20/C).

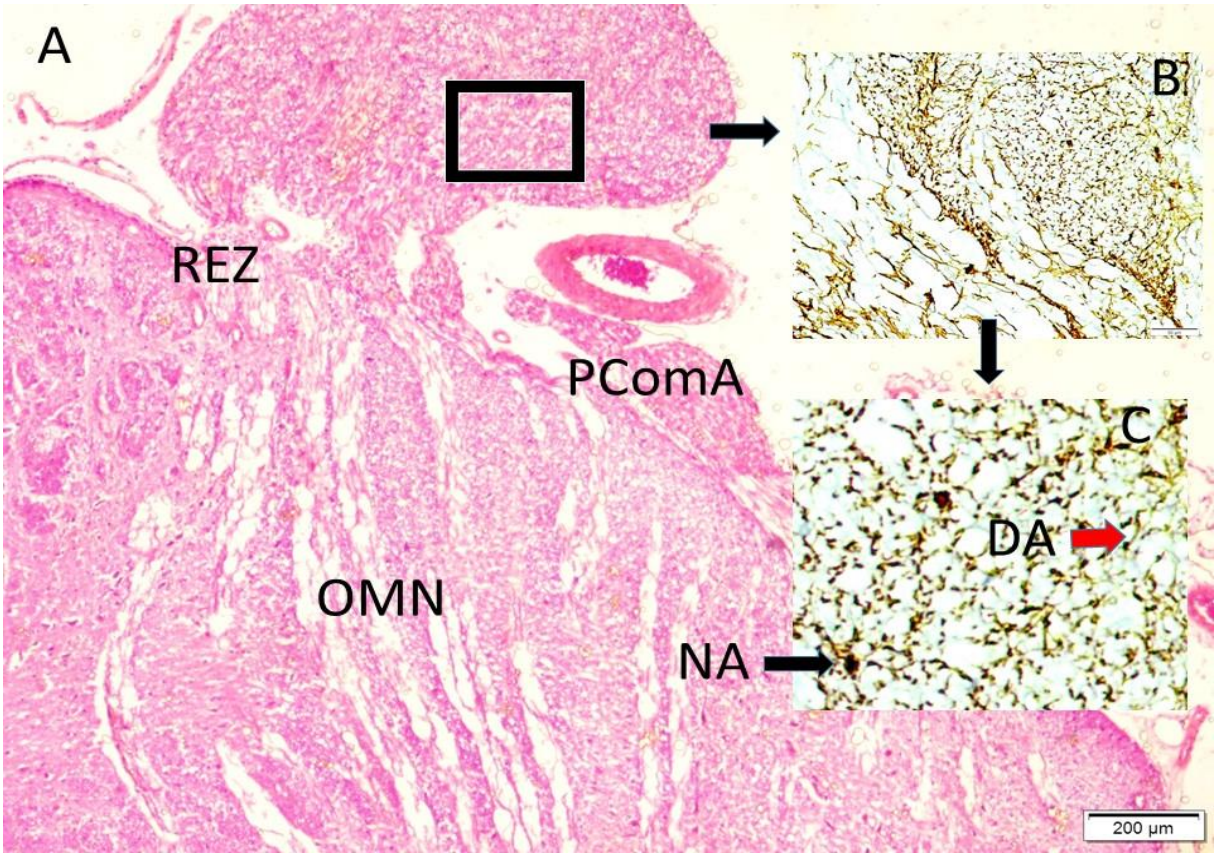


Figure 10: Oculomotor nerve (OMN) with their root entry zone (REZ) and PComA is seen in cisternal part of OMN is seen in a normal animal (LM, H&E, x4/A). Please note that autonom and somatic parts are different each other. Also, REZ zone is seen in (LM, NSE, x20, B) and axon numbers estimation method is seen in c (LM, NSE, x20/C).

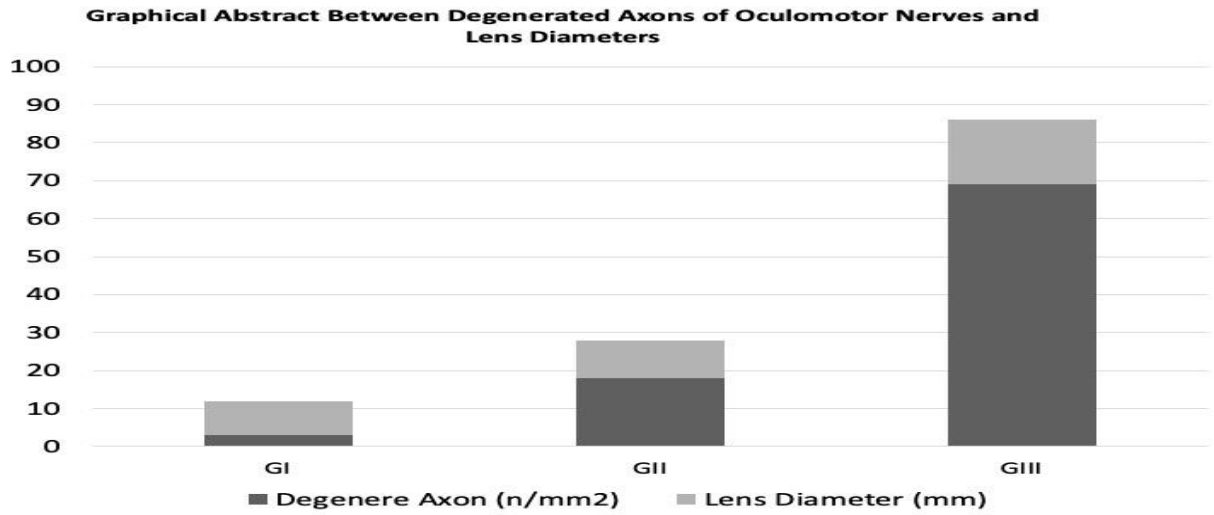


Figure 11: Lens diameter measurement graphical abstract is seen.

4. Discussion

The OcN network receives blood supply from the basilar, posterior cerebral, SCA, and PCom [1]. Primarily, the PCom supply the nerve roots. The OcN's are closely connected with various arteries, such as the SCA, posterolateral pontine artery, basilar artery, mesencephalic perforating arteries, the P1 segment, and the accessory collicular artery (3.7%) [2]. The nerve's dorsal surface has close connections with several arteries and their branches 3. Vascular penetration is observed in the third nerve, with the collicular artery and its branches being the most common penetrating vessels. The cisternal segment of the OcN is frequently supplied by mesencephalic perforators (88.9%) [2]. Clipping the PCom can result in third nerve palsy due to insufficient blood supply to the third cranial nerve root and mechanical nerve damage 3. Aneurysms of the PCom are the most common cause of third nerve palsy [4].

Mesencephalic infarcts can cause intra-axial involvement of the OcN with a fascicular lesion, leading to various eye dysfunctions [14]. Closed head traumas may result in diffuse axonal injury and OcN avulsion [5]. Ischemic OcN palsy can develop after non-aneurysmal subarachnoid hemorrhage, presenting symptoms such as mild headache, acute onset of blurry vision, and eye ptosis [15]. Recovery from OcN palsy caused by PCom aneurysm is possible [1]. Perimesencephalic subarachnoid hemorrhage can lead to third cranial nerve palsy, and focal subarachnoid hemorrhage may cause delayed oculomotor palsy [6, 16]. Aneurysmal subarachnoid hemorrhage localized to the interpeduncular cistern can result in permanent OcN palsy due to ischemic damage of the OcN web [17]. Facial ischemia can worsen PCom spasm and increase the risk of OcN ischemia [7].

Oculomotor nerves and ciliary ganglion relationships:

The ciliary ganglion is a type of parasympathetic ganglion associated with the OcN's. Postganglionic fibers are responsible for inducing miosis, which controls the light and accommodation reflex. Although the degenerated neuron density of the ciliary ganglion cannot be solely attributed to pupil dilation caused by parasympathetic pupilloconstrictor palsy, it is essential to consider the high neuron density present in the pupillodilatory superior cervical sympathetic ganglia as a significant contributing factor to pupil dilation, according to [8]. Photophobia results from denervation injury of the ciliary ganglion due to PCom spasm-induced OcN root insult [18]. Subarachnoid hemorrhage can cause denervation injury in the ciliary ganglion [10]. A study conducted by Aydin et al. revealed that meningitis often leads to the development of arachnoiditis and axonal degeneration at the cisternal segments of both oculomotor and optic nerves. These conditions subsequently result in neurodegenerative changes in the ciliary ganglion [9]. Aneurysmal compression of PCom causes axonal degeneration in OcN's and denervation degeneration in ciliary ganglia [11].

The ciliary muscles, which control the diameter and thickness of the lens, are largely controlled by the parasympathetic fibers of the oculomotor nerves and the cervical sympathetic fibers. These parasympathetic signals are transmitted to the pupillary and ciliary muscles of the eye by axons originating from the Edinger-Westphal nuclei in the brain stem. These fibers, which cause miosis as a result of contraction in the pupil, also contract the ciliary muscles, causing the lens to thicken and thus reduce its diameter. Thus, the suspension bonds are loosened, and the lens thickens and adjusts the accommodation for near-far vision. Thus, the lens with increased refractive power can focus better on nearby objects [19]. On the other hand, semaptics do the opposite of these functions [20]. Damage to the oculomotor nerve and the ciliary muscle formation it modulates leads to disruption of the accommodation reflex and visual defects [21]. In subarachnoid hemorrhages, axonal degeneration of the oculomotor nerve causes paresis or paralysis in the ciliary muscles, preventing their contraction. As a result, since the ciliary muscles cannot contract, the

lens cannot expand towards the periphery and becomes thicker, that is, its equatorial diameter decreases. This leads to near vision defects

5. Conclusion

In summary, the OcN network and its relationship with the ciliary ganglion are critical for understanding various neurological pathologies affecting the light reflex and other eye functions. The interactions between blood supply, nerve damage, and denervation injury in the ciliary ganglion can provide valuable insights into the development of mydriatic pupils in both normal and pathological conditions. In the eyes, there is often a condition called PCOM spasm, which can result in paresis or paralysis of the ciliary muscles that control the thickness of the lens, as they are fed by the radix of the oculomotor nerve. As a result of paresis or paralysis of the ciliary body, the muscles are unable to contract, causing an increase in the diameter of the lens, which results in myopia. A comprehensive understanding of these factors can help guide the development of targeted treatments and preventive strategies for oculomotor nerve-related disorders.

Limitations of the Study

This study does not include electrophysiological data.

Future Insight

Trigeminal nerve stimulation may be used to dilate PCom spasm.

Acknowledgement

We would like to thank to Prof. Dr. Mehmet Dumlu Aydın for his scientific vision.

Conflict of Interests

Author declares no conflict of interest with this study and manuscript.

Financial Support

There is no financial support in this study.

Author Contributions

The entire text of the work is attributed to the responsible author and the second author.

Ethical Approval

This experimental investigation was approved by the Health Research Ethics Committee of the Medical Faculty at Ataturk University (E-42190979-000-2200225459).

Data sharing statement

Authors declares; All data generated by this work are publicly available as long as reference rights are not violated if any part of the work is used.

Consent to participate

All authors participated equally to this study.

Informed Consent

Scientific works undertaken with this study did not require any informed consent.

References

1. Zhang WG, Zhang SX, Wu BH. A study on the sectional anatomy of the oculomotor nerve and its related blood vessels with plastination and MRI. *Surg Radiol Anat.* 2002; 24(5):277-84.
2. Marinković S, Gibo H. The neurovascular relationships and the blood supply of the oculomotor nerve: the microsurgical anatomy of its cisternal segment. *Surg Neurol Dec.* 1994; 42(6):505-16.
3. Wong GK, Ng SC, Tsang PK, Poon WS. Clipping vs coiling of posterior communicating artery aneurysms with third nerve palsy. *Neurology*. 2006; 66(12):1959-60.
4. Renowden SA, Harris KM, Hourihan MD. Isolated atraumatic third nerve palsy: clinical features and imaging techniques. *Br J Radiol.* 1993; 66(792):1111-17.
5. Yamada S, Mizutani T, Nagura H, Yamanouchi H, Matsuo Y. A case of closed head injury with diffuse axonal injury, and oculomotor nerve avulsion and midbrain infarction. *Rinsho Shinkeigaku*. 1995;35(3):267-71.
6. Shih TY. Delayed oculomotor palsy from focal subarachnoid hematoma. *J Clin Neuroophthalmol*. 1993; 13(3):218-19.
7. Tanriverdi O, Aydin MD, Onen MR, et al. Understanding of Dry Eye in Subarachnoid Hemorrhage: An Experimental Study on the Role of Facial Nerve Ischemia. *Turk Neurosurg*. 2019; 29(3):362-68.
8. Onen MR, Yilmaz I, Ramazanoglu L, et al. Uncovering the Forgotten Effect of Superior Cervical Ganglia on Pupil Diameter in Subarachnoid Hemorrhage: An Experimental Study. *Turk Neurosurg.* 2018; 28(1):48-55.
9. Aydin MD, Onder S, Ulvi H, Onder A, Baykal O. Histopathological alterations in ciliary ganglions in meningitis: an experimental study. *Minim Invasive Neurosurg.* 2005; 48(5):297-301.
10. Aydin MD, Akyol-Salman I, Sahin O. Histopathological changes in ciliary ganglion of rabbits with subarachnoid hemorrhage. *Int J Neurosci.* 2005; 115(11):1595-602.
11. Ozkan U, Aydin MD, Gündoğdu C, Onder A. Histopathologic changes in oculomotor nerve and ciliary ganglion in aneurysmatic compression injuries of oculomotor nerve. *Minim Invasive Neurosurg.* 2004; 47(2):107-10.
12. Cruz-Orive LM, Weibel ER. Recent stereological methods for cell biology: a brief survey. *Am J Physiol*. 1990; 258(4 Pt 1):L148-56.
13. Lanzino G, Andreoli A, Limoni P, Tognetti F, Testa C. Vertebro-basilar aneurysms: does delayed surgery represent the best surgical strategy? *Acta Neurochir (Wien)*. 1993; 125(1-4):5-8.
14. Bogousslavsky J, Regli F. Intra-axial involvement of the common oculomotor nerve in mesencephalic infarctions. *Rev Neurol (Paris).* 1984; 140(4):263-70.
15. Mehta S, Bathini A, Dubey A, et al. Isolated oculomotor nerve palsy secondary to non-aneurysmal subarachnoid hemorrhage. *J Cerebrovasc Endovasc Neurosurg*. 2022; 24(3):267-75.

16. Reynolds MR, Vega RA, Murphy RK, Miller-Thomas MM, Zipfel GJ. Perimesencephalic subarachnoid hemorrhage associated with a painless, pupillary-involving third cranial nerve palsy: case report and literature review. *Clin Neurol Neurosurg.* 2012; 114(8):1168-71.
17. Meyer YJ, Paine JT, Batjer HH. Focal subarachnoid hematoma: an unusual cause of delayed third cranial nerve paralysis. *Surg Neurol.* 1990; 34(3):169-72.
18. Aydin N, Kotan D, Keles S, et al. An experimental study of the neurophysical mechanisms of photophobia induced by subarachnoid hemorrhage. *Neurosci Lett.* 2016; 630:93-100.
19. Westheimer G, Blair SM. The parasympathetic pathways to internal eye muscles. *Invest Ophthalmol.* 1973; 12(3):193-97.
20. Heermann S. Neuroanatomy of the Oculomotor System. *Klin Monbl Augenheilkd.* 2017; 234(11):1334-43.
21. Levy NS, Kramer SG, de Barros T. Pupillary and accommodative abnormalities in the Vogt-Koyanagi-Harada syndrome. *Am J Ophthalmol*. 1970; 69(4):582-88.

NTMS New Trends in Medicine Sciences
https://dergipark.org.tr/tr/pub/ntms

ISSN: 2717-8161 RESEARCH ARTICLE

New Trend Med Sci 2023; 4(2): 83-88. https://dergipark.org.tr/tr/pub/ntms

The Neutrophil/Lymphocyte and Platelet/Lymphocyte Ratios of Pregnant Women Who Underwent the 75-g Oral Glucose Tolerance Test to Predict Gestational Diabetes

Yunus Emre Topdagi[1], Cagdas Demiroglu[2], Ahmet Ziya Sahin[3]

[1]Department of Gynecology and Obstetrics, Faculty of Medicine, Ataturk University, Erzurum
[2]Department of Gynecology and Obstetrics, Faculty of Medicine, SANKO University, Gaziantep
[3]Department of Nephrology, Faculty of Medicine, Gaziantep University, Gaziantep

Article History
Received 15 Jan 2023
Accepted 11 Apr 2023
Published Online 25 May 2023

****Corresponding Author***
Yunus Emre Topdagi
Department of Gynecology and Obstetrics
Faculty of Medicine
Atatürk University
Erzurum, Turkey.
Phone: +90 5358234656
E-mail: emr-topdagi@hotmail.com

Doi: 10.56766/ntms.1199230

Authors' ORCIDs
Yunus Emre Topdagi
http://orcid.org/0000-0003-0656-0765
Cagdas Demiroglu
http://orcid.org/0000-0002-7011-3890
Ahmet Ziya Sahin
http://orcid.org/0000-0001-5853-8709

Abstract: Gestational diabetes mellitus (GDM) is one of the most common medical complications of pregnancy. Early diagnosis and treatment are important; the condition can cause both maternal and foetal complications. Today, single-/double-bolus oral 50-100-g glucose tolerance tests (OGTTs) are preferred. We explored whether the peripheral blood platelet/lymphocyte ratio (PLR) and/or neutrophil/lymphocyte ratio (NLR) could guide diabetes screening of a target group (rather than all pregnant women). This retrospective study was conducted at the Obstetrics and Gynecology Clinic of Sanko University Hospital from January 2010 to January 2020. Pregnant women in gestational weeks 24 to 28 who underwent 75-g OGTTs were included. Patients were evaluated by dividing them into two groups. Group 1 included 300 women with GDM. Group 2 included 300 healthy pregnant women who were negative on the OGTT test. We retrieved patient ages, gestational weeks, all blood count data derived during pregnancy, fasting blood glucose levels, heights and weights, and body mass indices. Leukocyte and neutrophil counts were significantly higher in the diabetic patient group than in the control group (both $p<0.01$). The NLR and PLR differed significantly between the two groups (both $p<0.01$), but the demographic data did not. Increase in white blood cell count, and elevations in the PLR and NLR, independently predicted GDM. Blood NLR and PLR can also be used as a GDM screening test. The NLR and PLR (markers of inflammation) were significantly increased in pre-diabetic and diabetic patients. The NLR and PLR may usefully predict pre-diabetes and GDM. ©2023 NTMS.

Keywords: Gestational diabetes; Platelet-to-lymphocyte ratio; Pregnancy; Neutrophil-to-lymphocyte ratio.

1. Introduction

Gestational diabetes mellitus (GDM), a common medical complication in pregnancy is a glucose metabolization disorder that develops in the second trimester and disappears after pregnancy [1].

GDM affects 10–15% of all pregnant women; there is some regional/country variability [2]. The cases are divided into those who were diabetic before pregnancy but were first diagnosed with diabetes only during

Cite this article as: Topdagi YE, Demiroglu C and Sahin AZ. The Neutrophil/Lymphocyte and Platelet/Lymphocyte Ratios of Pregnant Women Who Underwent the 75-g Oral Glucose Tolerance Test to Predict Gestational Diabetes. *New Trend Med Sci.* 2023; 4(2):83-88. Doi:10.56766/ntms.1199230

pregnancy, and cases who develop diabetes during pregnancy (pregestational and gestational diabetes, respectively) [3]. To ensure that the foetus receives the glucose it requires, placental secretion of cortisol, growth hormone, oestrogen, progesterone, prolactin, and (especially) human placental lactogen all increase; triggering hyperinsulinemia, insulin resistance, fasting hypoglycemia, and postprandial hyperglycemia. This enhances the need for insulin; pancreatic hypertrophy and hyperplasia develop when the need is met [4, 5]. Foetal macrosomy, neonatal hypoglycemia, hyperbilirubinemia, and shoulder dystocia increase the frequencies of operative birth and birth trauma. Gestational hypertension, pre-eclampsia, a need for caesarean delivery, related complications, and type 2 diabetes are common. Early diagnosis and the treatment of gestational diabetes is vital; the condition can trigger maternal and foetal complications [6, 7].

Screening programmes for gestational diabetes are in place in many countries worldwide. Screening tests are performed in the second trimester (at gestational weeks 24-28) after the ingestion of 75 g (one bolus) or 50-100 g (two boluses) of glucose; venous plasma glucose levels are calculated [8, 9]. It is appropriate to use the tolerance test using 75-g oral glucose (OGTT) to evaluate all pregnant women in Turkey (the type 2 diabetes prevalence is high in our country). The test is well-tolerated, performed only once, and yields a single value [10]. We thus applied this test.

The platelet/lymphocyte ratio (PLR) and neutrophil/lymphocyte ratio (NLR) are simple markers of systemic inflammatory response (SIR) obtained from full blood count examined from peripheral blood [11]. Recent studies have shown that these markers are of prognostic utility in cancer patients; those with bowel and ischemic heart diseases; and patients with endometriosis, pre-eclampsia, and hyperemesis gravidarum [12-17]. Today, screening for diabetes is routine for all pregnant women. NLR and PLR have been studied in many diseases such as inflammatory bowel diseases, ischemic heart diseases, endometriosis, many malignancies, endometriosis. Based on past knowledge, inflammation in the etiology of GDM has always been investigated. In many studies, SIR markers such as NLR and PLR have been studied in GDM or DM patient groups. In some studies, significant statistics were obtained, while in others no significant statistics were found. The main purpose of our study is to conduct a study for the particularly prone group among all pregnancies screened for GDM, so that there will be no need to perform glucose loading tests on all pregnants [13-18]. Here, we explored whether the NLR and PLR could be used to screen a target group (thus not all pregnant women) in terms of gestational diabetes.

2. Material and Methods

This retrospective study was conducted in the 10-year period covering the dates of January 2010 and January 2020 at Sanko University Hospital Obstetrics and Gynecology Clinic between 24 weeks and 28 weeks. Pregnant women who apply between 24 and 28 weeks gestational weeks and have undergone a 75g oral glucose tolerance test included. In our study, 300 pregnant women diagnosed with gestational diabetes and 300 healthy pregnants with negative OGTT test were included as a control group. Patients' ages, gestational weeks, complete blood count parameters during pregnancy, fasting blood glucose, height and weight, BMI (body mass index) was scanned in patient files. The patient and control group included patients who applied to the obstetrics and general internal medicine clinic for routine control. Patients and control groups with a diagnosis of malignancy, patients with any infection, patients receiving steroid or immunosuppressive therapy, patients receiving chemotherapy or radiotherapy, patients with hematological diseases, patients with type 1 or type 2 diabetes mellitus were excluded from the study. The protocol of the study was approved by Sanko University Non-Interventional Clinical Research Ethics Committee (File no:07/07/2020, 2020/09) and written informed consent was obtained from all participants.

Patients were classified into two groups. Group 1 included 300 pregnant women diagnosed with gestational diabetes. Group 2 included 300 healthy pregnant women with negative OGTT test. The blood tests examined in all pregnant groups were taken in the outpatient clinic.

2.1. Statistical Analysis

Demographic distribution and statistical comparison of the data made in our study by SPSS (23. Version) program. Data are presented as mean, standard deviation, median, minimum, maximum, percentage and number. The normal distribution of continuous variables was analyzed using the Shapiro Wilk test. In the comparisons betten two groups with numerical variables, the Independent Samples T test was used when the normal distribution condition was met, and the Mann Whitney U test was used if it was not. In the comparison of continuous variables with more than two groups, the ANOVA test was used when the normal distribution condition was met, and the Kruskal Wallis test was used when it was not. The comparison between categorical variables was made with Chi-square test and Fisher's Exact test. In the comparison of two continuous variables, Pearson correlation test was used if the normal distribution condition is met, and the Spearman correlation test was used if it was not, and the statistical significance level was accepted as $p<0.05$.

3. Results

A total of 600 pregnant women with 300 pregnant OGTT tests positive and 300 pregnant OGTT tests negative were included in the study. The sociodemographic charecterictics of the patients and controls according to the diagnosis is shown in table 1. There were no significant differences between the

groups in terms of age, gravida, parite and body mass index (BMI). There was a significant difference between group 1 and the group 2 in terms of neutrophil and platelet counts.

In terms of lymphocyte count, group 1 were found to be higher when compared with the control group. There was no significant difference in lymphocyte count between group 1 and group 2 (Table 2).

Table 1: The sociodemographic charecterictics of the patients and controls according to the diagnosis.

	GDM group n=300	Control group n=300	p
Age	35.58±1.56	34.41±1.90	0.152
Gravida	3.14±0.21	2.98±0.52	0.321
Parity	3.02±0.18	2.78±0.39	0.187
Live Birth	2.98±0.16	2.64±0.28	0.110
BMI	27.5±1.21	26.9±1.14	0.210

Table 2: Comparison of groups according to neutrophil, lymphocyte and platelet levels.

	GDM group n=300	Control group n=300	p
Neutrophil	5750.26±312.7	2870.46±265.5	<0.001
Lymphocyte	2215.72±90.1	2045.51±65.2	0.121
Platelets	321458.21±7451.2	254129±9564.7	<0.001

There was a significant difference between patients and control group in terms of NLR and PLR, ($p<0.001$ for both). The NLR and PLR value were significantly higher in patients than control group (Table 3)

A significantly positive correlation was found between neutrophil count and patients ($p=0.242$), platelet count and patients ($p=0.313$) and a significantly negative correlation was found between lymphocyte count and patients ($p=-0.201$).

Table 3: Comparison of groups according to NLR and PLR.

	GDM group n=300	Control group n=300	p
NLR	2.78±1.4	1.59±1.2	<0.001
PLR	149.65±70.2	89.10±31.3	<0.001

4. Discussion

Subclinical inflammation and insulin resistance are the principal pathophysiological features of diabetes [18]. Several previous studies have reported correlations between subclinical inflammation and insulin resistance [19, 20]. Current studies have shown that inflammation, endothelial dysfunction and procoagulation disorder play a role in the occurrence of diabetes, insulin resistance and diabetes-related complications [21]. NLR, PLR and platelet index are low-cost, practical laboratory tests that are calculated from full blood counts examined during routine controls and are studied in most centers. Since there is no easy way to predict maternal GDM in pregnancy, inflammatory and platelet count detection by studying complete blood count in pregnant women in the first half of pregnancy contributes to maternal health in early detection of GDM. Pattanathaiyanon et al. showed that higher leukocyte numbers at early in the gestation process belonged with a greater risk of developing GDM [23]. However, Gorar et al. [23] reported that white blood cell, neutrophil, or lymphocyte parameters did not correlate significantly with GDM.

We found that the NLR and PLR indicated whether the OGTT test for gestational diabetes was required by all pregnant women or only a high-risk subgroup thereof. The NLR and PLR are simple, rapid, and convenient biological indicators of systemic inflammation. A study of 2753 pregnant women showed that women with gdm had a significant increase in the number of leukocytes in the first trimester.(compared to normoglycemic women) [24]. In another study, there was no significant difference between the GDM group and the normal healthy pregnant group in terms of NLR and PLR [25]. In the study conducted by Sahbaz et al., PLR and NLR increases were found to be significant between pregnants with gdm and healthy groups [26]. Friis et al. [27] study that inflammation markers (CRP, IL1-Rα, IL-6, TNF receptor II, monocyte-chemoattractant protein-1 and IL-10) increased from early- to mid-pregnancy, but not toward the end of pregnancy. Liu et al. [28] reported similar results.

NLR has been observed as an example of increased complications such as hearing loss in diabetic patients [29]. Indices of the systemic inflammatory response (the NLR and PLR) were associated with the the

development of diabetic retinopathy in patients lacking relevant family histories [30]. We showed earlier that, in T2DM patients, the serum CRP level/blood NLR combination served as a biomarker of Escherichia coli of β-lactamase-producing in urinary tract infections [31]. The PLR reflects the chronic inflammatory response; many studies have shown that the PLR usefully estimates the status of patients with tumors, diabetes, and neurological diseases [32, 33].

Fashami et al. [34] found that increases in the platelet and inflammatory indices of the complete blood count during the second trimester reflected the risk of GDM. Our results support this proposition. Onalan et al. [35] suggested that haematological parameters (the haematocrit and mean platelet volume), the PLR, and the NLR (they can be easily calculated from the exact count taken from the patients.) might seve as its cost-effective is appropriate in predicting microvascular complications of diabetes.

Today, gestational diabetes screening is performed on all pregnant women. However, many pregnant women oppose this screening test by drinking glucose. The aim of our study is to develop a method for glucose loading test by determining the risk group instead of all pregnants. The NLR and PLR values in our study were significantly higher in the gestational diabetic group. We recommend that glucose loading test should definitely be performed for patients in this group. We think that pregnant women who do not want to have the glucose loading test should insist on having a glucose loading test if at least the NLR and PLR values are high. The limitations of our study include the retrospective nature there of and there are no records of insulin levels and insulin resistance. The sample size was relatively small. Additional prospective studies are required to evaluate changes in the levels of inflammatory markers and platelet counts from the first trimester of the pregnancy to the end of pregnancy.

5. Conclusions

We found that an increased white blood cell count and a higher PLR and NLR independently predicted GDM. We recommend that PLR and NLR can be used as screening tests to distinguish pregnant women who may have GDM. An increased leukocyte count is very important marker for GDM; an elevation reflects subclinical inflammation. It is important to diagnose GDM early. Future studies focusing on the first trimester may improve patient outcomes by facilitating early interventions. Additional randomised controlled studies evaluating the relationships among the PLR and NLR, and GDM status, are required

Limitations of the Study

The limitations of the study is small sample size

Acknowledgement

None.

Conflict of Interests

We declare that we have no confict of interest.

Financial Support

The authors declared that this study received no financial support.

Author Contributions

Constructing the idea or hypothesis for research – Topdagi YE, Sahin AZ; Planning the design of the work- Topdagi YE, Demiroglu C; Execution of the experiments, patient follow-up - Topdagi YE, Sahin AZ; Analysis and interpretation of data - Topdagi YE, Demiroglu C; Providing financial support, tools and instruments – none; Biological materials, reagents and referred patients - Topdagi YE; Literature Review - Topdagi YE; Critical Review - Demiroglu C, Sahin AZ; Final approval of hthe cersion to be published - Topdagi YE, Demiroglu C, Sahin AZ.

Ethical Approval

Ethics committee approval was received for this study from the ethics committee of SANKO University.

Data sharing statement

All data relevant to the study are included in the article.

Consent to participate

All participants read the consent form and understand the study being described.

Informed Statement

Informed consent was obtained from all individual participants included in the study.

References

1. Practice Bulletin No. 137: Gestational diabetes mellitus. *Obstet Gynecol.* 2013; 122(2 Pt 1):406-16.
2. Li Y, Cooper A, Odibo IN. et al. Discrepancy in Insulin Regulation between Gestational Diabetes Mellitus (GDM) Platelets and Placenta. *J Biol Chem.* 291(18):9657-65. (Retraction published J Biol Chem. 2019 Jun 14;294(24):9656).
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2014; *37*(Supplement_1):S81-S90.
4. Pridjian G, Benjamin TD. Update on gestational diabetes. *Obstet Gynecol Clin N Am*. 2010; 37(2):255-67.
5. Aviram A, Yogev Y. Metabolic and hormonal changes in normal and diabetic pregnancy. In: Langer O, editor. The Diabetes in Pregnancy Dilemma: Leading Change with Proven Solutions. Shelton: People's Medical Publishing House. 2015:56-57.
6. Practice Bulletin No. 137: Gestational diabetes mellitus. *Obstet Gynecol.* 2013; 122(2 Pt 1):406-16.
7. Salzer L, Yogev Y. Complications of gestational diabetes. In: Petry CJ, editor. Gestational Diabetes: Origins, Complications, and Treatment. Boca Raton: Taylor & Francis Group. 2014:97-107.
8. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, et al. International association of diabetes and pregnancy study groups recommendations on the

diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care.* 2010; 33(3):676-82.

9. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin. Clinical Management Guidelines for Obstetrician-Gynecologists, Number 69, December 2005 (replaces Practice Bulletin Number 25, March 2001). Emergency contraception. *Obstet Gyneco*. 2013; 122:406-16.

10. Şen C, Yayla M, Api O. ve ark. Gebelikte diabet: Tanı ve tedavi. Türk Perinatoloji Derneği Uygulama Rehberi. *Perinat J*. 2016; 24(2):110-27.

11. Yavuzcan A, Cağlar M, Ustün Y, et al. Evaluation of mean platelet volume, neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in advanced stage endometriosis with endometrioma. *J Turk Ger Gynecol Assoc*. 2013; 14(4), 210-15.

12. Wang D, Yang JX, Cao DY. et al. Preoperative neutrophil-lymphocyte and platelet-lymphocyte ratios as independent predictors of cervical stromal involvement in surgically treated endometrioid adenocarcinoma. *OncoTargets Ther*. 2013; 6:211-16.

13. Topdagi Yilmaz EP, Topdagi YE, Al RA, Kumtepe Y. The relationship between C-reactive protein, carbohydrate antigen 125, and hematological parameters to endometriotic nodule localization in pelvis. *Journal of the Chinese Medical Association: JCMA*, 2020; 83(6):577-81.

14. Celikbilek M, Dogan S, Ozbakır O. et al. Neutrophil-lymphocyte ratio as a predictor of disease severity in ulcerative colitis. *J Clin Lab*. 2013; 27(1):72-76.

15. Aygün F, Efe D. Association of neutrophil/lymphocyte ratio with obstructive coronary artery disease and coronary artery calcium score detected by multislice computed tomography in type 2 diabetes mellitus patients. *Patient Prefer Adher*. 2015; 9:1023-31.

16. Caglayan EK, Engin-Ustun Y, Gocmen AY. et al. Is there any relationship between serum sirtuin-1 level and neutrophil-lymphocyte ratio in hyperemesis gravidarum? *J Perinatal Med*. 2016; 44(3):315-20.

17. Kurtoglu E, Kokcu A, Celik H, Tosun M, Malatyalioglu E. May ratio of neutrophil to lymphocyte be useful in predicting the risk of developing preeclampsia? A pilot study. *J Matern Fetal Neonatal Med.* 2015 Jan;28(1):97-9.

18. Pantham P, Aye IL, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta*. 2015; 36(7):709-15.

19. Pivari F, Mingione A, Brasacchio C, Soldati L. Curcumin and Type 2 Diabetes Mellitus: Prevention and Treatment. *Nutrients*. 2019; 11(8):1837.

20. Mertoglu C, Gunay M. Neutrophil-Lymphocyte ratio and Platelet-Lymphocyte ratio as useful predictive markers of prediabetes and diabetes mellitus. *Diabet Metab Synd.* 2017; 11(Suppl 1):S127-S131.

21. Lim AK, Tesch GH. Inflammation in diabetic nephropathy. Mediators Inflamm. 2012;146154.

22. Pattanathaiyanon P, Phaloprakarn C, Tangjitgamol S. Comparison of gestational diabetes mellitus rates in women with increased and normal white blood cell counts in early pregnancy. *J Obstet Gynaecol Res*. 2014; 40(4):976-82.

23. Gorar S, Abanonu GB, Uysal A, aet al. Comparison of thyroid function tests and blood count in pregnant women with versus without gestational diabetes mellitus. *J Obstet Gynaecol Res.* 2017; 43(5);848-54.

24. Wolf M, Sauk J, Shah A, et al. Inflammation and glucose intolerance: a prospective study of gestational diabetes mellitus. *Diabetes Care*. 2004; 27(1):21-27.

25. Sargın MA, Yassa M, Taymur BD, Celik A, Ergun E, Tug N. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios: are they useful for predicting gestational diabetes mellitus during pregnancy? *Ther Clin Risk Manag*. 2016; 12:657-65.

26. Sahbaz A, Cicekler H, Aynioglu O, Isik H, Ozmen U. Comparison of the predictive value of plateletcrit with various other blood parameters in gestational diabetes development. *J Obstet Gynaecol*. 2016; 36(5):589-93.

27. Friis CM, Paasche Roland MC, Godang K, et al. Adiposity-related inflammation: effects of pregnancy. *Obesity (Silver Spring, Md.)*. 2013; 21(1):E124-E130.

28. Liu W, Lou X, Zhang Z, Chai Y, Yu Q. Association of neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, mean platelet volume with the risk of gestational diabetes mellitus. *Gynecol Endocrinol.* 2021; 37(2):105-107.

29. Ulu S, Bucak A, Ulu MS, et al. Neutrophil-lymphocyte ratio as a new predictive and prognostic factor at the hearing loss of diabetic patients. *Eur Arch Otorhinolaryngol*. 2014; 271(10):2681-86.

30. Wang JR, Chen Z, Yang K, et al. (2020). Association between neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and diabetic retinopathy among diabetic patients without a related family history. *Diabetol Metab Synd.* 2020; 12:55.

31. Saheb Sharif-Askari F, Saheb Sharif-Askari N, Guella A, et al. Blood Neutrophil-to-Lymphocyte Ratio and Urine IL-8 Levels Predict the Type of Bacterial Urinary Tract Infection in Type 2 Diabetes Mellitus Patients. *Infect Drug Resis*. 2020; 13:1961-70.

32. Wang D, Yang JX, Cao DY, et al. Preoperative neutrophil-lymphocyte and platelet-lymphocyte ratios as independent predictors of cervical stromal involvement in surgically treated endometrioid adenocarcinoma. *Oncotarg Ther.* 2013; 6:211-16.

33. Mathur K, Kurbanova N, Qayyum R. Platelet-lymphocyte ratio (PLR) and all-cause mortality in general population: insights from national health and nutrition education survey. *Platelets*. 2019; 30(8):1036-41.

34. Fashami MA, Hajian S, Afrakhteh M, Khoob MK. Is there an association between platelet and blood inflammatory indices and the risk of gestational diabetes mellitus? *Obstet Gynecol Sci.* 2020; 63(2):133-40.

35. Onalan E, Gozel N, Donder E. Can hematological parameters in type 2 diabetes predict microvascular complication development? *Pak J Med Sci.* 2019; 35(6):1511-15.

NTMS New Trends in Medicine Sciences
https://dergipark.org.tr/tr/pub/ntms

ISSN: 2717-8161 CASE REPORT

New Trends in Medicine Sciences

New Trend Med Sci 2023; 4(2): 89-94. https://dergipark.org.tr/tr/pub/ntms

International Travel-Related COVID-19 Infection and Outbreak from Wedding Ceremony: First Case in Turkey

Esra Çınar Tanrıverdi[1*], Zulal Ozkurt[2]

[1]Department of Medical Education, Faculty of Medicine, Atatürk University Erzurum, Turkey.
[2]Department of Department of Infectious Diseases, Faculty of Medicine, Atatürk University Erzurum, Turkey

Article History
Received 17 Aug 2021
Accepted 13 Dec 2021
Published Online 25 May 2023

****Corresponding Author***
Esra Çınar Tanrıverdi
Department of Medical Education
Faculty of Medicine
Atatürk University,
Istanbul, Turkey
Phone: +90 5333233047
E-mail: esracinart@yahoo.com

Doi: 10.56766/ntms.983693

Authors' ORCIDs
Esra Çınar Tanrıverdi
http://orcid.org/0000-0001-8857-3986
Zulal Ozkurt
http://orcid.org/0000-0001-5554-8768

Abstract: The SARS-CoV-2 virus is a new and highly contagious respiratory virus that is mainly transmitted by air droplets. Here we reported the event in which the case that came to the wedding with a trip abroad led to SARS CoV-2 transmission and then an epidemic. Two index passenger groups include three families arriving in İstanbul from Paris to attend the wedding ceremony that infected Turkey with the COVID-19 infection. They were in close contact with about 350 people during and after the wedding ceremony. Clinically, the picture of COVID-19 infection was seen in 53 relatives. PCR positivity was found in 35 hospitalized cases. The infection spread to four cities in Turkey. The most frequent symptoms of 35 confirmed cases were dizziness (77%), fever (57.1%), joint and muscle pain (57.1%), loss of smell (45.7%), loss of taste (42.9%), shore throat (37.1%), dry cough (34.3%), diarrhea (25.7%), rhinorrhea (14.3%), and dyspnea (8.5%). The severity of 48 cases (90.5%) were mild-moderate. Severe pneumonia developed in five cases (9.4%), requiring intensive care and intubation, and four died (7.5%).
COVID-19 virus can be easily picked up during air travel and transmitted to other people through unprotected contact. The infection prevention rules should be strictly applied for the protection from disease. Persons should avoid attending meetings, even with their family or relatives, and should stay at home. The wedding and other social activities should be postponed until after the pandemic. © 2023 NTMS.

Keywords: Coronavirus; Travel; Weeding; Spread; Source.

1. Introduction

SARS-CoV-2 virus is a new and highly contagious respiratory virus from the coronavirus family. SARS-CoV-2 causes a wide range of diseases from asymptomatic diseases to severe fatal pneumonia characterized by ground-glass opacity. Multisystem involvement can also be seen in the infection 1.

Identified in January 2020, SARS-CoV-2 virus spreads rapidly and caused a pandemic in four months. The number of confirmed cases reached nearly 170 billion and death 3.5 billion in June 2021 [2]. All global regulations and social life have changed to prevent infection transmission. Some countries have banned or controlled international travel from endemic

Cite this article as: Çınar Tanrıverdi E, and Ozkurt Z. International Travel-Related COVID-19 Infection and Outbreak from Wedding Ceremony: First Case in Turkey. *New Trend Med Sci*. 2023; 4(2):89-94. Doi:10.56766/ntms.983693

to non-endemic areas.
We report here that the source of the epidemic is due to international travel for the wedding ceremony.

Outbreak
First index cases (A) were members of a family [mother (A1), father (A2), and daughter (A3)], who came to Turkey from France to attend his relatives' wedding on 7 March 2020. During the travel, the mother had conjunctivitis and the father had a sore throat. Daughter had no symptoms. Second index families (B and C) were 4 persons consisting of two couples (B1, B2, C1, C2), they arrived by plane from Paris on 8 March and attended the wedding ceremony in Istanbul the same night (Figure 1).

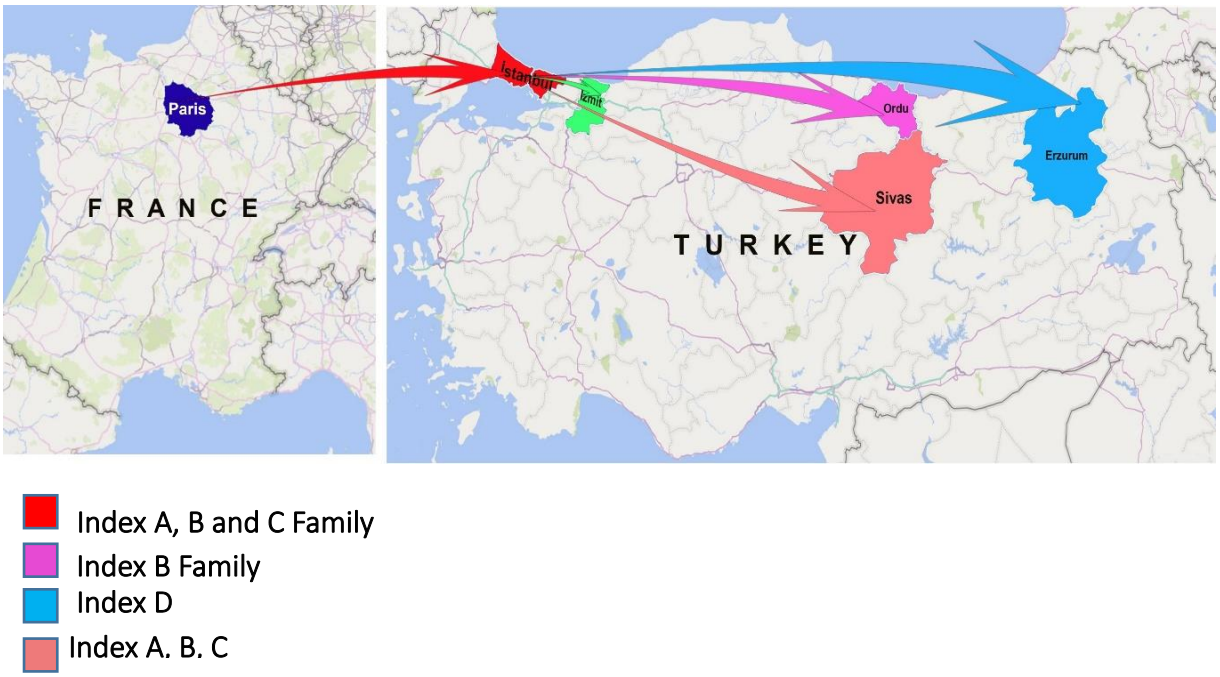


Figure 1: The travel map of index cases.

There were no complaints related to COVID-19. The index families and their relatives had close contact for hours, such as hugging, handshaking, dancing, and sharing the same environment in the wedding ceremony that 250 persons attended. At night, many relatives stayed at the same houses in a family apartment include three-floor, together with the index families (Figure 2).
Since there were no cases of COVID-19 in Turkey yet, they did not wear masks or follow social distancing. The next day vomiting started at index A1 and A2.
Turkish Public Health Department called B and C index families and said that there was a COVID-19 positive passenger on the plane departing from Paris and died, so they should be quarantined 14-days. But they did not comply with the isolation, and the next day, Index B family traveled to Sivas and Ordu cities of Anatolia (Figure 1 and 3).
Moreover, one another relative (index D) from Erzurum city was attended the same wedding ceremony and contacted them. Thus, the infection spread to four cities in Turkey (Figure 1).

There were approximately 350 people in contact with the A and B index cases during the wedding ceremony and subsequent travel period. In the following weeks, 53 relatives (8 children) became ill. 35 of them were diagnosed with COVID-19 with RT-PCR test positivity and were hospitalized. Other 18 persons in close contact had complaints but tested negative. They were probable COVID-19 infections.
Only one woman did not become ill, despite household contact (staying in the same home) who used traditional wearing “yaşmak” to cover her nose and mouth similar to mask.
There were 53 cases (26 females, 27 male) in this outbreak. The mean age was 51 years (range: 8-79).
Eight cases (15.0%) were children. Symptoms of confirmed cases showed in Table 1. The complaints of the patients were dizziness, fever, sore throat, dry cough, back and chest pain, myalgia, vomiting, diarrhea, loss of taste, and sense of smell (recovered in one-month period). Conjunctivitis was seen in two cases. Enuresis and sensory loss occurred in the legs in two cases but improved one month later. Forty-eight cases (90.5%) were mild-moderate. Five cases

(9.4%) had severe pneumonia, monitored and intubated in the intensive care unit. One of the intubated cases was the A1 index case, a 60-year-old woman with asthma. Another one was a 35-year-old woman, who has a breast cancer history andstayed at the same home with A1. Four cases (7.4%) died: A diabetic man (79), a man with chronic obstructive pulmonary disease (COPD) (63), a 70-year-old man, and a 70-year-old woman (Figure 3).

Among intubated patients, only young women recovered. Pericardial effusion, prolonged QT as a drug side effect, and arrhythmia were developed in an intubated young woman. Two weeks after discharge, she was hospitalized again with dyspnea and treated again with favipiravir, and hallucinations were detected during the last therapy period. In this case, cough continued for one month, similar to bronchial activation syndrome. All of the other cases improved after one month. In this outbreak, the secondary attack rate was estimated as 15%.

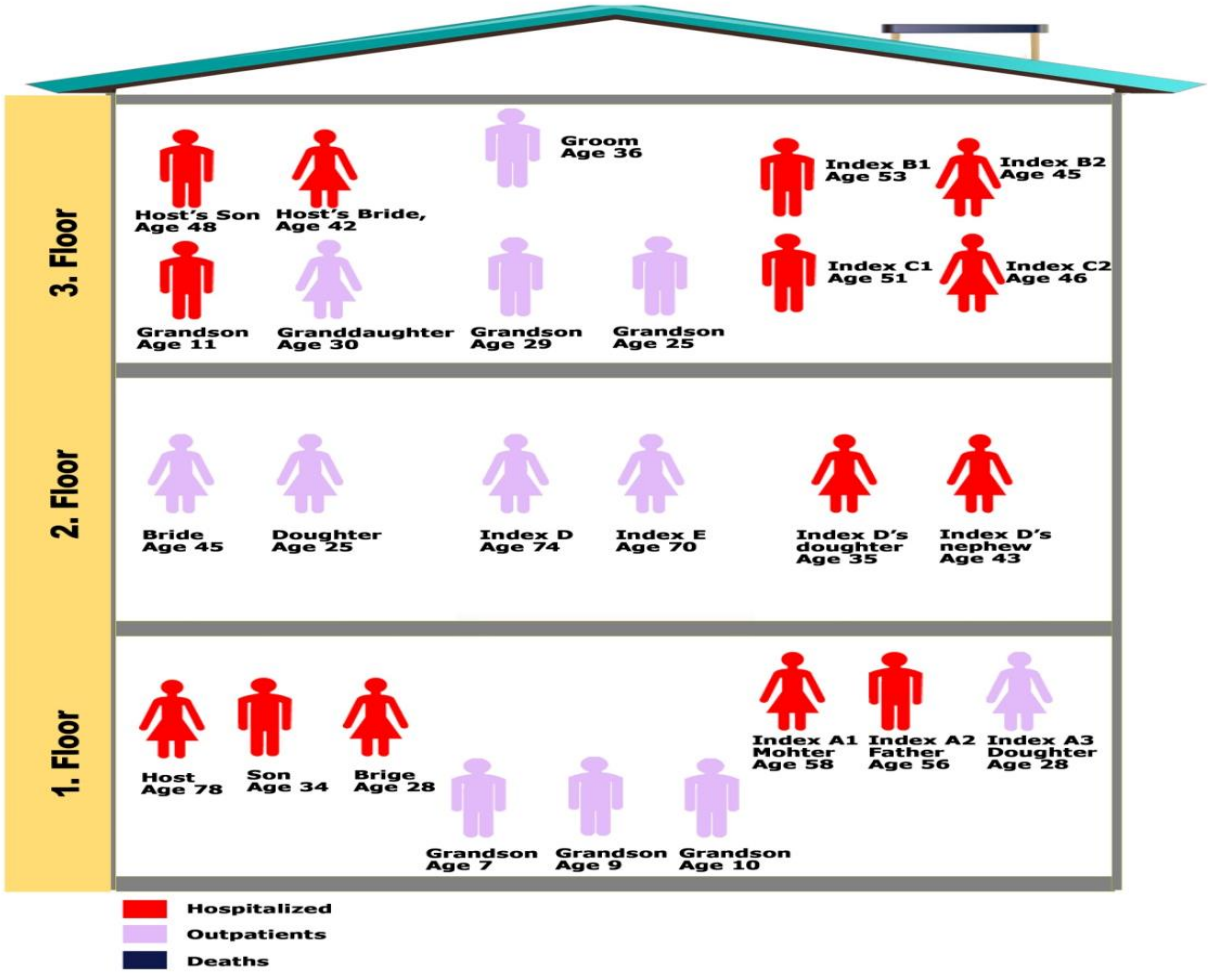


Figure 2: Household contact history of index cases in the family apartment in İstanbul after the wedding.

2. Discussion

Emerging infectious pathogens rapidly spread to distant areas by air travel such as the highly virulent respiratory pathogens SARS-CoV-1 and SARS-CoV-2. COVID-19 infection spread worldwide by international travelers and became a pandemic. International travel capacity has an important role in dispersing infection (3). Thus the travel restriction may have reduced the infection rate (4).

In this study, we described COVID-19 infection due to international travel between countries, and passengers caused an outbreak in Turkey. They were probably one of the first patients in Turkey. Since they arrived before the COVID-19 cases were detected in our country, they had unprotected contact with many people without taking any precautions.

Turkey is one of the countries where the COVID-19 pandemic emerged later, due to the limited international travel, a strict 14-day quarantine was imposed for people returning to the country from international travel, regardless of the presence of COVID-19 symptoms. The Turkish authorities, to prevent COVID-19, banned all meetings and weddings, closure of schools, closure of workplaces and markets, etc. They quickly implemented such broad prevention policies. Public training and relevant guidelines were published for infection prevention rules such as social distancing,

hand washing, and mask use. But the index cases in our study came just before or at the beginning of these measures. The first cases have not yet been identified in our country. So they probably didn't follow the isolation rules. Thus, they caused the start of the COVID-19 epidemic in four different cities of our country and the death of their close relatives.

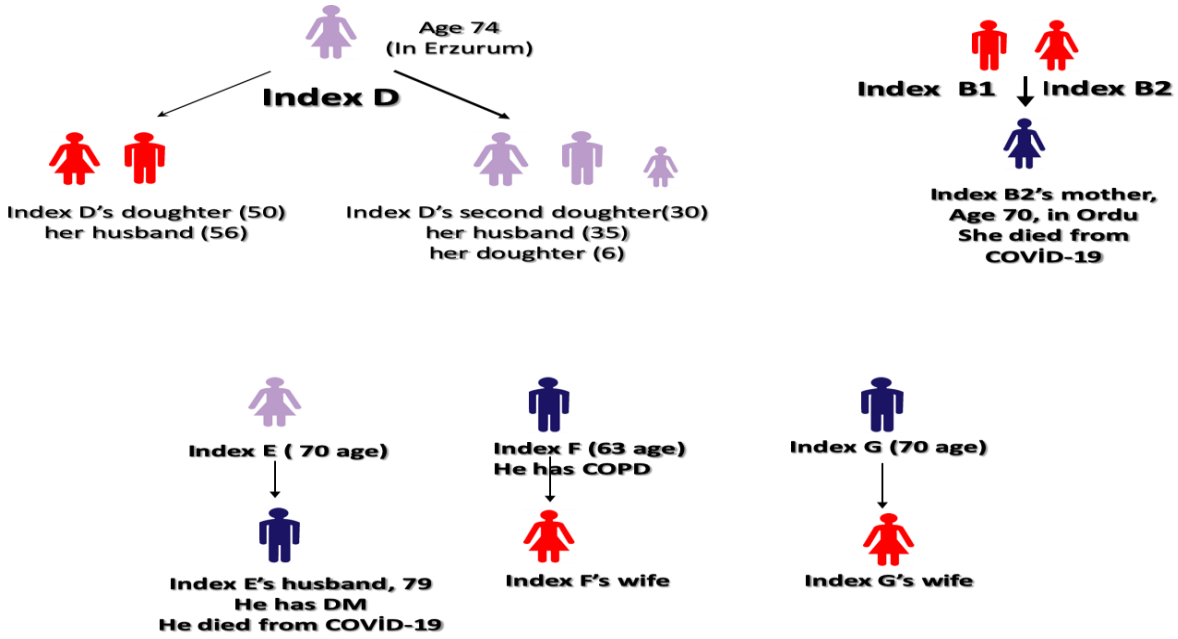


Figure 3: Persons contact with index cases at other cities.

Table 1: Table 1: Symptoms of COVID-19 cases.

Symptoms	Number (n=35)	Percent (%)
Dizziness	27	77.1
Fever	20	57.1
Myalgia	20	57,1
Joint Pain	20	57.1
Loss of Smell	16	45.7
Loss of Taste	15	42.9
Sore Throat	13	37.1
Dry Cough	12	34.3
Diarhea	9	25.7
Rhinorrhea	5	14.3
Dyspnea	3	8.5
Conjuctivitis	2	5.7
Sensory Loss in Legs	2	5.7
Enuresis	2	5.7
Hoarseness	1	2.8
Headache	1	2.8
Hemoptysis	1	2.8
Rash	1	2.8

This wedding ceremony took place before the official quarantine and prohibition. Since there were no cases in Turkey at the beginning of March, it was not necessary and/or obligatory to wear a mask and resort to social distance. All these measures were taken at a later period. So this wedding was one of the last meetings that caused an outbreak. But there were isolation rules for international travelers. The index had to be subject to travel bans and 14 days of isolation. In addition, due to the lack of fever at the arrival, index cases were probably not detected by thermal cameras.

In this outbreak, index cases had symptoms of COVID-19 infection and relative warning to wear masks or apply social distancing rule. But they did not comply, because they considered their symptoms were not related to COVID-19 infection. Additionally, mask-wearing has not been a rule in that period, yet. Therefore, the infection was transmitted to many persons at weddings and later in household contact.

COVID-19 virus is transmitted through the large respiratory droplets, and both mask usage and distancing between persons are important for protection. Index cases should be quarantined themselves, wearing masks, apply social distancing, and make proper handwashing and environmental cleaning and disinfection.

The risk of secondary transmission of SARS CoV-2 is greater in closed and crowded areas [5].

It was reported that the number of flashes increased with the loudness of speech and singing [6, 7].

Analyzing high-precision laser light scattering, the authors found that it could linger through the air for minutes without speaking aloud, emitting thousands of oral liquid droplets per second [6]. For this reason, index cases caused the spread of the virus to many unmasked people during the wedding held indoors. Therefore, in closed areas, precautions should be applied to protect SARS CoV-2 transmission [7].

The secondary attack rate (SAR) has been estimated as 0.55%-35% in COVID-19 infection [8-1]1. Liu et al. reported SAR was 35% in present close contact [9]. Li et all detected SAR was 4% in children and 17% in adults [8]. It was reported that SAR-related index cases were reported as 0% when index case applies self-quarantine versus as 16.9% if not applied. Additionally, SAR was found to be higher in between spouses (27.8%) than others (17.3%) [8]. It was reported that males' SAR rate is higher than females [9]. In the same study, the SAR rate of households was found as 7.5% and it was reported more than work or health care worker contact [9].

Symptomatic durations were found as important in the transmission of COVID-19 infection, and first 5 days reported more contagious and high attack rate than later period [12]. In another study, SAR in close contact was found 17-18% with a short incubation period and a very high rate (46%) in family members [13]. In this outbreak, SAR was calculated as 15.1%. Index cases may be superspreaders. Superspreaders were described during SARS, MERS, and COVİD-19 infection. Superspreaders were estimated to have higher viral load and usually in asymptomatic persons or immunosuppression. Another explanation is super spreaders have extensive social interaction [14]. In this outbreak, index cases had close contact with many relatives, living in four different cities. So, they were super spreaders with extensive social interaction.

Recently, an outbreak includes 16 people whose confirmed and probable cases with three deaths, reported from Chicago-Illinois after a funeral and a birthday party [15]. Similarly, after a funeral, 43 new COVID-19 cases were reported in the city of Erzurum. Persons from various cities had attended a funeral and in the subsequent weeks, new cases in the village were admitted to hospitals in the city center (unpublished data) [16].

SARS CoV-2 infection is a real risk during travel and is a travel-related infection. COVID-19 infection risk in found 4.5-60.2% in a 2-hour airplane trip without the mask. Infection risk in plain travel without mask found between 4.5- 60.2% in 2-hour flying, and it is estimated to increase 24.1-99.6% in 12-hour flighting. If all persons wear masks the risk is reduced by 73% for high efficiency and 32% for low- efficiency masks. Removing masks during food service increases the risk of infection [17]. It was shown that cross-border travel restrictions during the pandemic provide a reduction of cases number between 26% to 90%, the deaths number, the time to outbreak, and the effective reproduction number [18]. Protection rules should be strictly implemented and enforced during airlines and in other travels. Risk assessment for travel health is essential. Many factors contribute to the passenger's health risk. Therefore, a multifaceted approach is needed to prevent the transmission of the disease [19]. Therefore, pre-flight screening, mask-wearing, social distance, hand disinfection, and disinfection of frequently touched surfaces should be applied. Infection transmission is high during eating and drinking; this activity should be prohibited on short trips. Time without a mask on long journeys should be minimized when eating [20]. Passenger movements should also be limited. Recently, some countries have added vaccine cards to these measures on international travel [21].

3. Conclusions

In conclusion, the COVID-19 virus can be easily picked up during air travel and transmitted to other persons with unprotected household contact. Additionally, persons understood the importance of travel restriction, social distancing, and the usage of masks to prevent transmission of COVID-19. The infection prevention rules should be strictly applied for the protection from disease. Persons should avoid meetings, even with their family or relatives, and should stay at home. Quarantine of index cases is important to prevent disease transmission. All recommendations released by international or governmental health departments should be strictly applied.

Limitations of the Study

The most important limitation of the study is that the data is obtained from verbal data by talking to the patients.

Acknowledgement

We thank the patients who participated in the study.

Conflict of Interests

The authors declared no conflict of interest.

Financial Support

No funding was received to produce this article.

Author Contributions

ECT and ZO designed the research. ECT participated in data collection and data analysis. ECT and ZO wrote the manuscript, read and approved the final script.

Ethical Approval

Ethical permission was obtained from the Atatürk University Medical Faculty Clinical Research Ethics

Committee for this study (Date: 17.12.2020 Number: 10/15). The work is carried out in accordance with the rules of the Helsinki Declaration.

Informed Consent

Informed consent was obtained from all patients.

Availability of Data and Materials

All data of the study are contained in the article.

References

1. Dousari AS, Moghadam MT, Satarzadeh N. COVID-19 (Coronavirus Disease 2019): A New Coronavirus Disease. *Infect Drug Resist.* 2020; 13: 2819.
2. Worldometer. CU. https://www.worldometers.info/coronavirus/ 2021. Accessed 03.05.2021.
3. Wilson ME, Chen LH. Travellers give wings to novel coronavirus (2019-nCoV). *J Travel Med*. 2020; 27(2):1-3.
4. Chinazzi M, Davis JT, Ajelli M et al. The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID-19) outbreak. *Science.* 2020; 368(6489):395-400.
5. Nishiura H, Oshitani H, Kobayashi T et al. Closed environments facilitate secondary transmission of coronavirus disease 2019 (COVID-19). *MedRxiv* 2020; 20029272.
6. Anfinrud P, Stadnytskyi V, Bax CE, Bax A. Visualizing speech-generated oral fluid droplets with laser light scattering. *New Eng J Med*. 2020; 382(21):2061-63.
7. Bazant MZ, Bush JW. A guideline to limit indoor airborne transmission of COVID-19. *Proc Natl Acad Sci.* 2021; 118(17): e2018995118.
8. Li W, Zhang B, Lu J et al. The characteristics of household transmission of COVID-19. *Clin Infect Dis.* 2020; 71(8):1943-46.
9. Liu Y, Eggo RM, Kucharski AJ. Secondary attack rate and superspreading events for SARS-CoV-2. *The Lancet.* 2020; 395(10227):e47.
10. Bi Q, Wu Y, Mei S et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. *Lancet Infect Dis*. 2020; 20(8):911-19.
11. Kwok KO, Wong V, Wei VWI et al. Novel coronavirus (2019-nCoV) cases in Hong Kong and implications for further spread. *J Infect.* 2020; 80(6):671-93.
12. Cheng H-Y, Jian S-W, Liu D-P et al. Contact tracing assessment of COVID-19 transmission dynamics in Taiwan and risk at different exposure periods before and after symptom onset. *JAMA Internal Med.* 2020; 180(9):1156-63.
13. Jing Q, Li Y, Ma M et al. Contagiousness and secondary attack rate of 2019 novel coronavirus based on cluster epidemics of COVID-19 in Guangzhou. *Zhonghua Liu Xing Bing Xue Za Zhi.* 2020; 41(10):1623-26.
14. Al-Tawfiq J, Rodriguez-Morales A. Super-spreading events and contribution to transmission of MERS, SARS, and COVID. *J Hosp Infect*. 2020; 105(2):111-12.
15. Ghinai I, Woods S, Ritger KA et al. Community transmission of SARS-CoV-2 at two family gatherings-Chicago, Illinois, February-March. *MMWR Morb Mortal Wkly Rep.* 2020; 69(15):446-450.
16. https://www.ntv.com.tr/turkiye/cenaze-icin erzuruma-gelip-43-kisiyecorona-virus bulastirdilar,FwKv1PHvv0mp2vWx6kRfEg Accessed 02.03.2021.

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