e-ISSN: 2149-3189

European Research Journal

Volume 10 Issue 5 September 2024

Available at https://dergipark.org.tr/en/pub/eurj

The European Research Journal

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Abstracting and Indexing

The journal is abstracted and indexed with the following: ULAKBIM TR Index (ULAKBİM TR DİZİN), NLM Catalog (NLM ID: 101685727), Google Scholar (h-index: 12), Index Copernicus (ICV 2022: 100), EMBASE, ProQuest Central, EBSCO Academic Search Ultimate, ROAD, SciLit, MIAR (ICDS 2021: 3.8), J-Gate, SHERPA/RoMEO, BASE, EZB, CrossRef, JournalTOCs, WorldCat, TURK MEDLINE, Turkish Citation Index, EuroPub, OpenAIRE, ResearhGate, SOBIAD, Advanced Science Index, ScienceGate, OUCI, Publons, (Clarivate Web of Science)

Publisher

The European Research Journal (EuRJ)
Prusa Medical Publishing
Konak Mh. Kudret Sk. Şenyurt İş Mrk. Blok No:6 İç kapı no: 3
Nilüfer/Bursa-Turkey
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Table of Contents

Original Articles

Review

Case Report

The European Research Journal 2024;10(5):430-438 Original Article

DOI: https://doi.org/10.18621/eurj.1422897 *Neurology*

Fingolimod real life experience in non-naive multiple sclerosis patients

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ABSTRACT

Objectives: Fingolimod is approved in Turkey or the treatment of cases of multiple sclerosis (MS) which cannot be controlled with first-line treatments. There is limited information about its efficacy and safety in clinical practice in Turkey. The aim of this study was to evaluate the efficacy and safety of fingolimod treatment in patients with relapsing-remitting multiple sclerosis who were prescribed fingolimod by the Multiple Sclerosis specialists of Bursa Uludağ University Department of Neurology.

Methods: This is a single-center observational study evaluating 142 patients using fingolimod who were followed up for at least 12 months in our center between April 2015 and October 2022. Efficacy results were evaluated in terms of mean number of attacks, annualized relapse rate, relapse-free patient rate, disease progression, clinical and radiological disease activity, and no evidence of disease activity (NEDA-3). The safety outcomes are the rates of treatment-related severe adverse events and patients' continuation rates.

Results: Over 12 months of treatment with fingolimod, the average number of attacks decreased by 94.6%, the annual relapse rate decreased by 87%, and most patients did not relapse (83.1%). Alongside this, in 76.4% of cases, there was no disability progression and in 83.3% of cases, magnetic resonance imaging (MRI) activation was not observed. Excluding replacement due to ineffectiveness, 89.4% of patients continued fingolimod therapy. Cardiac events, treatment-related infections and a decreased lymphocyte count were observed as side effects.

Conclusion: In our center, switching from first-line treatments to fingolimod was effective in reducing disease activity in patients with multiple sclerosis.

Keywords: Fingolimod, real world, observational study, relapsing-remitting multiple sclerosis

MS is a chronic autoimmune, neurodegenerative disease of the central nervous system characterized by inflammation, demyelination, and loss of axons [1]. Clinically, the disease often progresses with recurrence and improvement of neurological symptoms; this form is classified as "relapsing-remitting MS" (RRMS). The most common symptoms include visual defects, spasticity, weakness, ataxia, and sensory symptoms such as bowel and bladder incontinence, fatigue, depression, numbness, or pain. For the last two decades, treatment strategies for MS have been aimed at reducing the frequency of attacks and slowing the progression of the disease. Classical immunomodulatory treatments such as interferon

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How to cite this article: Sarıdaş F, Koç ER, Özkaya G, Turan ÖF. Fingolimod real life experience in non-naive multiple sclerosis patients. Eur Res J. 2024;10(5):430-438. doi: 10.18621/eurj.1422897

Received: January 22, 2024
Accepted: April 29, 2024
Published Online: May 14, 2024

beta (IFN β) and glatiramer acetate (GA) used in primary care (which have been found to positively change the course of the disease in the treatment of RRMS), reduce the annualized relapse rate (ARR) by approximately 30% [2]. Fingolimod is the first oral MS drug approved by multiple countries for second-line therapy and it was first used in Turkey after a license was obtained in April 2011. Fingolimod prevents lymphocytes from leaving the lymph node and thymus with lysophospholipid receptors known as sphingosine-1 phosphate. Randomized clinical controlled trials and real-world data have demonstrated the efficacy of fingolimod on attack frequency and magnetic resonance imaging. Improvement was shown for relapse, radiologic activity, disability progression and brain atrophy compared with IFN β-1a and placebo [3-5]. Clinical experience in day-to-day practice will be beneficial for physicians and other healthcare professionals caring for people with MS. In this study, we present the outcomes of patients with RRMS treated with fingolimod for at least 12 months as a single-center clinical trial.

METHODS

Study Design

This study is a single-center, observational, retrospective review of the medical records of 142 patients treated with fingolimod 0.5 mg as part of standard clinical practice in our center between April 2015 and October 2022. Patients who started fingolimod at least 12 months ago and had no missing data were included were included in the study. The local ethics committee (Uludag University Faculty of Medicine Clinical Tesearches Ethics Board) reviewed and approved the study protocol (2022-16/35).

Study Population

The study population consists of patients with RRMS over 18 years under the treatment of neurologists specializing in MS. Patients were included in the study if they were diagnosed with RRMS according to the revised 2017 McDonald or earlier criteria at least 12 months before initiation of fingolimod and if they had continued fingolimod treatment for at least 12 months without interruption (<4 weeks). Before starting fingolimod, patients had to have been under follow-up for at least six months.

Patients were divided into two subgroups based on disease-modified therapy (DMT) use before fingolimod initiation: those who had received one DMT before and those who had received two or more prior DMTs. Those who had switched from induction therapies to fingolimod were not grouped due to the low number of patients. The patients were evaluated in four similarly distributed groups (12-23, 24-35, and ≥48 months) according to the duration of use. The groups were similarly distributed when grouped according to the number of previous treatments (one or two and above), age at diagnosis, first EDSS, and previous ARR.

Outcome Criteria

As outcome measures under fingolimod, the following were evaluated: ARR, rate of patients without relapse, rate of patients without disability progression (one point increase above Expanded Disability Status Scale (EDSS) baseline level, 1.5 point increase if EDSS 0), proportion of patients without new t2 lesion or new enhancement lesion on MRI and proportion of patients without both relapse and disability progression, and MRI activation. Relapses were defined as the appearance of new neurological deficits occurring at least 30 days after the onset of a previous event and lasting more than 24 hours in the absence of fever or infection. The ARR was calculated as the number of relapses divided by the exact (decimal) number of years on fingolimod treatment.

Sex and age were evaluated as demographic data. Clinical features included reasons for starting fingolimod treatment, DMTs used before fingolimod, final EDSS scores at the start, end, or if ongoing, disease duration before fingolimod initiation, the total number of attacks before and after fingolimod, and MRI activation. In terms of safety data, serious adverse events that required discontinuation, change or interruption of treatment were evaluated.

Statistical Analysis

The Shapiro-Wilk test was used to examine the data to determine whether or not it was normally distributed. The results were presented as mean±standard deviation, median (minimum-maximum), or frequency and percentage. Normally distributed data were compared using independent samples t-test or

one-way ANOVA. Kruskal Wallis and Mann-Whitney U tests were used for non-normally distributed data. The Bonferroni test was used as a multiple comparison test. Paired data were analyzed using the Paired t-test or Wilcoxon signed rank test. Categorical variables were compared using Pearson's chi-square test and Fisher's exact test between groups. P<0.05 was considered as the significance level. Statistical analyses were performed with IBM SPSS ver.28.0 (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.).

RESULTS

Population and Pre-Fingolimod Data

A total of 142 patients, 106 women (74.6%) and 36 (25.4%) men, were included in the study. The mean age at which the patients were diagnosed was 31.26±8.83 (18-60) years and the mean time from diagnosis to start of fingolimod treatment was 76.55±51.58 (8-244) months.

Different DMTs were used in 142 patients before fingolimod use. 98 patients (69%) used one DMT and 41 patients (28.9%) used 2 or more. In three patients was switched to fingolimod for de-escalation. The most commonly used DMT is IFN β. Their average usage time was 48.97±32.1 (5-189) months. The mean total number of attacks was 2.64±1.32(1-12), and the number of attacks was 1.33±0.92(0-5) when using DMT at the last use (at least six months). The ARR was 0.61±0.50 (0.11-3), and the ARR in the latest DMT was 0.47±0.48 (0-2.4). Disease onset was mean EDSS 1.18±0.92 (0-6.5), and mean fingolimod onset was 2.02±1.38 (0-6). The most frequently cited reasons for switching to fingolimod are as follows: the ineffectiveness of previous therapy, followed by adverse events or patient request, and de-escalation therapy (Table 1).

Usage Process and Subgroups

The mean duration of fingolimod use was

Table 1. Baseline data before fingolimod

		n (%) / mean (SD)
All previous disease-modifying therapies*	Interferon beta	98 (50.7%)
	Glatiramer acetate	58 (30.1%)
	Teriflunomide	28 (14.5%)
	Dimethyl fumarate	6 (3.1%)
Most recent previous disease-modifying therapy*	Interferon beta	57 (41.3%)
	Glatiramer acetate	50 (36.2%)
	Teriflunomide	25 (18.1%)
	Dimethyl fumarate	6 (4.4%)
Total number of attacks (mean)	Total	2.64±1.32
	In the last previous DMT	1.33±0.92
Annualized relapse rate	Total	0.61±0.50
	In the last previous DMT	0.47±0.48
EDSS	Disease onset	1.18±0.92
	Fingolimod onset	2.02±1.38
Reason for switch	Ineffectiveness	115 (81%)
	Adverse events or patient request	26 (16.2%)
	After induction therapy	3 (2.1%)

*Except for induction treatments

Descriptive statistics were given as mean±standard deviation or frequency (n) with percentage.

34.15±16.17 (12-85) months. In contrast, the distribution according to age at diagnosis (P=0.851) and first EDSS (P=0.201) was similar in the subgroups according to the time from diagnosis to fingolimod use (<24 months, 24-48 months, 48-72 months, 72-96 months, and >96 months), as expected, the number of attacks per previous year (P<0.001) and the number of previous attacks (P<0.001) increased as the time between diagnosis and start of fingolimod treatment increased.

Efficacy Results

The mean number of attacks after starting fingolimod treatment in all patients was 0.15±0.40 (0-2) compared to previous DMT use (1.33±0.92(0-5)) with an 88.8% reduction (P<0.001), total mean before fingolimod (2.64±1.32(1-12)) compared to 94.6% (P<0.001). The rate of patients who did not have an attack during fingolimod use was 83.1% in all patients. The mean ARR of all patients decreased from 0.61±0.5 to 0.08±0.22 before and after fingolimod by 87% (P<0.001). EDSS mean at disease onset was 1.18±0.92, 2.08 ± 1.35 at fingolimod onset and 2.28 ± 1.72 at the end of fingolimod treatment. There was a significant effect on slowing down the progression of the disability when these were compared (P=0.015). No disability progression was observed after fingolimod in 76.4% (n=109) of all patients (Fig. 1).

MRI follow-ups of 28 patients were unsuitable for

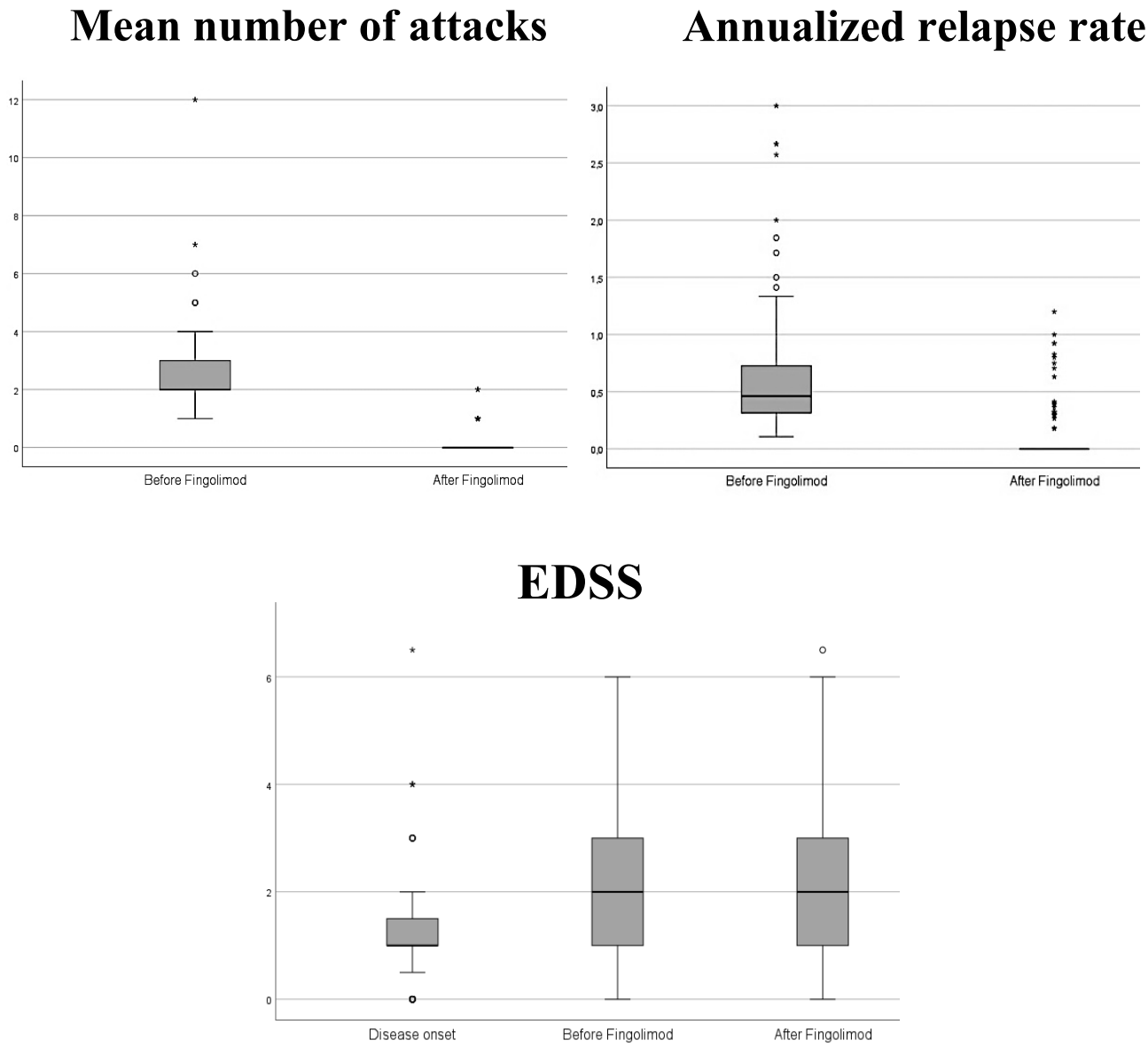

Fig. 1. Number of attacks, ARR and EDSS changes before and after fingolimod.

Table 2. Subgroup analyzes

		n	Annualized relapse rate Median (Minimum-Maximum)	Relapse-free % n (%)	EDSS progression-free % n (%)	MRI activity-free % n (%)	NEDA-3 % n (%)
Sex	Female	106	0.00 (0.00-1.20)	89 (84%)	80 (75.5%)	70 (83.3%)	55 (51.9%)
	Male	36	0.00 (0.00-0.80)	29 (80.6%)	29 (80.6%)	25 (83.3%)	19 (52.8%)
	P value		0.664	0.637	0.533	1.000	0.926
Disease onset age (year)	<25	40	0.00 (0.00-0.92)	35 (87.5%)	32 (80%)	25 (78.1%)	23 (57.5%)
	25-35	59	0.00 (0.00-1.20)	46 (78%)	46 (78%)	37 (78.7%)	28 (47.5%)
	>35	43	0.00 (0.00-1.00)	37 (86%)	31 (72.1%)	33 (94.3%)	23 (53.5%)
	P value		0.423	0.382	0.667	0.113	0.603
Number of previously used DMT*	1	98	0.00 (0.00-0.80)	83 (84.7%)	77 (78.6%)	66 (86.8%)	52 (53.1%)
	≥2	44	0.00 (0.00-1.20)	35 (79.5%)	32 (72.7%)	29 (76.3%)	22 (50%)
	P value		0.360	0.449	0.446	0.155	0.743
EDSS before fingolimod	<3	100	0.00 (0.00-0.92)	84 (84%)	81 (81%)	67 (84.8%)	45 (45%)
	≥3	42	0.00 (0.00-1.20)	34 (81%)	28 (66.7%)	28 (80%)	23 (54.8%)
	P value		0.518	0.658	0.065	0.525	0.288
Usage Period (months)	12-23	47	0.00 (0.00-1.20)	39 (83%)	35 (74.5%)	27 (75%)	22 (46.8%)
	24-35	30	0.00 (0.00-0.83)	25 (83.3%)	22 (73.3%)	22 (88%)	17 (56.7%)
	36-47	35	0.00 (0.00-0.32)	26 (74.3%)	26 (74.3%)	31 (93.9%)	22 (62.9%)
	≥48	30	0.00 (0.00-0.18)	28 (93.3%)	26 (86.7%)	15 (75%)	13 (43.3%)
	P value		0.270	0.243	0.551	0.109	0.344
Diagnosis-fingolimod onset time (months)	12-23	19	0.00 (0.00-0.40)	16 (84.2%)	17 (89.5%)	12 (92.3%)	9 (47.4%)
	24-47	29	0.00 (0.00-1.00)	25 (86.2%)	22 (75.9%)	25 (96.2%)	18 (62.1%)
	48-71	25	0.00 (0.00-0.92)	21 (84%)	18 (72%)	18 (81.8%)	16 (64%)
	72-95	27	0.00 (0.00-0.92)	24 (88.9%)	21 (77.8%)	13 (68.4%)	17 (63%)
	≥96	42	0.00 (0.00-1.20)	32 (76.2%)	31 (73.8%)	27 (79.4%)	14 (33%)
	P value		0.640	0.738	0.686	0.110	0.260

*Three patients who were switched to fingolimod as de-escalation therapy were excluded. (2 of these three patients did not have an attack)

evaluation. Of 114 patients, 83.3% (n=95) had no MRI activity. New/growing T2 lesions were detected in 16.4% (n=19). New Gd(+)T1 lesions were detected in 11 (10%) of them. NEDA-3 was achieved in 52.1% (n=74) of patients in terms of the number of attacks, EDSS progression, and absence of all MRI activity.
In terms of effectiveness evaluation, five parameters (ARR, relapse-free rate, EDSS progression-free rate, MRI activity-free rate, and achievement rate of NEDA-3) were evaluated in terms of five different group characteristics (sex, disease onset age, number of previously used DMTs, usage period, diagnosis-fingolimod onset time). No statistically significant difference was found in the outcome parameters specified in all subgroup analyses (Table 2).

Sustainability and Safety Implications

One hundred and nine (76.8%) patients continued the treatment. Treatment was discontinued due to side effects in 4.2%, ineffectiveness in 14.1%, both 1.4%, pregnancy in 2.1%, and patient request in 1.4% of cases. Excluding 11 patients whose treatment was changed due to ineffectiveness at follow-up up to 24 months, 80.5% of patients continued fingolimod treatment. The rates of adverse events leading to treatment discontinuation and treatment continuation were 10.6%; 61.7% at the end of the first year, 6.5%; 71.4% at the end of the second year, 4.5%; 72.3% at the end of the third year, and 4.2%; 76.8% at the end of the fourth year, respectively. Treatment non-compliance and side effects were most common in the first year. Serious adverse event rates were as follows: cardiac events 3.5% (three bradycardia, one atrioventricular arrhythmia, one acute coronary syndrome; one patient with bradycardia continued after treatment discontinuation, others discontinued), infection 1.4% (two herpes zoster infections; treatment discontinued), transient lymphopenia (<200/µL) 7% (all continued after treatment interruption), macular edema 2. 1% (treatment discontinued), transient elevation of liver transaminase levels 0.7% (three-fold or more increase from baseline) (all continued after treatment interruption), grade 2 or higher high blood pressure 2.8% (three discontinued, one continued after interruption) and other 2.8% (flushing, etc.; all discontinued). Except for two herpes zoster infections, no opportunistic infections, including progressive multifocal leukoencephalopathy (PML), were observed.

DISCUSSION

The efficacy of fingolimod treatment in MS disease has been demonstrated in randomized controlled studies [3-5]. Real-world data on fingolimod obtained after use is very instructive in clinical practice. In multicenter studies, variable cohort number of cases and fingolimod exposure time were reported. The following are examples of data on countries, case numbers and follow-up periods from a selection of studies: UK 209, 12 months; Poland 253, 42 months; Germany 4229, 7 years; Spain 804, 26.4 months; Italy 414, 22.7 months; Hungary 570, up to 5 years; Czech Republic 237, 3.5 years [6-13]. In our country, there are multicenter studies evaluating the 5-year effect on cognition, comparing natalizumab (NTZ) and ocrelizumab treatment in the first and second year, and reporting two-year efficacy and safety results [14-16].

MS most commonly affects young adults aged 20-30, with a male to female ratio of approximately 3:1 [17]. The gender distribution in our study was similar to the general distribution of the disease. The gender factor did not affect our preference towards fingolimod treatment.
DMTs used before fingolimod in our cohort were mostly IFN β (50.7%), followed by GA (30.1%) as in many other studies [6, 7, 10-12]. Some studies have evaluated the efficacy of fingolimod after induction therapy at different rates or in naive patients [10-12]. However, there were no naive patients included in this study and three patients used fingolimod after induction therapy. In our study, the rate of previous single DMT use was 69%. The most important reason for switching to fingolimod in our cohort was the ineffectiveness of previous DMTs, with a higher rate (81%) than in other studies. These rates may vary between populations, depending on regional drug reimbursement policies and the preferences of clinicians or patients. [6, 10, [12].

The rate of patients who did not relapse during fingolimod use was 83.1%, which is similar to the literature. [3][6-12] Studies with naive and post-NTZ patient groups have shown that these results are higher in naive patients and lower in post-NTZ patients [10, 12].

Compared to pre-treatment, the ARR decreased by 87%, similar to other studies (65%-95.6%) [6, 7, 9, 11, 12]. Fingolimod significantly reduces the ARR regardless of age at diagnosis, age at onset of fin-

golimod, gender, number of previous DMTs used, and duration of fingolimod exposure. ARR reduction rates increase with duration of use in studies and generally range from 74.1% to 90%. With the addition of patients who had previously received NTZ treatment, these rates were lower (65% [11], 77% [12]) and higher in naive patient groups (91.1%-95.6%) [10]. In our study, it was not possible to evaluate these rates due to the small number of patients receiving NTZ treatment and the lack of a naive patient group. However, switching to fingolimod treatment in the early stages of the disease may have been more effective in the slightly higher reduction rate in ARR compared to other studies (6.4 years).

MRI activity was absent in 83.3%. There was no significant difference between MR activity and gender, age at disease onset and other sub-parameters. Compared to other studies, the positive effect on radiologic activation in this study was slightly higher than expected [6, 10, 11, 15].

EDSS scores were slightly lower than in other studies (2.08 ± 0.2) and the proportion of patients with no increase in EDSS scores was similar (76.4%) [7, 12].

In the analysis of patients with complete MRI data, the rate of NEDA-3 was 52.1%, slightly below the literature data (52.4%-67.9%). [7, 11].

Considering reasons other than ineffectiveness (14.1%), the rate of adherence and persistence was lower than in the literature at 80.5% (81.2%-93.9%) [6-8, 10, 13]. The rate of treatment ineffectiveness has generally been reported as approximately 10% in studies, and these are usually patients who have been treated with more than one DMT previously. However, we did not detect a significant relationship between previously used DMTs and treatment ineffectiveness. The most common reasons for temporary interruption are adverse effects and patient decisions. In comparison, the reasons for permanently stopping treatment are side effects and lack of effectiveness [10, 13]. We determined the reasons for permanent discontinuation as ineffectiveness (14.1%) and side effects (4.2%). The most common side effects reported are cardiac event, infections, liver enzymes increase, and lymphopenia < 200 cells/µl. PML or macular edema are usually unspecified or minor [10, 12, 13, 18]. Side effects requiring discontinuation or interruption of treatment in our cohort were similar to the literature. Basal cell skin cancer was detected in one patient.

Our cohort is slightly above the expected range for the overall RRMS diagnosis age in terms of disease-onset distribution. However, compared to many other studies, patients in our cohort were switched to fingolimod at a younger age: 31.2 (32.6 to 42.8), had a lower EDSS before: 2.08 (2.1 to 3.6) and a shorter time from diagnosis or first clinical symptom to drug exposure: 6.4 (7.5 to 10.3) [6, 9, 10, 12, 13]. Despite all this, no significant difference was found in the outcome parameters, except for MRI activity and ARR [6, 9, 10, 12]. In addition, when the first year from the disease diagnosis was excluded, the effect did not decrease as the time from the diagnosis to fingolimod extended. The FREEDOM and FREEDOM II studies have demonstrated the importance of early initiation of therapy to protect against long-term disease-related disability [4, 5]. When comparing early fingolimod treatment with delayed treatment, there was a significant 45% reduction in ARR and a 24% reduction in the risk of confirmed disability progression. In addition, fingolimod efficacy is higher in naive patients and lower in de-escalation therapy [10-12]. Due to the variable health policies in Turkey, fingolimod treatment is not paid for the first year of diagnosis and special permission is required for its use. Therefore, it was not possible to follow a naive patient group in our study. Alongside this, since the number of patients who de-escalation was too small, this data was not included in the evaluation.

Limitations

It was not possible to evaluate NEDA-4 because the cognitive evaluation records were not complete. Since the MRIs were not optimal for 28 patients, they could not be assessed radiologically. Aside from the patients whose treatment was interrupted or discontinued, we could not reveal the complete profile because the side effect records were not detailed.

CONCLUSION

MS is a progressive disease, and starting the right DMT at the right time is very important in preventing the progression of the disease. Turkish patients across the entire cohort and across all subgroups evaluated saw significant benefits from fingolimod therapy, regardless of age, gender, or having previously used

DMTs. The most significant disease-stopping power of fingolimod is seen in untreated patients, but it was not possible to evaluate this situation in our study due to certain limitations. That being said, when considered together with other studies, there was no difference in efficacy. However, patients who had previously received different DMTs were younger, had a lower disability, or were switched to fingolimod treatment in a shorter time. This suggests that although fingolimod has the most significant disease-stopping power in naive patients, it is also effective in patients who have used other DMTs before, regardless of the number of DMTs used, age at diagnosis, and disease duration. Real-world data studies with larger subgroup analyses are required in this regard. Analysis of fingolimod adverse events in a real-world setting confirmed a long-term, favorable profile of both efficacy and safety from study start to study end. Our results support the ever-increasing evidence proving the long-term effectiveness and positive benefit/risk balance of fingolimod in RRMS in a real-life setting.

Authors' Contribution

Study Conception: FS; Study Design: FS; Supervision: ÖFT; Funding: ERK, ÖFT; Materials: FS, ERK; Data Collection and/or Processing: FS, GÖ; Statistical Analysis and/or Data Interpretation: FS, GÖ; Literature Review: FS; Manuscript Preparation: FS and Critical Review: ERK, ÖFT.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2024;10(5):439-447 Original Article

DOI: https://doi.org/10.18621/eurj.1453664 *Neurosurgery*

Changes in canal diameter and cervical lordosis in patients who underwent en-bloc laminoplasty for the treatment of cervical spinal stenosis

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ABSTRACT

Objectives: Cervical spinal canal-expanding laminoplasty has been recognized as an alternative to cervical laminectomy, especially in multilevel cervical myelopathy due to spinal stenosis. This study aimed to determine the effects of En-block laminoplasty on cervical sagittal balance, cervical spine biomechanics and whether it is sufficient to preserve cervical canal diameter and lordosis and whether it causes additional lordosis or kyphosis in cases of cervical narrow spinal canal.

Methods: Thirty-eight cervical narrow canal patients operated on between 2008 and 2020 were retrospectively evaluated and the results of 24 laminoplasty patients were evaluated. Spinal tomography results for the evaluation of canal diameter changes and X-ray results for cervical sagittal balance evaluation were compared with each other in the early postoperative period and 3 years later. For cervical lordosis assessment, Cobb and C2-C7sagital vertical axis measurements were performed using the PACS system.

Results: Fifteen patients were male and 9 were female. The mean age was 65.55±11.56 years (min-max: 58-84) and the mean follow-up period was three years. Two patients had one level stenosis, 4 had two levels, 7 had three levels, and 11 had four or more levels. Radiculopathy was present in 17 patients (71.8%) and myelopathy in 7 patients (29.1%). There was no statistically significant difference in the frequency of myelopathy/radiculopathy between genders. When all distances were considered, no statistical difference was observed in the early postoperative period (median ten days) and late postoperative period (median 3 years) in terms of canal diameters. No measurement value could be obtained for any level diagnosed as restenosis or requiring reoperation. Loss of lordosis was measured in only one patient. Otherwise, lordosis was preserved in all cases at late conversion.

Conclusions: The results of our study showed that en-block laminoplasty after the cervical narrow spinal canal was sufficient to maintain the cervical canal diameter in the long term with appropriate patient and surgical technique.

Keywords: Cervical spinal stenosis, en-bloc laminoplasty, cervical canal diameter, cervical lordosis, laminoplasty

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How to cite this article: Meral M, Koç RK. Changes in canal diameter and cervical lordosis in patients who underwent en-bloc laminoplasty for the treatment of cervical spinal stenosis. Eur Res J. 2024;10(5):439-447. doi: 10.18621/eurj.1453664

Received: March 15, 2024
Accepted: May 9, 2024
Published Online: May 28, 2024

Compression of the cervical spinal cord from the anterior side by the vertebral corpus and degenerated intervertebral disc protruding posteriorly and from the posterior side by thickening of the ligamentum flavum and lamina is called cervical spinal stenosis. Between the C3 and C7 levels, the diameter of the spinal canal in the sagittal plane (anteroposterior) is 15-25 (mean 17) mm. There is no consensus on the value required for the diagnosis of cervical stenosis. It is possible to define 10-13 mm as relative and 9 mm and below as definite cervical stenosis [1].

Impairment in neurologic functions may vary from mild to severe and the modified Japanese Orthopedic Association (mJOA) [2] and Nurick [3] scoring systems are most commonly used in neurologic functional evaluation. Patients with an mJOA score above 15 points are considered mildly affected patients. Patients with an mJOA score of 12-15 are considered moderately affected patients. Patients who are considered severely disabled have a mJOA score below 12. While multi-level pathologies are more common in men, single-level pathologies are more predominant in women [4].

The disease progresses with symptoms and signs related to spinal cord and root compression. Neck pain and restriction in neck movements are present. As the condition progresses, sensory changes such as dysesthesia, clumsiness in the hands, and impaired sensation of vibration and joint position may be observed. Atrophy and weakness in the small muscles of the hands are common. In the lower extremities, myelopathy findings such as spastic gait, hyperreflexia, and babinski signs are typically added [5].

Cervical spinal canal widening laminoplasty has been accepted as an alternative to cervical laminectomy, especially in multilevel cervical myelopathy due to spinal stenosis [6, 7]. The purposes of the method are to widen the cervical spinal canal, to preserve posterior bone structures and cervical motion, to prevent instability and deformity, to prevent the development of postlaminectomy membrane seen after laminectomy, to be applicable at multiple levels, and to avoid complications of anterior surgery such as dysphagia, hoarseness, instrument failure, and lack of fusion [8]. Cervical laminoplasty is preferred in cases such as cervical spondylotic myeloradiculopathy, congenital cervical narrow canal, and especially multi-level posterior compression. Significant anterior compression, active posterior infection, history of radiotherapy, and kyphosis are contraindications for laminoplasty [9].

Laminoplasty in cervical spinal stenosis was first described by Hirabayashi [6]. It was developed by Shaffrey's and O'Brien using implants. Recently, en-bloc C3-6 laminoplasty has been adopted and popularised by preserving the nuchal ligament attached to the C7 spinous process to prevent axial neck pain [10]. In this method, if C6-7 levels are also affected, C7 archocristectomy is recommended.

In this study, the results of 24 cervical spinal stenosis patients operated with En-Block Lamino-

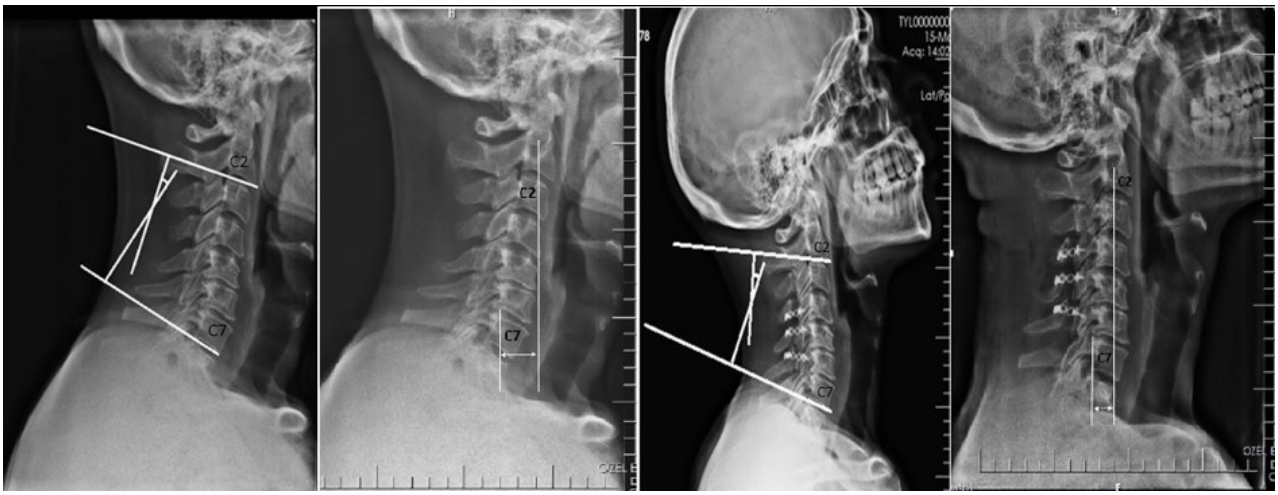

Fig. 1. Cervical Cobb angle and C2-C7 sagittal vertical axis measurement.

plasty were evaluated. Changes in cervical sagittal balance and enlarged canal diameter in the late postoperative period were evaluated. Late cervical canal diameter changes and cervical biomechanical changes were investigated.

METHODS

The results of 24 patients who underwent en-bloc laminoplasty for cervical spinal stenosis between 2008 and 2020 in our clinic were evaluated retrospectively. All patients in our series were operated with the hardware-assisted en-bloc laminoplasty technique [10, 11]. Patients who underwent postoperative revision surgery received additional treatments such as infection, malignancy, and radiation therapy, and whose radiologic images could not be obtained completely were excluded from the study. One side of the lamina is cut with a high-speed drill, preferably on the side where the clinical finding is dominant. On the other side, only the outer cortex is cut and the inner cortex of the inner lamina is left untouched. The lamina is then lifted with small angled curettes. The ligamentum flavum, interspinous, and supraspinous ligament are preserved. The removed lamina is then fixed with mini-plates.

In the study, axial cervical spinal tomography results were evaluated to assess postoperative late changes in cervical canal diameter. For the evaluation of the cervical sagittal balance, the measurement values of the early and late postoperative radiographs were compared. Standard cassettes and radiographs taken with the same device (Multifunctional Radiograph Unit Siemens-Germany) were used for radiologic evaluation. For sagittal plane imaging, radiographs were obtained from 1.8m in the lateral direction, in the neutral position, standing, and with C4 centralized. Picture Archiving and Communication Systems (PACS) software was used. For the evaluation of cervical lordosis and axial cervical balance, measurements were made with the Cobb angle and C2-C7 sagittal vertical axis method. In the Cobb method, the angle between the lower-end plateau of the C2 vertebra and the lower-end plateau of the C7 vertebra is calculated. For the C2-C7 sagittal vertical axis measurement, the distance between the C2 plump line and the posterior-superior end-plate of C7 is measured (Fig. 1) [12]. The study was approved by the Erciyes University institutional ethics committee (2023/279).

Statistical Analysis

Statistical analysis IBM SPSS Statistics 21.0 (SPSS Inc, Chicago, IL, USA) software was performed. The conformity of the data to normal distribution was analyzed by the Shapiro-Wilk test, and histogram and Q-Q graphs were evaluated. Data were expressed as median (1^{st}-3^{rd} quartile). Wilcoxon test was used for comparisons between periods (early and late postoperative period). The relationship between categorical variables was evaluated using Pearson's chi-square (χ^2) test (and Fisher's exact test). A value of $P<0.05$ was considered statistically significant.

RESULTS

Of a total of 24 patients, 15 (62.5%) were male and 9 (37.5%) were female. The mean age of the patients was 65.55±11.56 (min-max: 58-84) years. The mean age of males was 62.86±9.75 (min-max: 58-84) and the mean age of females was 61.53±14.01 (min-max: 59-81). Radiculopathy was present in 17 patients (71.8%) and myelopathy in 7 patients (29.1%). Two patients (8.3%) had one level stenosis, 5 patients (16.6%) had two levels, 7 patients (29.1%) had three levels and 11 patients (45.8%) had four or more levels. In total, 24 patients underwent laminoplasty at a total of 78 distances.

When the canal diameters were evaluated in individual distances, no statistically significant difference was found in the early and late canal diameter measurements in a total of 62 distances in C3-4/ C5-6/ and C6-7 distances (P=0.822, P=0.732, and P=0.509, respectively). In the C4-5 distance, a significant difference was found in a total of 16 distances (P=0.003) (Table 1).

Although statistically significant narrowing was observed in some distances in tomography measurements performed after an average of three years considering all distances, no measurement value could be obtained for any level diagnosed as restenosis or requiring reoperation. However, the median canal diam-

Table 1. **Postop early and late mean values of canal diameters according to distances**

Levels	Channel diameter (mm) (Postop 10. day)	Channel diameter (mm) (Postop 3. year)	P value
C3-4 (n=6)	13.85 (13.28-14.20)	13.80 (13.40-14.03)	0.822
C4-5 (n=16)	14.00 (12.98-14.53) a	13.90 (13.03-14.43) b	**0.003**
C5-6 (n=30)	14.10 (13.55-14.28)	14.00 (13.45-14.53)	0.732
C6-7 (n=26)	14.00 (13.70-14.53)	14.00 (13.23-14.90)	0.509

Data was shown as median (1st-3rd quartile). Postop=postoperative
The different lowercase letters indicate the difference between groups.

Table 2. **Early and late postoperative averages of canal diameters**

	Channel diameter (mm) (Postop early)	Channel diameter (mm) (Postop 3. year)	P value
Channel diameters (n=24 patients) (n=78 levels)	14.00 (13.40-14.35)	13.90 (13.28-14.53)	0.906

eter measurements of all distances at the 10th postoperative day and 3rd-year follow-up were 14.00 (13.40-14.35) and 13.90 (13.28-14.53), respectively. There was no statistically significant difference in canal diameter between early and late postoperative values (P=0.906) (Table 2).

The median preop/postop jobb angle values were 9.80 (4.20-13.55) degrees 9.70 (4.40-13.10) degrees (P=0.974) and the median C2-C7 sagittal vertical axis values were 41.10 (22.20-54.10) mm - 40.20 (21.50-55.80) mm (P=0.937) (Table 3).

No lordosis loss was observed in the early postoperative period and three years after the operation except in one patient. Cervical lordosis loss was observed in one patient in the late postoperative period (Jobb angle changed from 10.40 to 4.10, sagittal vertical axis changed from 46.20 mm to 20.10 mm). In the early postoperative period, axial neck pain was observed in 2 patients, which lasted for several weeks and was controlled with medical treatment (Figs. 2 and 3).

Table 3. **Cervical lordosis evaluation averages**

	Channel diameter (Postop early)	Channel diameter (Postop 3. years)	P value
Cervical jobb angle (degree) (n=24)	9.80 (4.20-13.55)	9.70 (4.40-13.10)	0.974
Sagittal vertical axis (mm) (n=24)	41.10 (22.20-54.10)	40.20 (21.50-55.80)	0.937

Data was shown as median (1st-3rd quartile). Postop=postoperative

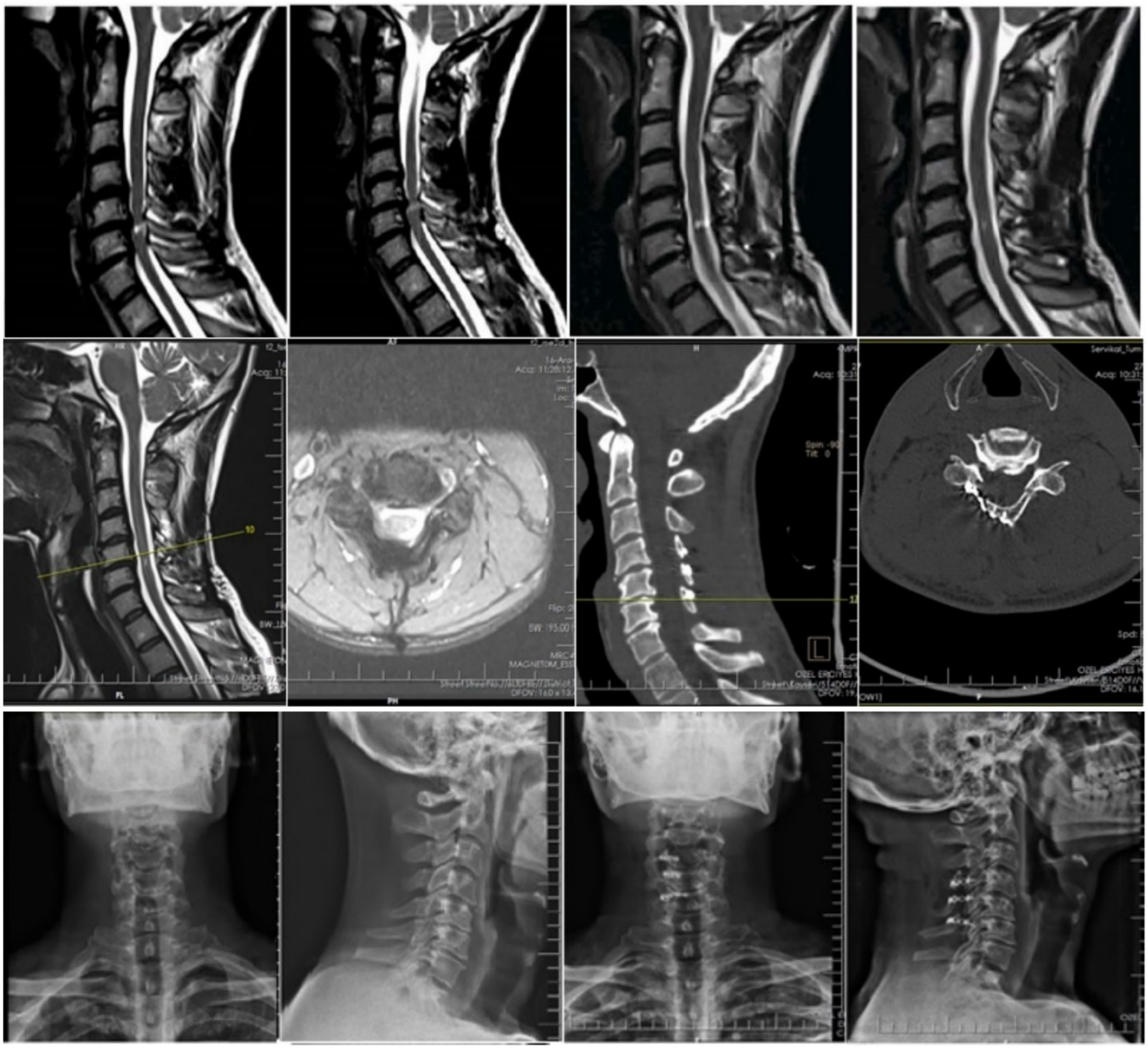

Fig. 2. Preoperative (left two) – postoperative (right two) MR/CT/CR (in order from top to bottom) results of a patient with cervical stenosis.

DISCUSSION

Laminoplasty is a method considered as a smooth transition between laminectomy and laminectomy and instrumentation and fusion. Krita first described the use of laminoplasty in cervical myelopathy in 1968 [13]. There are studies that the method is sufficient to maintain the diameter of the cervical canal and does not cause additional lordosis or kyphosis, preserves the cervical sagittal balance, and does not disrupt the biomechanics of the cervical spine. Expanding Z-laminoplasty was the first type of laminoplasty described by Oyama and Hattori [14] in 1972. Hirabayashi *et al*. [7] described a simpler method, open-door laminoplasty, in 1977. The most commonly used methods today are Hirabayashi's open-door laminoplasty and Kurokawa's double-door laminoplasty - spinous process separation.

One of the points to be considered here is to preserve the structure of the nuchal ligaments adhering to the C6 and C7 spinous processes to prevent instability and kyphotic deformity in the C6/7 segment. Protection of the attachment site of the erector spinea muscles to C2 and preservation of the integrity of the nuchal ligaments are very important in flexion deformity Protection of the semispinalis cervicis adhering to C2 is important in preserving cervical motion and lordotic structure in the postoperative period. It has also been reported that not including C7 in laminoplasty has a positive effect on axial pain in the post-

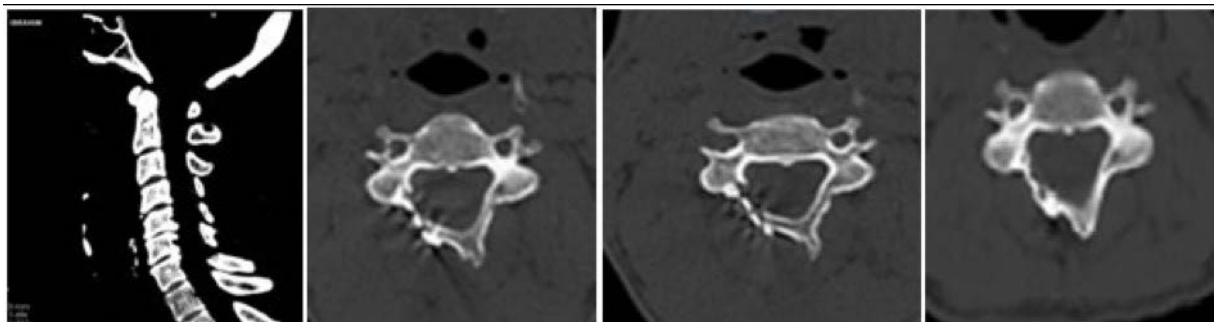

Fig. 3. Early and late canal diameter CT images.

operative period [15, 16]. In our series, when it was necessary to include C2 in the laminoplasty, laminoplasty was performed by dividing C2 in two over the spinous process. Thus, muscle attachment and ligament integrity were preserved. For C7, archocristectomy was performed to prevent axial neck pain. Although neurologic recovery after cervical laminoplasty is generally satisfactory, some problems are encountered. These problems can be categorized as early and late complications. The most important early complications are neurologic deterioration and C5 root deterioration. Neurologic deterioration may be due to inadequate decompression. There are series reporting C5 root paresis between 8-50% [17]. Instrumentation for lordosis with laminoplasty and the presence of C4 anterolisthesis are thought to be the factors in the formation of this complication and it is mostly temporary. Intraoperative electrophysiologic studies have reported that C5 root damage is not due to perop direct nerve or spinal cord damage [17]. In our series, postop C5 worsening was never observed.

In the late period, the issues reported and discussed are decreased neck movements, kyphotic deformity and axial neck pain. Two patients complained of axial neck pain lasting longer than six months. It was controlled with medication and exercise. Spinal cord sagittal diameter between C3-C7 is 8 mm and sagittal canal diameter is 17-18 mm. When the sagittal canal diameter is between 13 mm and 17 mm, symptomatic spondylosis is present, while myelopathy is very rare. Patients with a canal diameter of 10-13 mm have a risk of cervical spondylotic myelopathy, and patients with a canal diameter of less than 10 mm often have myelopathy [18]. The key point for a successful laminoplasty is the enlargement and preservation of the spinal canal. Preservation of motion and lordotic alignment are also the main goals. Although the classical open-door laminoplasty has satisfactory results and reclosure has not been demonstrated radiologically, several techniques, mostly implant-assisted, have been described to preserve canal width [19]. These methods provide stable fixation for the elevated lamina but are often complex and difficult procedures. Several studies have demonstrated the superiority of open-door laminoplasty over other techniques in terms of its effectiveness in increasing the sagittal diameter of the spinal canal and its low complication rate [20]. Yang *et al*. [21] reported that in the classical technique, loosening sutures may become dislodged and the raised lamina may reclose. On the other hand, this can be overcome by bone healing and/or uncomplicated secure fixation [22, 23]. Recurrent stenosis or early closure after laminoplasty has been reported with rates up to 10% most commonly at the C5 or C6 levels [24]. No recurrent stenosis was detected in our patient series. There was no statistically significant difference between the initial postoperative values and the values measured three years later, and there was no statistical difference between single or multiple distances in terms of re-closure. One patient developed stenosis in the upper adjacent segment.

The effects of laminoplasty on cervical lordosis were evaluated by direct radiography. Cobb, Tangent angles, effective cervical lordosis, and C2-C7 sagittal vertical axis measurements are the most commonly used methods to evaluate cervical lordosis by direct radiography. In the Cobb method, the angle between the lower-end plateau of the C2 vertebra and the lower-end plateau of the C7 vertebra is measured, and in the Tanjant method, the angle between the C2 pos-

terior corpus and the C7 posterior corpus is measured. The line drawn between the posterior lower end of the C2 vertebral corpus and the posterior lower end of the C7 vertebra is used to calculate the effective lordosis. Cobb and Tanjant's methods of cervical angle measurements have high reliability in the evaluation of cervical lordosis and are the most commonly used methods in practice [23]. However, the use of the lower-end plateaus of the vertebrae in the Cobb method and the diagnostic accuracy of this method due to degenerative diseases are lower than the Tanjant method using the posterior edges of the vertebral corpus [25]. C2-C7 sagittal vertical axis is used to measure the regional sagittal alignment of the cervical spine [12]. In a study, it was reported that effective lordosis measurement in patients was more sensitive in showing osteophytes extending into the spinal canal without disrupting cervical lordosis, in addition to showing cervical lordosis, and was therefore simpler and more reliable than the Cobb and Tanjant methods [26]. In recent studies, it has been found that cervical parameters with simpler features are more preferred and the detection error is lower [27]. In our study, Cobb angle and C2-C7 cervical vertical axis measurements were used in the evaluation.

In recent years, there has been increasing interest in the sagittal alignment of the cervical spine and its relationship with clinical outcomes. It is known that cervical lordosis is generally not physiological and should not be followed in all patients undergoing surgery. It is increasingly recognized how these angular parameters (lordosis or kyphosis) interact with translational parameters reflecting the overall cervical spine and spinal balance, which in turn influence patient outcomes. Cervical kyphosis associated with cervical sagittal imbalance is known to lead to worse postoperative outcomes, and this dichotomy in outcomes has not been shown to improve even with alignment correction [28].

Although loss of lordosis can occur with laminoplasty, it is usually not associated with the type of severe kyphosis that can be seen after multilevel laminectomy alone [23, 25]. In addition, cervical lordosis decreases with age, especially between 50-60 years. The disc physiologically loses its elasticity and flexibility due to dehydration during its normal life cycle. This changes the height and shape of the discs, which plays a role in the loss of lordosis. Therefore, this decrease in lordosis may be related not only to surgery but also to aging-related changes [10]. In addition, greater cervical lordosis (especially in patients with postoperative lordosis greater than 20°) has been reported to result in less neck pain in patients undergoing laminoplasty [29]. Lin *et al*. [30] studied preoperative patients with cervical spondylotic myelopathy (CSM) and investigated whether cervical sagittal parameters were related to the progression of patients with CSM. They reported that whether in the surgical or non-surgical group, the recovery of patients with C2-7 Flexion > 29° was better than the recovery of patients with C2-7 Flexion ≤ 29°. In another study, it was reported that preoperative cervical sagittal balance indices were related with the clinical outcomes of posterior longitudinal ligament ossification (OPLL) patients after laminoplasty [31]. Disruption of the posterior tension band, resection of 50% of the facet joint and capsule, and incorrect case selection (cases with a lordotic angle of less than 10° and kyphotic deformity) play an important role in postoperative kyphosis and related axial pain [23]. It has been reported that adverse radiological changes and axial neck pain after cervical laminoplasty are mostly caused by neck muscle destruction, especially separation of muscle insertions from the spinous processes of C2 and C7, diffuse atrophy of the muscles related to the nuchal ligament, ischemia of the shoulder muscles and delayed union of the facet joints [10]. Li *et al*. [32] analyzed the relationship between changes in cervical curvature, spinopelvic sagittal parameters and clinical efficacy after posterior laminoplasty and showed that it was correlated with changes in cervical sagittal alignment after laminoplasty. Therefore, they stated that patients should be examined preoperatively with a full-length spine film to evaluate cervical and spino-pelvic sagittal balance. There are also studies indicating that laminoplasty does not cause worsening axial neck pain in properly selected cases [25,26]. Stephens *et al*. [33] reported that laminoplasty did not cause worsening axial neck pain and was associated with significant improvements in other clinical and myelopathy outcomes in an appropriately selected group of patients with myelopathy who did not have significant diffuse axial pain preoperatively and had appropriate sagittal alignment (C2-7 neutral/lordotic). A systematic meta-analysis compared cervical sagittal parameters between patients with cervical spine disorders and asympto-

matic controls. The findings showed that the T1 slope was significantly lower among patients with cervical spine disorder compared to controls and higher for the spine cranial angle [34]. In this study, cervical lordosis loss was observed in only one patient in the three-year follow-up measurements of patients who underwent laminoplasty.

Limitations

The number of cases is not sufficient, patients could not be divided into subgroups due to the small number, and clinical findings could not be standardized. Some of the additional factors that may affect the picture could not be excluded (DM, Osteoporosis, Rheumatological diseases), The causes of stenosis could not be standardized.

CONCLUSION

The results of our study showed that en-block laminoplasty after the cervical narrow spinal canal was sufficient to maintain the cervical canal diameter in the long term with appropriate patient and surgical technique selection and did not cause additional lordosis or kyphosis, the cervical sagittal balance was preserved and the biomechanics of the cervical spine were not impaired. We think that a longer control series will be useful in this regard. We think that a more long-term control series will be useful in this regard.

Authors' Contribution

Study Conception: MM, RKÇ; Study Design: MM, RKÇ; Supervision: MM, RKÇ; Funding: N/A; Materials: N/A; Data Collection and/or Processing: MM, RKÇ; Statistical Analysis and/or Data Interpretation: MM, RKÇ; Literature Review: MM, RKÇ; Manuscript Preparation: MM, RKÇ and Critical Review: MM, RKÇ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2024;10(5):448-455 Original Article

DOI: https://doi.org/10.18621/eurj.1505012 *Obstetrics and Gynecology*

Could different cut-off values be used for 50-gram glucose tolerance test in low and high risk groups?

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ABSTRACT

Objectives: There are controversies about screening strategy and cut-off levels for gestational diabetes mellitus (GDM). Here, we aimed to identify optimal cut-off values for 50-gram oral glucose tolerance testing (OGTT) in high and low risk pregnant women.

Methods: A total of 500 patients who underwent two step OGTT were divided into two groups as GDM (n=31) and controls (n=469). Moreover, patients were grouped as high (n=114) and low risk (n=386) for GDM. Having≥2 risk factors such as family history of type-2 diabetes, obesity, glucosuria, previous history of GDM, macrosomia and diabetic complications were accepted as high risk. Demographic data, OGTT results, birth characteristics were recorded and compared between groups. A cut-off value for 50-gram OGTT was evaluated in low and high risk groups.

Results: The 50-gram OGTT value above 140 mg/dL discriminated GDM with 100% sensitivity and 92.11% specificity in all patients (AUC=0.969, P<0.001). The prevalence of GDM was 19.3% in high and 2.3% in low risk group. The 50-gram OGTT value above 140 mg/dL discriminated GDM with 100% sensitivity and 94.57% specificity in high risk patients (AUC=0.992, P<0.001). Furthermore, 50-gram OGTT value above 149 mg/dL discriminated GDM with 100% sensitivity and 93.63% specificity in low risk patients (AUC=0.976, P<0.001).

Conclusions: Although screening in low risk population is a debating issue worldwide, our local guidelines still recommend screening all pregnant women. We suggest that performing 100-gram OGTT only in patients who have higher values than 149 mg/dL in 50-gram OGTT can be an alternative screening strategy in low risk group.

Keywords: Cut-off, gestational diabetes mellitus, oral glucose tolerance test, threshold

Gestational diabetes mellitus (GDM) is one of the most common endocrine disorders of pregnancy. It is defined as any degree of glucose intolerance with first recognition during pregnancy [1]. Gestational diabetes mellitus have catastrophic effects for both mother and fetus during pregnancy. Moreover, it has long-term consequences. For this reason, accurate diagnosis and treatment has crucial role in maternal and fetal well-being [2, 3].

Screening programmes have been recommended by The American College of Obstetricians and Gynecologists (ACOG) and the American Diabetes Association (ADA) [4, 5]. Although 50-gram oral glucose tolerance testing (OGTT) followed by 100-gram OGTT is commonly performed all around the world for screening, there are still controversies about the

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How to cite this article: Dinçgez B, Özgen G, Özgen L. Could different cut-off values be used for 50-gram glucose tolerance test in low and high risk groups? Eur Res J. 2024;10(5):448-455. doi: 10.18621/eurj.1505012

Received: June 25, 2024
Accepted: July 17, 2024
Published Online: July 25, 2024

optimal screening strategy [6, 7]. Another debating issue about GDM screening is the threshold values of OGTT [8]. Previous studies have suggested different cut-off points due to the nutrition and ethnicity [6, 7, 9, 10]. Generally, a glucose value ≥140 mg/dL is accepted worldwide which can identify 80% of women with GDM. Besides this, ADA and ACOG claimed that a cut-off value of 130 mg/dL could be acceptable and identify 90% of GDM cases. Also, it has been suggested that GDM screening is not mandatory in low risk pregnant women for GDM. Having two or more risk factors including being above 45 years old, obesity, physical inactivity, high-risk ethnicity, family history of diabetes, previous GDM, macrosomia, pregestational diabetes, history of coronary artery disease, hypertension and medications leading to hyperglycemia is defined as high-risk population whereas patients who have no risk factor are defined as low risk [11]. Significant prevalance has been reported in low risk pregnant women all around the world and countries are still going on screening for this groups [4, 5, 9]. Unfortunately, there is no consensus in the literature about cut-off values in low risk pregnant women for GDM.

Considering that higher cut-off values leads to undiagnosed cases and lower threshold values cause performing more diagnostic tests, we grouped pregnant women to high and low risk groups and then aimed to identify optimal cut-off values for risk groups in Turkish pregnant women in the present study.

METHODS

This is a retrospective study performed at a high-volume university affiliated research and training hospital between January 2022 and December 2023. Local ethics committee approved this study with a decision number of 2024-TBEK 2024/06-11. Also, the present study complies with the declaration of Helsinki. Written informed consent was taken from all study participants for using data from medical records.

Study Population

During two year study period, OGTT screening for GDM was applied to 632 patients and these patients were obtained from medical records. Then, patients with unavailable perinatal data and who underwent 75-gram OGTT were excluded. Also, patients who have any contraindications and intolerance to OGTT, a history of pregestational diabetes, chronic diseases, and drugs affecting glucose metabolism such as corticosteroids noted in the medical reports of our hospital were excluded.

After selected according to the exclusion criteria, a total of 500 consecutive patients who were admitted to our obstetrics and gynecology outpatient clinic for prenatal visits, screened with two step OGTT for GDM and who had given birth in our hospital were enrolled in the study. The participants were divided into two groups based on 100-gram OGTT results as GDM (n=31) and controls (n=469). Moreover, patients were grouped as high-risk (n=114) and low-risk (n=386) groups for GDM.

In our clinic, pregnant women were routinely screened for GDM by one step protocol of 75-gram OGTT or a two-step protocol of 50-gram OGTT followed by 100-gram OGTT. Since 75-gram OGTT is both a diagnostic and screening test and our aim in the study is to determine the cut-off for the screening test, only patients screened with a two-step protocol were included. In two step protocol, accordance with recommendations by ADA and ACOG, 1 hour 50-gram OGTT was performed followed by 3 hours 100-gram OGTT if plasma blood glucose levels at first hour exceeds 140 mg/dL. According to the Carpenter and Coustan criteria, GDM was established with two abnormal values of 95 mg/dL for fasting, 180 mg/dl for first hour, 155 mg/dL for second hour and 140 mg/dL for third hours in 100-gram OGTT. Also plasma glucose ≥200 mg/dL after OGTT was accepted as GDM [4, 12].

High risk patients for GDM was diagnosed according to these criteria: (a) Family history of type-2 diabetes, (b) obesity (prepregnancy body mass index [BMI) ≥30 kg/m^2), (c) previous history of GDM, (d) previous history of macrosomia (fetal weight ≥4500 gram), (e) history of diabetic complications, and (f) Glucosuria. Patients who had two or more risk factors accepted as high risk for GDM. In high risk group, 50-gram OGTT was done in initial antenatal visit and if it was negative then the test repeated between 24-28 gestational week.

Demographic data such as age, BMI, gravida, par-

ity, OGTT results, birth characteristics such as birth weight, delivery week, delivery mode, baby gender, and Apgar scores were recorded for each patient for further analysis and compared between groups. A cut-off value for a 50-gram OGTT screening test was detected by ROC analysis both in low and high-risk groups.

Statistical Analysis

Shapiro Wilk test was used to determine whether the variables were distributed normally or not. All continuous variables were distributed non-normally and the Mann Whitney-U test was used for comparisons between two groups. Categorical variables were compared with the Chi-square test. Variables were presented as median (minimum-maximum) values for continuous variables and frequency (percentages) for categorical variables. The ROC analysis was used to evaluate the discriminative role of 50-gram OGTT values for GDM and Youden index was used to determine the cut-off values. Analyzes were carried out by using SPSS version 22.0 and MedCalc 18 programs, and P value ≤0.05 was considered as statistically significant.

RESULTS

The prevalance of GDM was found to be 6.2%. The sociodemographic, laboratory and birth characteristics of GDM and control groups were presented in Table 1. There was no statistically significant difference between two groups in terms of age, gravida, parity, gestational week, cesarean section rate, birth weight, baby gender, Apgar scores of first and fifth minutes, and 100-gram OGTT values at third hour. Gestational diabetes mellitus patients have significantly higher BMI and lower birth week. Moreover, GDM group had higher fasting blood glucose, 50-gram OGTT and 100-

Table 1. The sociodemographic, laboratory and birth characteristics of GDM and control patients

	GDM (n=31)	Control (n=469)	P value
Age (years)	29 (18-43)	26 (18-43)	0.090
BMI (kg/m^2)	32 (24-42)	29 (20-41)	**<0.001**
Gravida (n)	3 (1-6)	3 (1-9)	0.474
Parity (n)	1 (0-5)	1 (0-4)	0.079
Gestational week (week)	26 (24-28)	27 (24-29)	0.077
Delivery week (week)	37 (31-41)	38 (32-41)	**0.017**
Cesarean section, n (%)	22 (71)	297 (63.3)	0.391
Birth weight (gram)	3300 (1760-4690)	3185 (1720-4250)	0.463
Baby gender, n (%)			0.990
Female	19 (61.3)	288 (61.4)	
Male	12 (38.7)	181 (38.6)	
Apgar scores of first minutes	9 (8-10)	9 (8-9)	0.725
Apgar score of fifth minutes	10 (9-10)	10 (9-10)	0.117
Blood glucose (mg/dL)	90 (61-125)	84 (61-121)	**0.001**
50-gram OGTT values (mg/dL)	158 (141-218)	119 (61-171)	**<0.001**
100-gram OGTT values (mg/dL)			
1st hour	185 (161-235)	171 (153-178)	**<0.001**
2nd hour	160 (131-205)	145 (109-154)	**<0.001**
3th hour	123 (81-164)	110 (89-140)	0.088

BMI=body mass index, OGTT=oral glucose tolerance testing

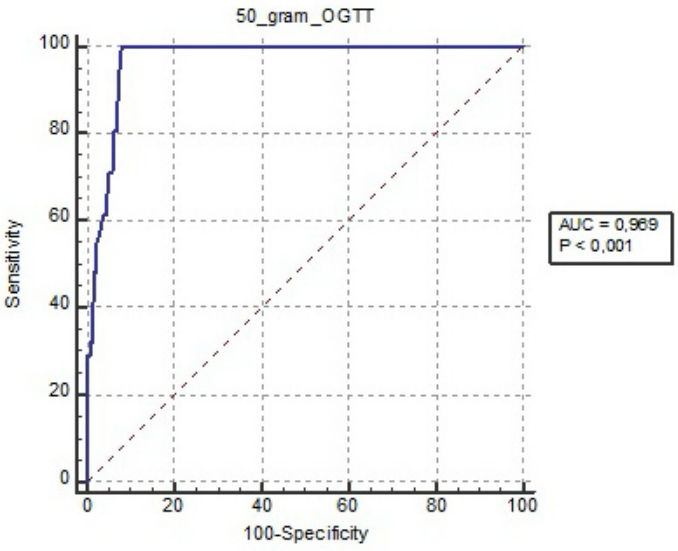

Fig. 1. A 50 gram OGTT for GDM for all patients.

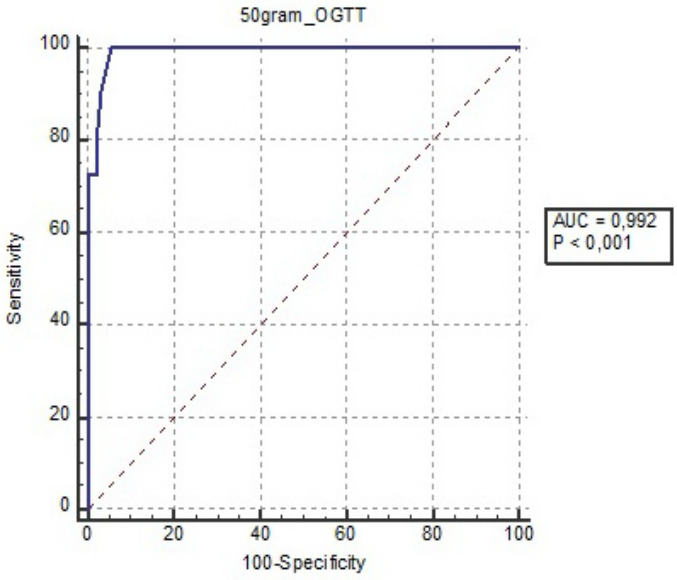

Fig. 2. A 50 gram OGTT for GDM for high risk patients.

Table 2. The sociodemographic, laboratory and birth characteristics of high and low risk for GDM

	High risk for GDM (n=114)	Low risk for GDM (n=386)	P value
Age (years)	28 (18-43)	26 (18-43)	0.170
BMI (kg/m^2)	32 (30-41)	28 (20-42)	**<0.001**
Gravida (n)	3 (1-9)	2 (1-9)	0.199
Parity (n)	1 (0-4)	1 (0-5)	0.118
Gestational week (week)	26 (24-28)	27 (24-29)	0.611
Delivery week (week)	38 (31-41)	38 (32-41)	0.926
Delivery mode, n (%)	73 (64)	246 (63.7)	0.953
Birth weight (gram)	3245 (1720-4500)	3185 (1720-4690)	0.222
Baby gender, n (%)			0.511
Female	73 (64)	234 (60.6)	
Male	41 (36)	152 (39.4)	
Apgar scores of first minutes	9 (8-10)	9 (8-9)	0.607
Apgar score of fifth minutes	10 (9-10)	10 (9-10)	0.240
Fasting blood glucose (mg/dL)	81 (64-112)	81 (61-125)	0.735
50-gram OGTT values (mg/dL)	113 (61-210)	122 (85-218)	0.273
100-gram OGTT values (mg/dL)			
1st hour	182 (171-235)	171 (153-214)	**<0.001**
2nd hour	155 (131-205)	145 (109-205)	**0.004**
3th hour	110 (81-145)	110 (81-164)	0.877
GDM diagnosis, n (%)	22 (19.3)	9 (2.3)	**<0.001**

BMI=body mass index, GDM=gestational diabetes mellitus, OGTT=oral glucose tolerance testing

gram OGTT values at first and second hours.

A ROC analysis was performed to determine the cut-off value for GDM for all patients. The 50-gram OGTT value >140 mg/dL discriminated GDM with 100% sensitivity and 92.11% specificity in all patients (AUC=0.969, P<0.001) (Fig. 1).

The sociodemographic, laboratory and birth characteristics of high and low risk patients for GDM were shown in Table 2. No significant difference was found between high and low risk groups according to age, gravida, parity, gestational week, delivery week, delivery mode, birth weight, baby gender, Apgar scores of first and fifth minutes, fasting blood glucose, 50-gram OGTT values and 100-gram OGTT values at third hours. GDM was diagnosed at 19.3% of high risk patients whereas the ratio of GDM was 2.3% in low risk group (P<0.001). BMI, 100-gram OGTT values at second and third hours were significantly higher in high risk group as compared to low risk group.

The ROC curve analysis revealed that 50-gram OGTT value >140 mg/dL discriminated GDM with 100% sensitivity and 94.57% specificity in high risk patients (AUC=0.992, P<0.001) (Fig. 2). Furthermore, 50-gram OGTT value >149 mg/dL discriminated GDM with 100% sensitivity and 93.63% specificity in low risk patients (AUC=0.976, P<0.001) (Fig. 3).

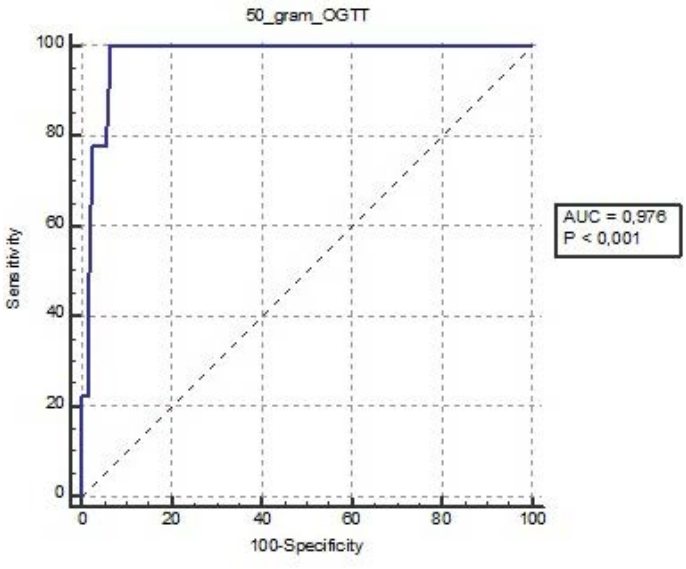

Fig. 2. A 50 gram OGTT for GDM for low risk patients.

DISCUSSION

The prevalence of GDM is gradually increasing worldwide and reaching 10-20% in high-risk populations [13]. The prevalence of GDM tightly depends on ethnicity, obesity, and medical and family history. Asians are known to have higher GDM rates as compared to other ethnicities [14]. In a study from Turkey, the prevalence of GDM was reported as 8.1% using Carpenter and Coustan criteria [15]. Similarly, in a systematic review and meta-analysis, the prevalence of GDM was reported as 7.7% which varies from 1.9 and 27.9% in different forty-one Turkish articles [16]. Consistent with the literature, we found GDM prevalence as 6.2% in our study.

Another factor affecting the prevalence of GDM is the threshold values for OGTT [17]. In a study of Juntarat *et al*. [18], the prevalence of GDM was reported as 4.14% when the 50-gram OGTT cut-off level was accepted as 130 mg/dL and 4.08% when it was accepted as 140 mg/dL. Kösüs *et al*. [15] revealed the prevalences as 8.1% and 7.1% for the same cut-off levels in a study performed in Turkey [15]. Tan *et al*. [19] found the prevalence 11.4% for 130 mg/dL and 9.5% for 140 mg/dL threshold levels. These higher rates can be related to the different risk statuses in study populations. Having risk factors is too important for GDM prevalence. Basnet *et al*. [20] reported a 7.07% prevalence in high-risk pregnancies for GDM while Juntarat *et al*. [18] reported this prevalence as 20.16%. Consistent with the literature, we found the GDM prevalence as 19.3% in the high-risk group in our study.

There is not enough data searching the prevalence of GDM in low-risk patients. Jimenez Meleon *et al*. [21] reported the prevalence as 0.6% in low-risk pregnant women. In another study using the cut-off levels of 130 mg/dL, the prevalence was 2.1% among low-risk patients [22]. When 75-gram OGTT was performed, the incidence was detected as 3.4% in low-risk pregnant women [23]. In our study, we performed 50-gram OGTT, accepted the cut-off level as 140 mg/dL, and found the prevalence as 2.3%.

Considering these data, higher cut-off values lead to undiagnosed cases, and lower threshold values cause more diagnostic tests. Thus, defining an appropriate cut-off level for both high and low-risk groups

would be logical. In the literature, there is little data about this issue. Basnet *et al*. [20] suggested that 130 mg/dL could identify extra cases of GDM in high-risk women [20]. Similarly, many studies have shown that 10% of GDM cases may be missed by using a 140 mg/dL cut-off level for all risk groups [15, 19]. Juntarat *et al*. [18] concluded not adopting lower cut-off values for low-risk groups. To the best of our knowledge, there are not many studies evaluating a new cut-off value for GDM screening. In a study of Vitoratos [24], a cut-off value of 126 mg/dL was offered while Mahasukontachat *et al*. [25] suggested the threshold of 176 mg/dl with 58.5% sensitivity and 88.2% specificity for 50-gram OGTT. However, they recommended this value for screening during the first antenatal visit [24,25]. Punthumapol *et al*. [26] searched cut-off values for each trimester and recommended 177 mg/dL with 60.78% sensitivity and 75% specificity in high-risk populations. In the study of Eslamian and Remazani [27], a cut-off value of 135 mg/dl was suggested with a sensitivity of 91.7% and 83.6% specificity. In a study by Kösüs *et al*. [15], 808 pregnant women were searched and the optimal cut-off level was reported as 132 mg/dL for 50-gram OGTT. They reported no GDM for patients who have glucose levels below 130 mg/dL [15]. Different from our study, patients who have a previous history of GDM, family history, macrosomia, hypertension, glucosuria, polyhydramnios, multiple pregnancies, previous unexplained fetal loss, and delivery before the 24th gestational week were excluded. So, we could not make any conclusion about cut-off levels in high-risk group. Miyakoshi *et al*. [28] identified the cut-off value as 140 mg/dL with 96% sensitivity and 76% specificity. Similar to this study, we suggested a cut-off value of 140 mg/dL with 100% sensitivity and 92.11% specificity in all patients. Also, we found that a 50-gram OGTT value >140 mg/dL discriminated GDM with 100% sensitivity and 94.57% specificity in high-risk patients. This value could be higher but we only performed 100-gram OGTT for patients who have a 50-gram result above 140 mg/dL. For low-risk patients, a study from Turkey suggested that 50-gram OGTT can be omitted up to a threshold value of 147.5 mg/dL [29]. Similarly, we propose that a cut-off value of 149 mg/dl could be used for 50-gram OGTT screening in low-risk pregnant women. Due to the sensitivity of 100% and specificity of 93.63%, performing 100-gram OGTT only in patients who have higher values than 149 in 50-gram OGTT can be an alternative option to the present screening strategy.

Limitations

The present study has some limitations. It has a retrospective design and the datas obtained from single center and prone to selection bias. More importantly, cut-off level was accepted as 140 mg/dL for 50-gram OGTT for all groups but it could be more appropriate to accept it as 130 mg/dL because the cut-off level for high risk group can be below 140 mg/dL. The main reason of this limitation is due to the screening recommendations of our Ministry of Health. Thus we do not have any suggestions about patients who have 50-gram OGTT values between 130 mg/dL and 140 mg/dL. Lastly, the present study was lack of showing the relationship between adverse neonatal outcomes and 50-gram OGTT cut-off values.

CONCLUSION

Gestational diabetes mellitus screening with OGTT is an appropriate approach for our population. Although screening in low risk population is a debating issue worldwide, our local guidelines still recommend screening all pregnant women for GDM. Accordingly, we suggest using higher cut-off points in low risk group would be more appropriate than not screening in Turkish population.

Authors' Contribution

Study Conception: BD, GÖ, LÖ; Study Design: BD, GÖ; Supervision: LÖ, GÖ; Funding: N/A; Materials: N/A; Data Collection and/or Processing: GÖ; Statistical Analysis and/or Data Interpretation: BD; Literature Review: LÖ, BD; Manuscript Preparation: BD and Critical Review: GÖ, BD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgement

The study was carried out University of Health Sciences, Bursa Yuksek Ihtisas Research and Training Hospital, Department of Obstetrics and Gynecology, Bursa, Turkey.

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The European Research Journal 2024;10(5):456-464 Original Article

DOI: https://doi.org/10.18621/eurj.1509117 *Pediatric Hematology and Oncology*

Comparison of augmented Berlin-Frankfurt-Münster (BFM) and BFM 2000 treatment protocols in children diagnosed with high-risk acute lymphoblastic leukemia

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ABSTRACT

Objectives: The main purpose of this study is comparing the augmented Berlin-Frankfurt-Münster (BFM) and BFM 2000 treatment protocols applied to pediatric patients diagnosed with high-risk acute lymphoblastic leukemia (ALL) in our clinic in different years in terms of relapse incidence and survival rates.

Methods: When evaluated all patients considering the Children's Oncology Group (COG) criteria, 53 of our patients who were in the medium or high risk group according to the BFM 2000 protocol and were in the high risk group received treatment with Augmented BFM protocol and 17 of them received the BFM 2000 protocol. Age, gender, bone pathology, physical examination, hepatomegaly, splenomegaly, lymphadenopathy, presence of bleeding, hemogram values, immunophenotype, 8th, 14th and 33rd day treatment response, presence of translocation, central nervous system (CNS), extramedullary involvement, risk group, presence of relapse, time to relapse, follow-up period and hospital stay until maintenance treatment were examined.

Results: Event-Free Survival (EFS) and Overall Survival (OS) values of patients were 83.6% and 90.1%, respectively. While EFS was 89.4% and OS was 90.6% in the group receiving the Augmented BFM treatment protocol, EFS was calculated as 71.7% and OS was 88.2% in those receiving the BFM-2000 treatment protocol. Accordingly, when the EFS values of those who received the Augmented BFM treatment protocol were compared with those who received BFM-2000, statistically significant values were found (P<0.01).

Conclusions: It was observed that the augmented BFM treatment protocol was more protective against relapses and shortened the duration of hospitalization compared to the BFM 2000 treatment protocol.

Keywords: Acute lymphoblastic leukemia, child, treatment protocol

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy and accounts for around 30-35% of all types of malignancies. The incidence of leukemia among all cancer cases in Türkiye has been reported to be 31.3%. ALL is most commonly diagnosed between the ages of 2 and 5 years and is more prevalent in males [1, 2]. The life expectancy for ALL, which used to be considered an incurable disease, has increased dramatically with the discovery of new drugs, the

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How to cite this article: Özer S, Ağaoğlu FL, Karakaş Z, Ünüvar A, Devecioğlu Ö. Comparison of augmented Berlin-Frankfurt-Münster (BFM) and BFM 2000 treatment protocols in children diagnosed with high-risk acute lymphoblastic leukemia. Eur Res J. 2024;10(5):456-464. doi: 10.18621/eurj.1509117

Received: July 5, 2024
Accepted: August 4, 2024
Published Online: August 6, 2024

determination of the combinations in which these drugs can be used and the development of treatment protocols [3]. In the past two decades, expected 5-year Overall Survival (OS) rates in children with ALL have reached 90% in developed countries [3, 4, 5]. Moreover, relapse rates in particular have been significantly reduced. The improvement in outcomes over the last few decades can be attributed mainly to changes in the use of drug combinations and intensification of treatment for patients diagnosed with more difficult-to-treat disease [6]. The optimization of standard cytotoxic chemotherapy has resulted in significant improvements in Event-Free Survival (EFS) and OS outcomes, particularly in high-risk ALL patients, although it has not brought about a notable enhancement in EFS and OS rates in some ALL patients [7]. Numerous cancer study groups worldwide have contributed to the development of treatment protocols to increase the expected EFS and OS. In particular, the Children's Oncology Group (COG), which unites the Children's Cancer Group and the Pediatric Oncology Group, and the Berlin-Frankfurt-Munster (BFM) study group are pioneers in the development of these effective treatment protocols [8]. Up to date, various prognostic factors have been identified in ALL. Some prognostic factors vary according to treatment protocols; however, age, leukocyte count at the time of initial diagnosis, and response to treatment remain unchanged. With the development of diagnostic and treatment centers and advancing research, it has been realized that both clinical and laboratory findings are effective on prognosis, and thus, treatment by risk class has been brought to the agenda. Recent studies have also focused on defining new prognostic factors to intervene in patients with poor prognosis with new treatment protocols [9, 10]. The COG and the BFM study group treat ALL by stratifying it into risk groups. However, they do not use the same risk classification. This leads to some variations in the treatment protocols of the two groups. In the present study, all patients were divided into two groups: patients in the high-risk group according to COG criteria, and patients in the intermediate-risk or high-risk group according to the BFM study group criteria, but in the high-risk group according to COG criteria. The aim is to compare the effects of both treatment protocols on EFS and OS and the effects of prognostic factors on overall survival and event-free survival rates.

METHODS

Patients who were referred to our clinic with a prediagnosis of ALL or who were diagnosed with ALL upon examination were evaluated with bone marrow aspiration in the Department of Pediatrics, Division of Pediatric Hematology-Oncology, İstanbul University Istanbul Faculty of Medicine. Accordingly, the data of 70 patients diagnosed with ALL were retrospectively analyzed. The study included 53 patients who were classified in the high-risk ALL group according to COG criteria and treated with augmented-BFM treatment protocol, and 17 patients who were classified in the intermediate-risk or high-risk ALL group according to BFM study group criteria and in the high-risk ALL group according to COG criteria and treated with BFM 2000 treatment protocol. The following characteristics of all patients were analyzed: age and gender at presentation, presence of organomegaly on physical examination, complete blood count parameters, treatment responses to bone marrow aspiration on day 8, day 14, and day 33, FAB classification, immunophenotypes, presence of translocations, extramedullary involvement, risk group, presence of relapse, time of relapse, and duration of follow-up period and hospitalization. The patients receiving the two treatment protocols were compared according to the data obtained.

Bone marrow aspiration samples were evaluated under light microscopy. Typing was performed according to FAB criteria. Immune phenotyping was performed using the flow cytometry method at the Molecular Oncology and Hemopathology Research Center, İstanbul University Cerrahpaşa Faculty of Medicine. CD13, CD14, and CD33 were used as myeloid markers for immune phenotyping; CD19, CD20, CD22, CD24, and CD10 were used as lymphoid markers for the B cell line; CD3, CD5, and CD7 were used for the T cell line. The presence of CD34 above 10% and any data above 20% was considered positive. The presence of t(9;22), t(4;11), t(1;19), and t(12;21), translocations known to have positive and negative effects on prognosis, were investigated at the Genetic Department of İstanbul University Experimental Medicine Research Institute. The presence of mediastinal mass was evaluated by chest radiographs. Central nervous system (CNS) involvement was investigated by cytologic and biochemical evaluation of the cerebrospinal fluid sample. The high-risk criteria

used according to COG were 1<Age <10 and WBC ≥50,000/mm^3 or Age ≥10 and any WBC value, and testicular involvement. According to the BFM study group, for the intermediate risk group, criteria include a blast count in the peripheral blood of <1000/mm^3 on day 8, complete remission on day 33, negative t(9;22), and bcr/abl, absence of t(4;11) (MLL/AF4 recombination), and not meeting any high-risk criteria. For the high-risk group, criteria include a blast count in the peripheral blood of ≥1000/mm^3 on day 8, positive t(9;22) and/or bcr/abl, positive t(4;11), and MLL/AF4, and bone marrow classified as M2/M3 on day 33 (M2: blasts 5-25%, M3: blasts >25%).

Statistical Analysis

All statistical analyses were performed using SPSS 12.0 for Windows software package. Chi-Square and Fisher's exact Chi-Square tests were used for comparisons between groups. Student-T tests were used for response to treatment, relapse, and survival. Overall survival and event-free survival between the groups receiving the Augmented BFM protocol and BFM 2000 protocol were analyzed using Kaplan-Meier survival analysis. Long rank and Breslow tests were used to compare the overall survival and event-free survival rates of both groups. Factors that may cause relapse were analyzed using Cox-Regression analysis.

RESULTS

The combined evaluation of patients who received treatment according to the two treatment protocols resulted in an EFS of 83.6% and OS of 90.1%. EFS was 89.4% and OS was 90.6% in patients treated with the augmented BFM treatment protocol alone, while EFS

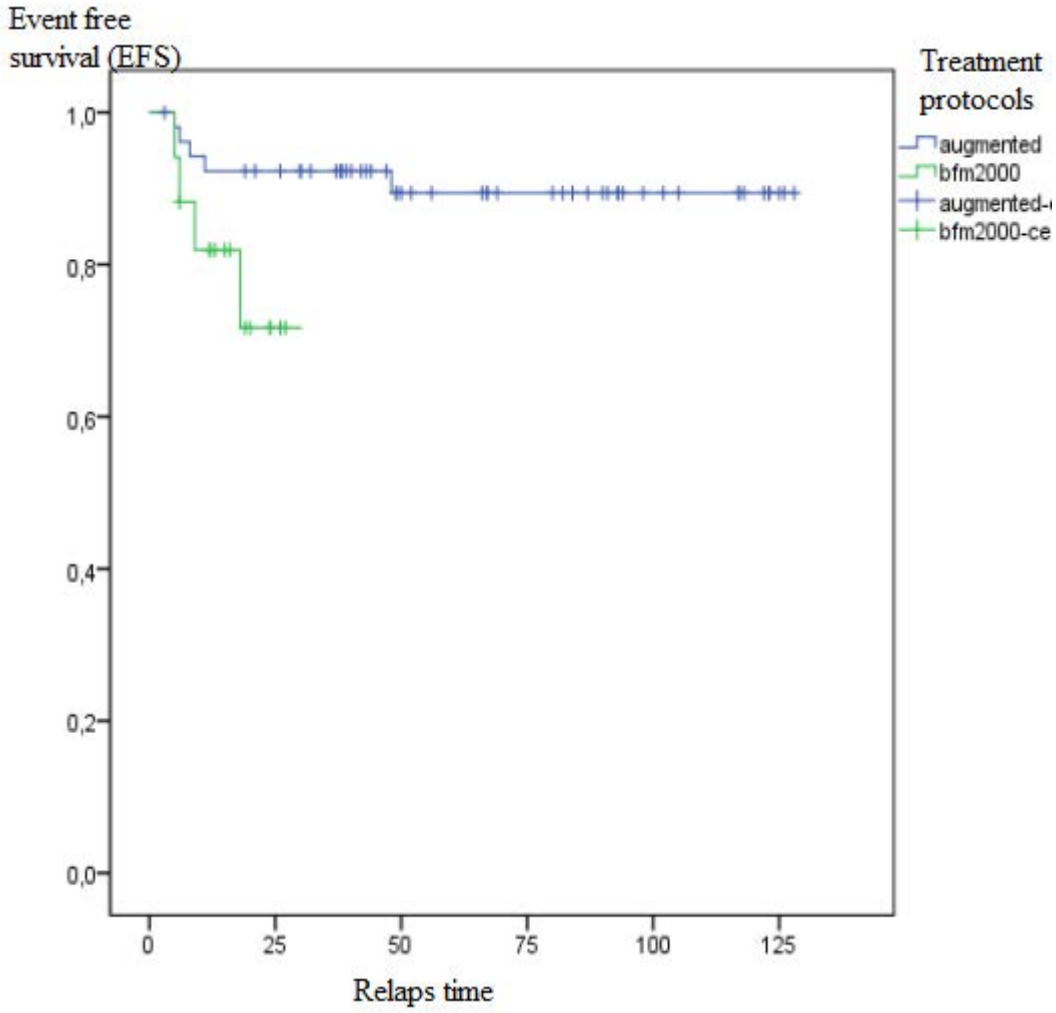

Fig. 1. Survival of patients according to event-free survival values.

was 71.7% and OS was 88.2% in patients treated with the BFM 2000 treatment protocol. The EFS values of the patients who received the augmented BFM treatment protocol were statistically significantly higher when compared to the patients who received the BFM 2000 treatment protocol (P<0.001). Patients treated according to the augmented BFM treatment protocol showed a lower rate of relapse. The augmented BFM treatment protocol was more protective against relapses than the BFM 2000 treatment protocol (Figs. 1 and 2).

Demographic data of the patients revealed that 47 (67.1%) of our 70 patients were male and 23 (32.9%) were female. The male/female ratio was 2/1. A total of 34 (48.6%) patients were 10 years and older, while 36 (51.4%) of the patients were between 1 and 9.99 years of age. Due to organ infiltration, 42 (60%) patients had hepatomegaly smaller than 2 cm and 25 (35.7%) patients had hepatomegaly larger than 2 cm at the time of admission. It was determined that 39 (55.7%) patients had splenomegaly smaller than 2 cm and 27 (38.6%) patients had splenomegaly larger than 2 cm. In the evaluation of complete blood counts, it was found that in 41 cases (58.6%), the hemoglobin (Hb) value was below 10 g/dL, while in 25 cases (35.7%), it was above 10 g/dL. the leukocyte count was less than 10,000/mm^3 in 21 cases (30%), between 10,000 and 50,000/mm^3 in 18 cases (25.7%), and above 50,000/mm^3 in 29 cases (41.4%). The platelet count was found to be below 20,000/mm^3 in 12 cases (17.1%), between 20-100,000/mm^3 in 30 cases (42.9%) and above 100,000/mm3 in 24 cases (34.3%). Bleeding was found in 12 patients with a platelet count below 100.000/mm^3 on admission. CNS involvement was found in 3 (4.3%) patients, mediastinal mass in 6 (8.6%) patients, and testicular involvement in 1 (1.4%) patient. One patient exhibited biphenotypic characteristics, while 44 patients (62.8%) exhibited B-cell characteristics and 16 patients (22.9%) exhibited T-cell characteristics. In the evaluation of the presence of translocation in bone marrow aspiration material, t(4;11) was determined in 4 (5.7%), t(9;22) in 6 (8.6%), and t(12;21) in 1 (1.4%) of the patients. When patients were compared based on the presence of

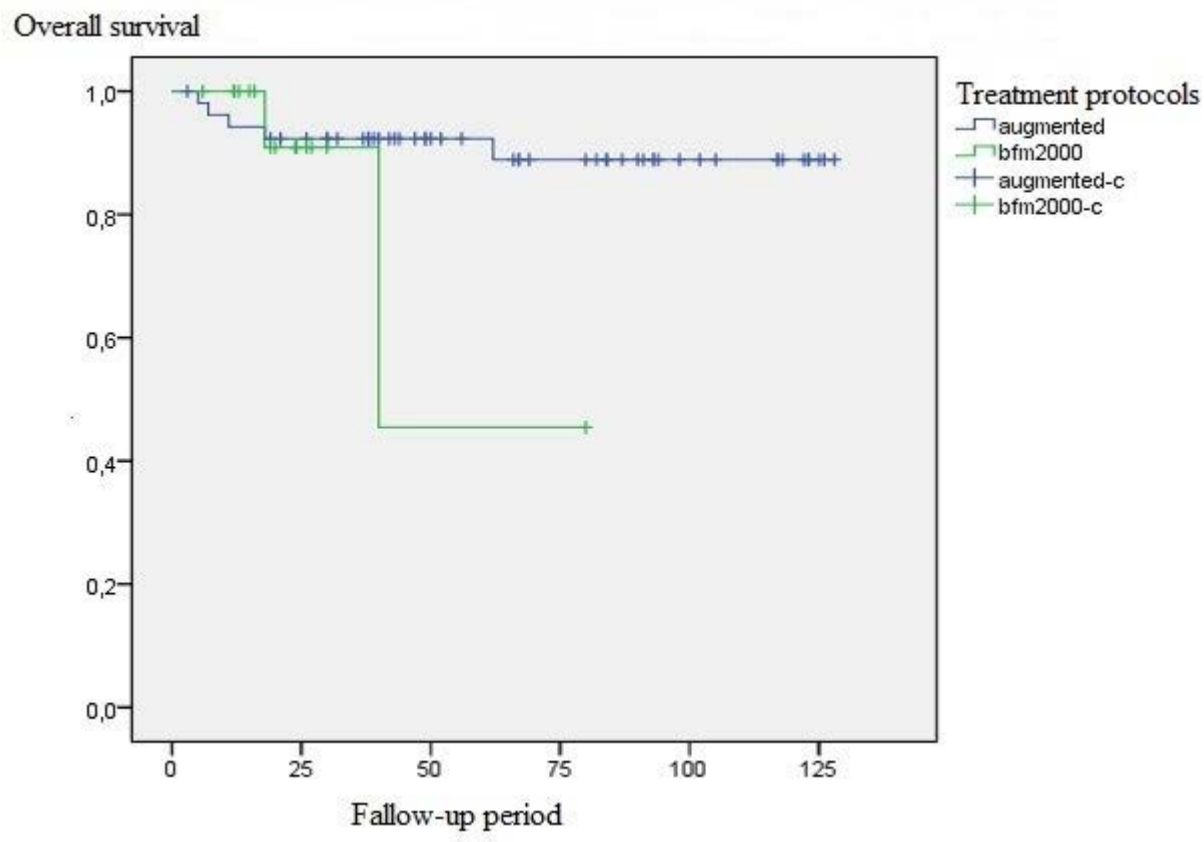

Fig. 2. Survival of patients according to overall survival values

Table 1. Comparison of the features of two treatment protocols groups

Features at diagnose	Augmented BFM treatment group			BFM 2000 treatment group			P value
	n	Event	EFS (%)	n	Event	EFS (%)	
Gender							
Male	35	4	86.6	12	4	74.7	0.398
Female	18	1	94.4	5	1	75	
Age							
1-9.9 year	30	1	96.6	6	3	41.7	0.446
≥10 year	23	4	80.3	11	1	81.8	
Hepatomegaly							
<2cm	32	1	100	10	3	80	**0.029**
≥2cm	19	4	77	6	2	55.6	
Splenomegaly							
<2cm	29	3	87.1	10	2	80	0.679
≥2cm	22	2	90.9	5	3	40	
Lenfadenopathy							
<2cm	45	5	87.1	11	3	80.8	0.921
≥2cm	7	0	100	4	2	37.5	
CNS involvement							
+	2	0		1	1		
-	51	5	88.9	1	1		
Mediastinal mass							
+	6	1	83.3				0.432
-	47	4	90	14	4	77.9	
Morphology							
L1	26	1	96.2	13	3	80.8	0.325
L2	24	3	87	2	0		
Hemoglobin							
<10 g/dL	33	3	89.9	8	3	56.3	0.755
≥10g/dL	17	2	87.5	8	2	87.5	
White blood count							
<50000/mm^3	27	3	87.7	12	3	83.3	0.709
≥50000/mm^3	25	2	91.7	4	2	50	
Platelets							
<100000/mm^3	32	3	90.6	10	4	83.3	0.858
≥100000/mm^3	18	2	85.6	6	1	64	
CALLA							
Positive	10	0					
Negative	34	5	84				
Translocations							
+	4	1	86.7	14	4	50	0.810
-	38	2	100	2	1	75	
8th day blast response							
Blast<1000	38		90.4	13		81.3	0.732
Blast>1000	4		9.6	3		18.7	
33rd day blast response							
Blast<5%	53		100	17		100	
Blast>5%	0			0			

CALLA=Common Acute Lymphoblastic Leukemia Antigen, CNS=Central Nernous System, EFS=Event-Free Survival

translocations, it was observed that relapse occurred in only one patient. Regarding the response to the treatment, relapse was detected in 5 of 53 patients (9.4%) treated according to the augmented BFM treatment protocol and in 5 of 17 patients (29.4%) who received the BFM 2000 treatment protocol. It is 5.6 times more common in patients receiving the BFM 2000 treatment protocol. (Table 1).

In both treatment groups, the mean duration of hospitalization during intensive chemotherapy before maintenance treatment was 4.4 months for the patients treated with the augmented BFM treatment protocol and 6.8 months for the patients treated with the BFM 2000 treatment protocol. The mean duration of hospitalization was statistically significantly shorter in those who received treatment according to the augmented BFM treatment protocol ($P<0.001$). The mean duration of hospitalization during the intensive chemotherapy period was 6.4 months in patients with relapse and 4.6 months in those without relapse before switching to maintenance treatment.

DISCUSSION

In the treatment of acute lymphoblastic leukemia, an almost complete cure can be achieved through chemotherapy, radiotherapy, bone marrow transplantation, targeted therapies and immunotherapy, and the establishment and development of leukemia centers. In the last 40 years, it has been observed that radiotherapy, intensified multiple chemotherapy, and the application of treatment options based on risk groups have significantly increased life expectancy. Five-year overall survival in children has increased to almost 80% [11]. Although hematopoietic stem cell transplants have contributed positively to survival, intensive chemotherapies given to improve overall survival in some ALL subtypes have failed to achieve the desired goal of survival. The researchers aim to improve treatment protocols in chemotherapy-resistant cases by better understanding the pathogenesis [12].

Numerous study groups have been established to date to achieve success in the treatment of pediatric cancers. However, over time, some of them came to the fore and incorporated other study groups into their group. Today, pioneering studies are carried out by the COG and the BFM Study Group, in which many European countries now participate. These two study groups have been developing treatment protocols by examining prognostic factors to ensure successful leukemia treatment for years. Although prognostic factors change over time, mainly the patient's age, leukocyte count on admission, and response to treatment remain unchanged. Almost all treatment protocols consider age and leukocyte count at diagnosis as the most common prognostic factors. It is known that a higher leukocyte count at the time of diagnosis indicates a poorer prognosis, and the leukocyte count at the time of diagnosis has been considered an unchanging prognostic factor for years [2]. The 5-year event-free survival was 79.4% in those who received treatment according to the treatment protocol of the BFM group. In a study conducted by the COG group in a large group of patients, the 5-year event-free survival rate was 81.2% in patients treated with the augmented BFM protocol. Patients are stratified into risk groups in all ALL chemotherapy protocols, and remission induction, consolidation, CNS eradication, and maintenance treatment schemes are applied with some modifications [12].

Of the 70 patients included in the study, 10 relapsed and the EFS was 86.3% in all patients regardless of the treatment protocol. Among these cases, 5 showed relapse while receiving treatment according to the augmented BFM protocol, and the other 5 showed relapse while receiving treatment according to the BFM 2000 protocol. The incidence of relapse in patients treated with the augmented BFM treatment protocol was found to be 9.4%. This was found to be consistent with the literature [13]. The incidence of relapse in patients treated according to the BFM 2000 treatment protocol was 29.4%. The blast count on day 8 was below 1000 may be considered to be a significant factor in the high incidence of relapse. The rate of patients who received augmented BFM with a blast count of less than 1000 on day 8 was 94.7%, while this rate was 84.6% in patients who received the BFM 2000 treatment protocol. Relapses were 5.6 times more common in patients treated according to the BFM 2000 treatment protocol compared to augmented BFM treatment.

The 5-year event-free survival rate in children diagnosed with ALL was around 50%, whereas today, with multiple chemotherapy, radiotherapy, and treatment according to the risk group, event-free survival

is achieved in 75-80% of patients for a much longer time [14]. In the present study, patients treated according to the augmented BFM treatment protocol had a median event-free survival of 116.7 months. This was determined as 56.2 months in patients who received treatment according to the BFM 2000 treatment protocol. However, the follow-up period of the patients who received the BFM 2000 treatment protocol was still shorter when compared to the other group. It can be concluded that the A-BFM treatment protocol is statistically significantly more protective against relapses than the BFM 2000 treatment protocol in high-risk ALL. However, researchers should take into account that the number of patients receiving treatment according to the BFM 2000 treatment protocol was limited. The literature suggests that the male/female ratio in ALL is usually in the range of 1.1-1.4/1 [15]. The prevalence of ALL in male children was 54-57% according to the study conducted in a large series of patients by the Pediatric Oncology Group, which evaluated numerous major studies to date, whereas the rate of male patients was 67.1% in the present study. Only high-risk ALL patients were included in this study. Multiple studies have shown that boys have a worse prognosis than girls receiving the same treatment [15]. The EFS was found to be 79.7% in boys and 91.1% in girls. However, in the present study, when all cases were evaluated together, the effect of gender on EFS was not found to be statistically significant. The literature shows that male gender is a poor prognostic factor. This inconsistency may be attributed to the fact that the study group included only patients diagnosed with high-risk ALL [16].

Hepatomegaly, splenomegaly, or lymphadenomegaly is observed in 30-40% of ALL due to infiltration of the liver, spleen, and lymph nodes. In the present study, hepatomegaly was present in 95.7% of cases and splenomegaly in 94.3% of cases. The high rate of organomegaly compared to the literature can be attributed to the fact that the study group consisted only of high-risk ALL patients. Hepatomegaly and splenomegaly increase the risk of relapse in ALL patients [17]. On physical examination, the EFS value was found to be 73.5% in cases with hepatomegaly larger than 2 cm and 90.7% in cases with hepatomegaly <2 cm. Hepatomegaly larger than 2 cm on physical examination has a statistically significant negative effect on EFS. Hepatomegaly was found in approximately half of the patients diagnosed with ALL and was found to be associated with a higher peripheral blast count in the periphery [18]. In contrast to hepatomegaly, splenomegaly had no effect on EFS.

Harousseau investigated the effect of hemoglobin level at diagnosis on prognosis in a series of 141 patients. It was found that the rate of complete remission was 63% in patients with Hb values above 8 gr/dl, 84% in patients with Hb values between 8-10 gr/dL, 70% in patients with Hb values between 10-12 gr/dL and 60% in patients with Hb values >12 gr/dL [19]. On the other hand, in a few studies, it was observed that the Hb level at the time of diagnosis was not effective on the duration of event-free survival [20]. In our study, EFS was 83.9% in patients with Hb <10 g/dL and 80.6% in patients with Hb ≥10 g/dL, and it was determined that hemoglobin values had no statistically significant effect on EFS.

In a study in which patients included in all leukemia groups were evaluated, the rate of patients with a white blood cell count >50.000/mm^3 was found to be 17% [20]. The effect of leukocyte count at the time of diagnosis on prognosis has been well-known for a long time. Many researchers have examined the relationship between leukocyte count at diagnosis and prognosis. In some patient groups, a leukocyte count of >50,000/mm^3 at the time of diagnosis was found to be a poor prognosis criterion, similar to that of large study groups developing childhood leukemia treatment protocols [21-23]. Since our study included patients with high-risk leukemia, 41.4% of the cases had a white blood cell count above 50,000/mm^3. In patients with a white blood cell count of more than 50.000/mm3, EFS was found to be 85.7%, while in patients with a white blood cell count < 50.000/mm^3, EFS was found to be 81.4%. However, the effect of leukocyte count on EFS was not statistically significant in our study. This variation can be attributed to the fact that the patients were in the high-risk group.

Zhang *et al*. [24] investigated the relationship between platelet count and prognosis and found that the prognosis was poor in patients with a platelet count <20.000/mm^3 at the time of initial diagnosis. In the present study, EFS was found to be 82.7% in patients with a platelet count of less than 100,000/mm^3 and 82.8% in patients with a platelet count of more than 100,000/mm3. Since the number of patients with a platelet count below 20,000/mm^3 was low, no compar-

ison could be made. However, it was observed that the frequency of bleeding decreased as the platelet count increased in our patient group.

Among the patients included in this study, 10 patients had relapse. The most prominent indicator of treatment failure in leukemia is the development of relapse. Approximately 15-25% of pediatric patients with ALL develop relapse. The majority of relapses occur in the bone marrow (80%), followed by the CNS (12-16%) and testis (8%) [25].

Limitations

The most important limitation of this study is the small size of the study group. In particular, the number of patients receiving the BFM 2000 treatment protocol is low. To increase the reliability of the study, data from a larger patient group and longer follow-up data need to be collected and analyzed.

CONCLUSION

In high-risk ALL patients who were followed up for more than 5 years in our clinic, the event-free survival rate was 83.6% and the overall survival rate was 90.1%. The EFS values of the different treatment protocols applied were 89.4% and 71.7% for the augmented BFM and BFM 2000 treatment protocols, respectively. The augmented BFM treatment protocol offers a better treatment option for high-risk ALL patients. However, it is essential to consider that the number of patients receiving treatment according to the BFM 2000 treatment protocol was low, and further comparisons with larger patient groups are required.

Authors' Contribution

Study Conception: SÖ, FLA; Study Design: SÖ, FLA, ZK; Supervision: SÖ, FLA, AÜ; Funding: N/A; Materials: SÖ, FLA, ZK, AÜ, ÖD; Data Collection and/or Processing: SÖ, FLA; Statistical Analysis and/or Data Interpretation: SÖ, FLA, AÜ; Literature Review: SÖ, FLA, ZK, AÜ, ÖD; Manuscript Preparation: SÖ and Critical Review: SÖ, FLA, ZK, AÜ, ÖD.

Ethical declaration

Since ethics committee approval is not mandatory for retrospective studies in the years this study was conducted, ethics committee approval was not obtained. The Dean of Istanbul University Faculty of Medicine, where the study was conducted and the article was prepared, was not notified and no negative feedback was received.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgements

This study was accepted as a poster presentation at the SIOP 2013- 45th Congress of the International Society of Paediatric Oncology, 25th-28th September 2013, Hong Kong, China and published as a poster presentation in the journal of the Pediatric Blood and Cancer.

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The European Research Journal 2024;10(5):465-473 Original Article

DOI: https://doi.org/10.18621/eurj.1488539 *Pediatric Cardiovascular Surgery*

The importance and prognostic effect of thyroid hormones in patients with transposition of the great arteries

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ABSTRACT

Objectives: Transposition of the great arteries (TGA) is a rare congenital heart disease that occurs in 3 in 10,000 newborns and is rapidly fatal (90%) within one year if left untreated. The prognosis of this pathology changed after introducing an early arterial switch operation (ASO), while the left ventricle could still adapt to systemic high-pressure conditions. Appropriate regulation of thyroid hormones positively impacts metabolism, cardiac function, and postoperative recovery. Therefore, regular thyroid hormone monitoring and thyroid function monitoring of TGA patients may help to improve the health status and prognosis of this group of postoperative patients.

Methods: In our study, 127 patients who underwent ASO at our pediatric cardiac surgery clinic between 01.01.2014 and 18.09.2021 were retrospectively analyzed and included. Among the patients, 43% (n=54) were females, and 57% (n=73) were males.

Results: The coronary arteries were normal in 89.7% (n=114) and abnormal in 10.3% (n=13) of the patients. Twenty-one of the patients exited, and mortality was calculated to be 16.5%. There were no significant differences in mortality or thyroid stimulating hormone (TSH), free thyroxine (T4), or free triiodothyronine (T3) values (P=0.674, P=0.345, P=0.478). In our study, in which we investigated the effect of thyroid hormone levels on prognosis in neonatal patients with TGA with normal free T3, T4, and TSH values, we found that TSH levels were greater in the group with advanced aortic regurgitation and exitus, although the effect of thyroid hormones on postoperative results was not statistically significant.

Conclusions: Congenital hypothyroidism is a common disease with cardiac effects. During the neonatal period, this disease may conceal itself. Careful, expert clinical follow-up and clinical trials are crucial to improve outcomes in the surgical treatment of transposition of the great arteries, a complex congenital heart disease.

Keywords: Transposition of the great arteries, arterial switch operation, thyroid hormones, thyroid stimulating hormone

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How to cite this article: Arslanoğlu E, Kara KA, Bakhshaliyev, et al. The importance and prognostic effect of thyroid hormones in patients with transposition of the great arteries. Eur Res J. 2024;10(5):465-473. doi: 10.18621/eurj.1488539

Received: May 22, 2024
Accepted: August 3, 2024
Published Online: August 6, 2024

Thyroid hormones have many effects on cardiovascular function. Hypothyroidism causes decreased heart rate, stroke volume, and contractility, resulting in decreased cardiac output. Thyroid hormone replacement helps to maintain adequate cardiac output by increasing heart rate, stroke volume, and contractility. Accordingly, there is an important relationship between thyroid hormone levels and cardiac function [1].

Transposition of the great arteries (TGA) is a rare congenital heart disease that occurs in 3 in 10,000 newborns and is rapidly fatal (90%) within one year if left untreated. The prognosis of this pathology has changed since the introduction of the early arterial switch operation (ASO), which was performed while the left ventricle could still adapt to systemic high-pressure conditions [2, 3]. The ASO was first successfully performed in Brazil in the mid-1970s by Dr. Adib Jatene. The ASO, which is usually performed during the neonatal period, initially has a high early mortality rate. However, mortality decreases as the operative experience increases and various technical maneuvers are performed [4].

Patients undergoing cardiopulmonary bypass (CPB) may have a significant reduction in circulating levels of thyroid hormones. It has been shown that CPB secondary to hemodilution, hypothermia, and ultrafiltration triggers a hypothyroid state [3]. Hypothyroidism affects myocardial energy metabolism and is associated with poor prognosis after cardiac surgery with CPB in patient groups such as at-risk neonates [2, 4].

Congenital hypothyroidism is defined by thyroid hormone deficiency and is the most common endocrine problem in newborn infants. It may be permanent or transient. The clinical findings of more than 90% of congenital hypothyroidism patients do not occur during the neonatal period. This depends on the degree of thyroid gland dysfunction and residual thyroid function, deiodinase adaptation and maternal thyroxine at the end of pregnancy. Early diagnosis and treatment of babies without clinical symptoms are based on newborn screening [5]. In our study, we investigated the effect of thyroid hormone level on the prognosis of ASO in our subclinical patients with thyroid stimulating hormone (TSH) values in the normal range.

METHODS

In our study, 127 patients who underwent ASO at our pediatric cardiac surgery clinic between 01.01.2014 and 18.09.2021 were retrospectively included. The study was conducted retrospectively with the permission of the hospital administration and in accordance with the Declaration of Helsinki and ethical rules (ID: E-22686390-050.99-41961). Patients who had not undergone surgery before were selected. Patients with thyroid hormone levels in the normal range who underwent ASO were included in the study. Patients who required aortic arch repair were not included in the study. Twenty-four patients underwent balloon septostomy preoperatively. TSH, free thyroxine (T4), and free triiodothyronine (T3) are routinely measured in all patients during the preoperative period. We did not routinely administer thyroid hormone replacement during the postoperative period.

The decision for surgical indication was made jointly by the pediatric cardiology and pediatric cardiac surgery team after evaluation of left ventricular geometry, thickness, and function. The coronary anatomy of all patients was evaluated intraoperatively, and appropriate reconstruction was performed. Aortabicaval cannulation was performed in all patients.

Preoperative hemodynamic monitoring via the radial artery or femoral artery was performed in all patients. A central venous pressure catheter was placed through the subclavian vein and monitored. A urinary catheter was inserted for urine monitoring.

In patients with ventricular septal defects, the defect was closed with an autologous pericardial patch. All patients underwent sternotomy. Patients were administered heparin (3 mg/kg) before CPB. The activated clotting time (ACT) was maintained above 450 seconds. Under CPB, arterial blood pressure was maintained between 40 and 60 mmHg with a pump flow of 150-175 mL/kg/min. The hematocrit value was maintained between 25 and 35%. Cold blood cardioplegia was used in 21 patients, Custodiol® was used in 14 patients, and del-nido blood cardioplegia was used in the remaining patients. An autologous pericardium was preferred as the pulmonary reconstruction material. Moderate hypothermia (28-32 °C) was induced. All patients underwent the Lecompte maneuver. Routinely, the sternum was exited with an open

sternum. The sternum was closed according to the patient's condition.

The need for extracorporeal membrane oxygenation (ECMO) was decided by the intensive care team after evaluating the patient's urinary diuresis, blood pressure, lactate parameters in blood gas, left ventricular (LV) status and ejection fraction (EF)% on echocardiography. All the ECMOs were placed centrally. In the postoperative intensive care unit (ICU), intensive care was provided by a team of pediatric cardiologists and pediatric cardiac surgeons. Decisions regarding inotropic support and ventilation were made jointly. Peritoneal dialysis was performed 24 h postoperatively when there was inadequate diuresis (1-2 mL/kg/h) and in patients without a diuretic response. The need for diuretics or peritoneal dialysis was determined by intensive care physicians.

Our study was a retrospective, single-center study in which the patient's age, gender, weight, diagnosis, previous operations, performed operations, and biochemistry results were recorded.

Statistical Analysis

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. In addition to descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, and maximum), the distribution of the data was evaluated with the Shapiro–Wilk test. The Mann-Whitney U test was used for two-group comparisons of quantitative data that did not show a normal distribution. The Friedman test was used for three or more periodic comparisons, and the Wilcoxon test was used for two-period comparisons. Spearman's correlation analysis was used to determine the relationships between quantitative data. ROC analysis was used to determine the predictive value of the quantitative data. Logistic regression analysis was used to determine the independent variables affecting the dependent variable. Significance was evaluated at the $P<0.01$ and $P<0.05$ levels.

RESULTS

The preoperative weight, age, patent ductus arteriosus (PDA) diameter, atrial septal defect (ASD) size, ventricular septal defect (VSD) size, aortic diameter, pulmonary artery diameter, TSH, free T4, free T3 values, total CPB time, aortic cross-clamp (ACC) time, operation time, CPB balance, intensive care unit time, length of stay in hospital and ventilation times are given in Table 1.

Among the patients, 43% (n=54) were females, and 57% (n=73) were males. The coronary arteries were normal in 89.7% (n=114) and abnormal in 10.3% (n=13) of the patients. Twenty-one of the patients exited, and mortality was calculated to be 16.5%.
The degree of postoperative aortic regurgitation was mild in 77.1% (n=98), severe in 2.3% (n=3), moderate in 3.1% (n=4), and absent in 17.5% (n=22) of patients (Table 2).

There was no statistically significant relationship between mortality and sex (P=0.572). There was no statistically significant relationship between mortality and postoperative degree of aortic regurgitation (P=0.072). Preoperative TSH, free T3 and free T4 values were not significantly different from those of postoperative aortic regurgitation. TSH values were calculated as 1.84 mIU/L in patients with moderate AR and 1.84 mIU/L in patients with advanced aortic regurgitation and 7.9 mIU/L in patients with mild and no aortic regurgitation (3.94 mIU/L). There were no significant differences in mortality or TSH, free T4 or free T3 values (P=0.674, P=0.345, P=0.478). However, TSH was greater in the mortality group than in the living group (Fig. 1).

The preoperative heart rate, postoperative heart rate, postoperative hematocrit (HCT) level after CPB, weight, age, PDA size, preoperative heart size, preoperative heart size, and preoperative aortic diameter did not significantly differ among the surviving group and the mortality groups (P>0.05). The difference in the CPB balance between the surviving group and the exitus groups was statistically significant (P=0.001). There was no statistically significant relationship between coronary artery circumference and sex (P>0.05). There was no statistically significant correlation between coronary artery pattern and postoperative degree of aortic regurgitation (P>0.05).

The preoperative heart rate, pump balance, HCT after CPB, weight, age, PDA size, preoperative ASD size, preoperative VSD size, preoperative aortic diameter, pulmonary artery diameter, ventilation time, intensive care unit time, length of stay in the hospital, ACC time, and total CPB time were not significantly

Table 1. Preoperative measurements

	Mean±SD	Min-Max (Median)
Weight (kilogram)	3.39±0.59	2.1-5.8 (3.2)
Age at the time of surgery (days)	15.9±25.66	2-155 (10)
PDA size (mm)	2.66±2.21	0-9 (2.3)
Preoperative ASD size (mm)	5.43±2.76	0-16.5 (5)
Preoperative VSD size (mm)	5.45±5.44	0-21.4 (4)
Preoperative aortic diameter (mm)	8.79±1.77	3.2-14.1 (8.5)
Preoperative pulmonary artery diameter (mm)	10.17±2.19	6.2-16.6 (9.6)
Preoperative aortic diameter (mm)/ Preoperative pulmonary artery diameter (mm) rate	0.87±1.15	0.5-1.35 (0.88)
Preoperative heart rate (min)	141±14.82	110-170 (140)
Preoperative TSH (mIU/L)	3.43±2.63	0.132-9.98 (2.39)
Preoperative free T3 (ng/dL)	2.70 ± 0.6	2.4-3.4 (2.8)
Preoperative free T4 (µg/dL)	12.7 ± 2.9	8.3-14.5 (11.5)
Total CPB time (min)	174.25±54.48	76-410 (173)
ACC (min)	120.78±39.76	31-272 (118)
Operation time (min)	266.37±76.14	150-570 (255)
CPB balance (cc)	-161.49±141.3	-520-495 (-155)
After CPB HCT value (%)	35.98±6.58	13.3-54.4 (36)
ICU time (day)	12±11.58	0.1-48 (9)
Length of hospital stay (days)	3.33±3.71	0-22 (3)
Ventilation (days)	3.93±5.3	0-28.6 (2)

ASD=Atrial Septal Defect, VSD=Ventricular Septal Defect, PDA=Patent Ductus Arteriosus, CPB=Cardiopulmonary Bypass, ACC=Aortic Cross Clamp HCT=Hematocrit, ICU=Intensive Care Unit TSH=Thyroid Stimulating Hormone, SD=standart deviation, min=minimum, max=maximum

different between those with usual and unusual pattern coronary arteries (P>0.05).

The Alanine aminotransferase (ALT) value significantly differed according to the period (P=0.001). The preoperative ALT value was significantly lower than that on postoperative day 2 (P=0.001). The postoperative day 1 ALT value was significantly greater than that on postoperative days 2 and 3 (P=0.001). The postoperative day 1 ALT value was significantly greater than that on postoperative day 2 (P=0.001). The aspartate aminotransferase (AST) value significantly differed according to the period (P=0.001). The preoperative AST value was significantly lower than the other measurements (P=0.001). The preoperative

Table 2. The degree of postoperative aortic regurgitation distribution

		n	%
The degree of postoperative aortic regurgitation	**Mild**	98	77.1
	Severe	3	2.3
	Moderate	4	3.1
	Absent	22	17.5

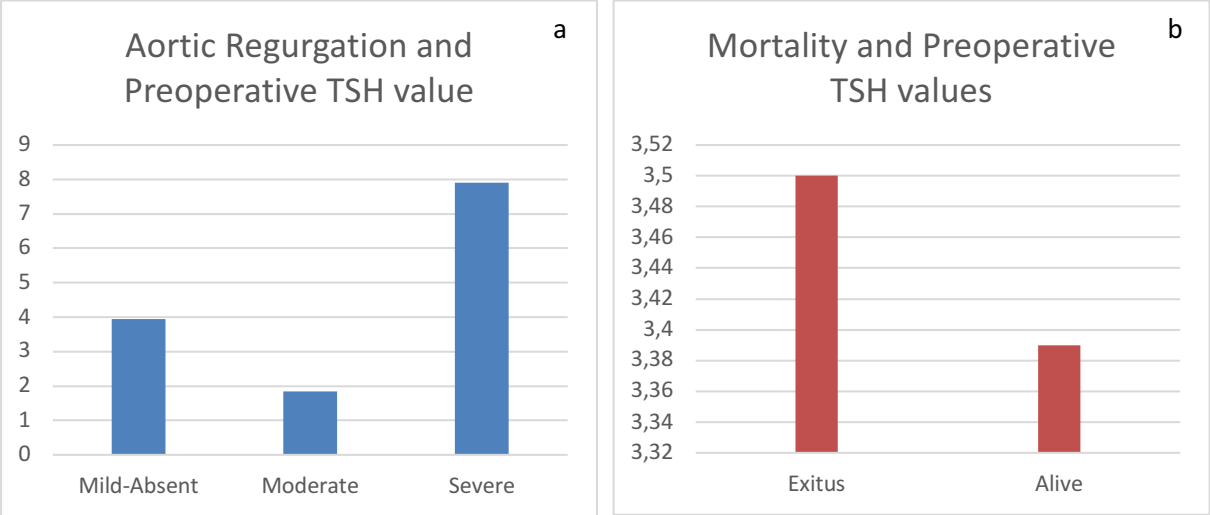

Fig. 1. (a) Aortic regurgitation and preoperative TSH values. (b) Mortality and preoperative TSH values. TSH= thyroid stimulating hormone.

urea value significantly decreased (P=0.001). The preoperative urea value significantly decreased compared with the other measurements (P=0.001). The preoperative creatinine value significantly decreased compared with the other measurements (P=0.001). The hematocrit value did not significantly differ according to the period (P>0.05). The platelet value significantly differed according to the period (P=0.001). The difference between the preoperative platelet value and the other measurements was statistically significant (P=0.001) (Table 3).

In the correlation analysis, there was no statistically significant correlation between preoperative heart rate and pump balance, post-pump heart rate, weight, age at surgery, PDA size, preoperative ASD size, preoperative VSD size, preoperative aorta, pre-

Table 3. ALT, AST, urea, creatinine, hematocrit, platelet measurements

	Preoperative	Postoperative (1st day)	Postoperative (2nd day)	Postoperative (3rd day)	P value
ALT (IU/L)	15.95±17.69 0.1-118.3 (12.1)	40.49±148.55 4.01-1205 (11.8)	37.49±118.62 0.01-734 (6.51)	34.63±130.15 0.01-725 (3.7)	**0.001****
AST (IU/L)	57.23±106.23 15-904.4 (37.2)	183.13±346.75 15-2798 (101.6)	224.47±571.28 10.2-3930 (72.65)	207.38±705.91 11.9-3995.3 (43.7)	**0.001****
Urea (mg/dL)	28.32±13.44 6.78-66.61 (29.26)	36.39±20.11 16-160 (32)	49.2±31.31 10.59-236 (43)	49.85±34.44 8.33-253 (43.2)	**0.001****
Creatinine (mg/dL)	0..6±0.25 0.17-1.49 (0.61)	0.73±0.28 0-1.31 (0.69)	0.8±0.38 0.22-1.84 (0.72)	0.76±0.41 0.18-1.96 (0.66)	**0.001****
Hematocrit (%)	42.12±15.84 26.5-156 (39.7)	37.63±7.02 20.2-60.5 (36.9)	38.84±6.3 26.3-54.1 (38.8)	37.88±5.42 28-49.2 (37.8)	**0.574**
Platelets ($\times10^3$/µL)	302.79±125.5 46.7-689 (288)	156.45±93.14 31-470 (132)	152.83±82.83 31.8-427 (131)	143.13±59.21 13.3-290 (142)	**0.001****

Data are shown as mean±standard deviation and median (minimum-maximum). ALT= alanine aminotransferase, AST=aspartate transferase

Friedman test: **P<0.01

operative pulmonary artery diameter, ratio to pulmonary/aorta size, total CPB, ACC, operation time, intensive care unit time, length of stay in hospital or ventilation days (P>0.05).

There was a positive and weakly significant relationship between hematocrit after CBP and length of service (r=.264, P<0.05). There was a positive and weakly significant relationship between the hematocrit after CBP and the number of days of ventilation (r=,250, P<0.05). There was no statistically significant correlation between hematocrit after CPB and weight, age at surgery, PDA size, preoperative ASD size, and preoperative VSD. size, preoperative aorta diameter, preoperative pulmonary artery diameter, pulmonary aorta, preoperative TSH, total CBP, ACC, operation time, or intensive care unit duration (P>0.05).

There was no statistically significant relationship between preoperative TSH or free T4 free T3 values and operation time, intensive care unit time, hospital stay time, or ventilation days (P>0.05). There was a positive and highly significant correlation between the length of service and the number of ventilation days (r=.687, P<0.01).

DISCUSSION

Congenital hypothyroidism is defined by thyroid hormone deficiency and is the most common endocrine problem in newborn infants. It may develop due to genetic factors such as mutations in the thyroid peroxidase (TPO) or TSH receptor (TSH-R) gene or immunological factors such as elevated anti-TPO levels or morphologic thyroid gland defects. Disorders that may occur in newborns with subclinical hypothyroidism may be difficult to detect due to the absence of sensitive markers. Lipid metabolism, myocardial function, linear growth, and cognitive abilities may be affected [6]. As a result of increasing surgical experience, the results of the ASO are encouraging. Although known factors affecting the success of the surgical operation include the patient's age and weight at the time of intervention, repair of a concomitant ventricular septal defect, coronary artery anomaly, CPB time, and time, there is no specific study on the patient's thyroid hormone levels. [10] In our study, in which we investigated the effect of thyroid hormone levels on the prognosis of neonatal patients with TGA with normal free T3 and T4 and TSH values, we found that the effect of thyroid hormones on postoperative results was not statistically significant, but we found that the TSH level was greater in the group with postoperative advanced aortic regurgitation and exitus.

Children undergoing cardiopulmonary bypass are known to develop low serum T3 levels during bypass and in the postoperative period [7]. The decrease in T3 levels is more profound and long-lasting than that observed in adults.

Portman *et al*. [8] compared patients treated with free T3 and placebo immediately after CPB among patients who underwent pediatric cardiac surgery in a pediatric population younger than 1 year. They found an increase in the peak systolic pressure ratio in patients treated with T3 and suggested that this improved cardiac performance and myocardial oxygen consumption. They suggested that cardiac output increased and pulmonary vascular resistance decreased after systemic T3 administration [8]. In our clinic, we do not routinely perform thyroid hormone replacement but rather only in patients with a diagnosis.

A study on the pharmacokinetic evaluation of T3 administration in the pediatric population revealed that the T3 hormone was cleared faster than it was in adults [9]. Therefore, we believe that the neonatal group is more sensitive to events that may occur in T3 metabolism.

Another important concern after ASO in patients undergoing ASO is the risk of NeoAVR. Neo-aortic valve insufficiency can develop due to the anastomosis site of the neo-aorta or the size difference of the large arteries [11]. In our series, 2.7% (n=2) of patients had advanced postoperative aortic regurgitation, and 2.7% (n=2) had intermediate postoperative aortic regurgitation. The higher mean TSH values in the advanced aortic regurgitation group, although not statistically significant, suggest that this group is more at risk for subclinical hypothyroidism.

Most of the time, compensated hypothyroidism is asymptomatic in newborns due to maternal thyroid hormones and is detected in the laboratory. The causes of compensated hypothyroidism are unclear. Autoimmunity has been hypothesized by several authors in the literature. The natural history of congenital hypothyroidism is not consistent, which is why many authors do not favor replacement unless a definitive

diagnosis is made. In TGA patients, having appropriate levels of thyroid hormones affects energy regulation in the body. Insufficient thyroid hormones can lead to slowed metabolism and reduced energy production. This may have negative effects on the growth, development and general health status of TGA patients [9, 10].

The effects of T3 on the heart are due to transcriptional regulation of several contractile and calcium-processing genes. These effects follow their role in postnatal heart development, and cardiomyocytes exit the cell cycle and differentiate terminally soon after birth, suggesting a role for thyroid hormones in postnatal heart development. Furthermore, recent evidence that cardiomyocytes retain their proliferative competence beyond that of newborns has shown that cardiomyocytes can revert to the proliferative phase [12]. In patients with cardiomyocyte, the levels and function of cardiac thyroid hormones may be affected. This is because TGA causes severe abnormalities in the cardiovascular system and affects systemic and pulmonary blood circulation. In addition, the blood supply to the thyroid gland may also be altered in TGA patients. Therefore, we believe that proper regulation and monitoring of thyroid hormones will be highly important in TGA patients. In the subclinical patient group in which we performed ASO, we did not find a statistically significant correlation between TSH values and intensive care unit stay or service time. We believe that this may be due to the lack of severe hypothyroidism in the selected patient group and compensatory mechanisms of the newborn.

Patients with TGA often have saturation largely dependent on the shunt between the right and left systems, despite being affected by adequate respiratory function, anemia, and low cardiac output. There is no definitive study on how hypoxia-induced inflammation and oxidative stress affect thyroid hormone sensitivity [13, 14]. Additionally, the lack of a statistically significant correlation in our results may be attributed to the possibility that inflammation in patients could vary in response.

The primary reason for perinatal iodine overload is the local application of iodine antiseptics to both the mother and the baby. Topical iodine applied to the mother during childbirth or to the baby, especially for umbilical cord care after birth, can lead to iodine overload in babies. Topically applied iodine can be easily absorbed through the skin and mucous membranes. Furthermore, the intake of iodine-containing compounds such as amiodarone and contrast agents can also lead to iodine overload. Topical iodine applied to newborns can inhibit thyroid hormone synthesis and secretion through the Wolff–Chaikoff effect. Newborns are the most sensitive group to iodine overload. Maternal iodine overload during pregnancy can result in increased TSH levels in babies [15-18]. We believe that the use of topical iodine during childbirth should be considered to prevent iodine overload in such a sensitive population. We also believe that examining individual sensitivities and genetic factors in interpreting babies' responses to iodine overload will be instructive.

Dopamine, a commonly used drug in pediatric cardiac intensive care units, acts through TSH, while amiodarone affects thyroid hormone metabolism by inhibiting the monodeiodinase enzyme that converts T4 to T3 [19]. In our practice with newborn patients, we avoid these drugs unless absolutely necessary and regularly monitor thyroid function. We believe that clinicians should be particularly mindful of thyroid problems in patients treated with dopamine and amiodarone, especially those with Down syndrome.

The ASO is considered the best surgical option for patients with transposition of the great arteries. Although newborn patients are sensitive, the success of surgery can be enhanced with increased experience and precautionary measures [2, 20]. We believe that larger studies are needed to assess the preoperative and postoperative changes in thyroid hormone levels, which may influence patient prognosis, especially considering the known cardiac effects of thyroid hormones.

When evaluating serum hormone levels, it is important to remember that TSH, sT3, and sT4 levels may vary according to the norm used in the kit [21]. We used the same kit used in our laboratory and did not change the results.

Thyroid hormone changes occurring in postoperative intensive care patients without primary thyroid disease are termed "nonthyroidal illness syndrome." Although characterized by a decrease in free T3 and an increase in reverse T3 without an increase in thyroid-stimulating hormone levels, these values can remain within the normal range in newborns. Although low free T3 levels have been associated with poor progno-

sis in studies involving these patient groups, the effect of replacement therapy has not been proven [22]. We believe that clinicians need to be aware of postsurgical hypothalamic-pituitary-thyroid axis changes and the effects of commonly used drugs on thyroid hormone metabolism and take necessary precautions.

Hemodilution and hypothermia associated with CPB can suppress enzyme activity involved in thyroid hormone synthesis and disrupt thyroid metabolism. The suppression of TSH release may also be associated with hypothalamic dysfunction (low levels of thyroid-releasing hormone) or a reduced response to TSH [23]. We used moderate hypothermia (28-32°C) during CPB and tried to keep the CPB lines as short as possible to avoid hemodilution.

CONCLUSION

Proper regulation of thyroid hormones has a positive impact on metabolism, cardiac function, and postoperative recovery. Therefore, regular monitoring of thyroid hormone levels and follow-up of thyroid function in patients with TGA can improve the health status and prognosis of this patient group. Congenital hypothyroidism is a commonly encountered disease with cardiac implications that can persist during the neonatal period. Careful expert clinical follow-up and further clinical research are crucial for improving outcomes in the surgical treatment of complex congenital heart diseases such as TGA.

Authors' Contribution

Study Conception: EA KAK; Study Design: EA KAK; Supervision: HC ET; Funding: EA; Materials: EA; Data Collection and/or Processing: EA, SH, FY; Statistical Analysis and/or Data Interpretation: DCT, EA, FY; Literature Review: EA, SH, KAK; Manuscript Preparation: EA, KAK and Critical Review: HC, NC.

Ethical declaration

Ethical permission was obtained from the Atlas University, Medical Faculty Clinical/Human Research Ethics Committee for this study with date 22.04.2024 and number 04/18 and Helsinki Declaration rules were followed to conduct this study.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgement

Thanks to all Kartal Kosuyolu Research Hospital Healthcare co-workers for their efforts.

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The European Research Journal 2024;10(5):474-481 Original Article

DOI: https://doi.org/10.18621/eurj.1509016 *Obstetrics and Gynaecology*

Can first trimester vitamin D levels predict adverse maternal outcomes in patients who do not take vitamin D supplements?

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ABSTRACT

Objectives: Vitamin D deficiency is claimed to be associated with adverse perinatal outcomes. Here, we aimed to compare adverse maternal outcomes according to vitamin D levels and determine the predictive role of first-trimester vitamin D for adverse maternal outcomes in patients not receiving vitamin D supplementation.

Methods: This study was designed as a retrospective study. A total of 232 patients were divided into three groups: vitamin D deficient (n=127), vitamin D insufficient (n=57), and vitamin D sufficient group (n=48). Then, the vitamin D deficiency group was divided into severe (n=72) and mild (n=55) vitamin D deficiency. Sociodemographic characteristics, first-trimester vitamin D levels, and adverse maternal outcomes such as gestational diabetes mellitus, preeclampsia, preterm birth, and intrauterine growth restriction were compared between the groups. The predictive role of first-trimester vitamin D levels for adverse maternal outcomes was evaluated by ROC analysis.

Results: Gestational diabetes was more common in the vitamin D deficient group than in the vitamin D insufficient group (P=0.024). However, preeclampsia, preterm delivery, and composite adverse outcomes were more common in the vitamin D deficient group than in the vitamin D sufficient group (P=0.044, P=0.008, and P=0.023, respectively). There was no difference in maternal outcomes between the mild and severe vitamin D deficiency groups. First-trimester vitamin D levels ≤19 ng/mL predicted adverse outcomes with 73.2% sensitivity and 49.2% specificity (AUC=0.630, P=0.006).

Conclusions: We recommend screening vitamin D levels in the first trimester, especially in high-risk groups, due to the increased incidence of adverse outcomes. We believe that more research is needed to clarify the relationship between vitamin D and adverse outcomes and the effect of supplementation on these outcomes.

Keywords: First trimester, maternal outcomes, supplementary nutrients, vitamin D

Micronutrients are essential for metabolic processes. Since metabolism becomes faster during pregnancy, pregnant women need more micronutrients and perinatal morbidities can easily occur in micronutrients deficiencies [1].

Vitamin D is a vital micronutrient for bones and calcium metabolism. Vitamin D plays an important role not only in calcium and bone metabolism, but also

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How to cite this article: Dinçgez B, Özgen G, Özgen L. Can first trimester vitamin D levels predict adverse maternal outcomes in patients who do not take vitamin D supplements? Eur Res J. 2024;10(5):474-481. doi: 10.18621/eurj.1509016

Received: July 6, 2024
Accepted: August 4, 2024
Published Online: August 6, 2024

in immune regulation, fertility and perinatal well-being [2]. In the literature, increased tumor necrosis alfa, interferon gamma and interleukin-6 levels were shown in vitamin D deficiency [3]. In gynecological practice, vitamin D has been found to be associated with ovarian reserve, menopausal response, polycystic ovary syndrome, endometriosis, ovarian carcinoma and breast cancer [4, 5].

Recent studies have shown that vitamin D deficiency or insufficiency is associated with adverse obstetric outcomes such as preeclampsia, gestational diabetes mellitus (GDM), intrauterine fetal growth restriction (IUGR), preterm birth, increased cesarean section rates and placental abnormalities [1, 2, 6]. In the future life, it has been found to be associated with hyperactivity disorders, attention deficits, type 1 diabetes, and asthma [7-9]. However, this relationship is still under debate.

However, the synthesis of vitamin D is an easy procedure, low dietary intake, skin diseases affecting synthesis, low sun exposure, and malabsorption syndromes can lead to vitamin D deficiency [1]. The cut-off levels for diagnosis are controversial worldwide and vary depending on ethnicity. In general, vitamin D deficiency is considered for vitamin D levels below 20 ng/ml (50 nmol/L), and vitamin D insufficiency is considered for vitamin D levels between 20-30 ng/ml (50-75 nmol/L) [10]. Vitamin D deficiency is an increasing problem worldwide. A systematic review reported a prevalence of vitamin D deficiency as 46-87% in different regions of the world [11]. Due to this high prevalence, researchers have focused on investigating the effects of vitamin D deficiency and supplementation on perinatal outcomes.

In this study, we aimed to compare adverse maternal outcomes among groups with vitamin D deficiency, insufficiency, and sufficiency. The outcomes were then compared between groups with mild and severe vitamin D deficiency. Additionally, we aimed to determine the predictive role of first-trimester vitamin D for adverse maternal outcomes in patients not receiving vitamin D supplementation.

METHODS

This is a retrospective study performed at a university affiliated Training and Research Hospital between March 2022 and March 2024. The study was in accordance with Helsinki Declaration and it was approved by the local ethics committee (2024-TBEK 2024/06-10). Written informed consent was taken from all study participants for using data from medical records.

Study Population

A total of 589 patients who have first-trimester vitamin D levels were included in the study. Singleton pregnant women between 16 to 45 years old and having available perinatal records and first-trimester vitamin D levels were included in the study. Exclusion criteria were as follows: multiple pregnancies, missing medical records of pregnancy outcomes, using tobacco products, having systemic disease before pregnancy, and using drugs affecting vitamin D levels such as steroids and anti-epileptics. Also, we excluded patients who take vitamin D supplements during pregnancy to prevent changes in maternal pregnancy outcomes. After selected according to the inclusion and exclusion criteria, a total of 232 patients were analyzed. The participants were divided into three main groups: patients who have vitamin D levels below 20 ng/mL were accepted as vitamin D deficient group (n=127), patients who have vitamin D levels between 20 and 30 ng/mL were accepted as vitamin D insufficient group (n=57) and patients who have vitamin D levels above 30 ng/mL were accepted as vitamin D sufficient group (n=48). Then, the vitamin D deficient group was divided into two groups: patients who have vitamin D levels below 12 ng/mL were accepted as severe vitamin D deficiency (n=72) while vitamin D levels between 12 ng/mL and 20 ng/mL were accepted as mild vitamin D deficiency (n=55).

Sociodemographic features such as age, parity, gestational week, body mass index, presence of insurance, economic status, seasonal change, educational status, first-trimester vitamin D levels, adverse maternal outcomes such as GDM, preeclampsia, preterm birth, and IUGR were retrospectively screened and obtained from medical records.

Pregnant women were routinely screened for GDM by two-step protocol which means performing 100-gram oral glucose tolerance test (OGTT) after 50-gram OGTT screening value exceeds 140 mg/dl at the first hour. According to the Carpenter and Coustan criteria, GDM was established with two abnormal values of 95 mg/dl for fasting, 180 mg/dl for the first hour,

155 mg/dl for the second hour, and 140 mg/dl for the third hours in 100-gram OGTT [12]. Preterm birth was defined as a live birth before the 37th gestational week, while preeclampsia was defined as the existence of hypertension after 20th gestational week accompanied by proteinuria or systemic symptoms such as visual symptoms, pulmonary edema, headache, renal insufficiency, impaired liver function, and thrombocytopenia [13].

IUGR was diagnosed based on the following criteria: a) fetal abdominal circumference/estimated fetal weight <3rd percentile, b) changes in umbilical artery Doppler, c) fetal abdominal circumference/estimated fetal weight <10th percentile with mean pulsatility index >95th percentile or pulsatility index >95th percentile [14]. In our clinic, vitamin D levels are routinely measured between 9 am to 12 am by chemiluminescent microparticle immunoassay technique with ARCHITECT i2000SR immunoassay analyzer (Abbott Diagnostics).

Statistical Analysis

The normality of distribution was tested by the Shapiro Wilk test. Since the data were not normally distributed, the Kruskal-Wallis test was used for comparisons of continuous variables between 3 groups, while the Mann-Whitney U test was used for comparisons between 2 groups. Chi-square, Fisher's Exact,

Table 1. Sociodemographic features and maternal outcomes of three groups

	Vitamin D Deficienct Group (n=127)	Vitamin D Insufficient Group (n=57)	Vitamin D Sufficient Group (n=48)	P value
Age (years)	28 (18-42)	28 (18-43)	29.5 (18-40)	0.687
Parity (n)	1 (0-5)	1 (0-5)	0.5 (0-5)	0.600
Gestational week (week)	10 (5-14)	11 (5-14)	11 (6-14)	0.358
Body mass index (kg/m^2)	27 (21-36)	27 (24-36)	27 (23-36)	0.166
Presence of insurance, n (%)	115 (90.6)	53 (93)	47 (97.9)	0.269
Economic status, n (%)				0.794
Low	53 (41.7)	25 (43.9)	21 (43.8)	
Middle	68 (53.5)	27 (47.4)	25 (52.1)	
High	6 (4.7)	5 (8.8)	2 (4.2)	
Season, n (%)				0.987
Spring	30 (23.6)	12 (21.2)	10 (20.8)	
Summer	61 (48)	28 (49.1)	22 (45.8)	
Autumn	25 (19.7)	11 (19.3)	12 (25)	
Winter	11 (8.7)	6 (10.5)	4 (8.3)	
Educational status, n (%)				0.442
Illiterate	28 (22)	8 (14)	8 (16.7)	
Literate	99 (78)	49 (86)	40 (83.3)	
Gestational diabetes, n (%)	15 (11.8)	1 (1.8)	1 (2.1)	**0.017**
Preeclampsia, n (%)	16 (12.6)	2 (3.5)	1 (2.1)	**0.032**
Preterm birth, n (%)	22 (17.3)	5 (8.8)	1 (2.1)	**0.015**
IUGR, n (%)	7 (5.5)	1 (1.8)	1 (2.1)	0.365
Adverse maternal outcome, n (%)	30 (23.6)	7 (12.3)	4 (8.3)	**0.029**
Vitamin D level (ng/mL)	11 (4-19)	25 (21-27)	35.3 (31-45)	**<0.001**

Data are shown as median (minimum-maximum) or n (%). IUGR=Intrauterine growth restriction.

and Fisher Freeman Halton tests were performed for group comparison of categorical variables. Variables were given as median (minimum-maximum) values for continuous variables and frequency (percentages) for categorical variables. The predictive role of first-trimester vitamin D levels for adverse maternal outcomes was evaluated with ROC analysis. SPSS version 22.0 and MedCalc 18 statistical software were used for statistical analysis. A P-value ≤0.05 was accepted as statistically significant.

RESULTS

Gestational diabetes mellitus was detected in 7.3% (n=17) of the patients and preeclampsia 8.2% (n=19) of patients. A total of 28 pregnant women had preterm birth (12.1%). Intrauterine growth restriction was present in 3.9% (n=9) of the patients. Composite adverse maternal outcomes were detected in 17.7% (n=41) of all participants.

The sociodemographic features and maternal outcomes of the three groups are shown in Table 1. There were no significant differences among the three groups in terms of age, parity, gestational age, body mass index, insurance, economic status, seasonal variation, and educational status. As expected, maternal first-trimester vitamin D levels were significantly different among the three groups. When maternal outcomes were examined, the rates of GDM, preeclampsia, preterm birth and composite adverse outcomes were statistically different in at least one group. There was no difference among the groups in terms of IUGR rates.

Comparison of maternal outcomes between groups is demonstrated in Table 2. Gestational diabetes was significantly common in the vitamin D deficient group as compared to the vitamin D insufficient group while preeclampsia, preterm birth, and composite maternal adverse outcomes were more common in the vitamin D deficient group as compared to the vitamin D sufficient group.

Sociodemographic features and maternal outcomes of severe and mild vitamin D deficiency groups were presented in Table 3. No difference was detected between the severe and mild vitamin D deficiency group concerning age, parity, gestational week, body mass index, presence of insurance, economic status, seasonal change, and educational status. Furthermore, there was no statistically significant difference between the two groups according to maternal outcomes.

The predictive role of vitamin D for adverse maternal outcomes was evaluated by ROC analysis. ROC analysis revealed that first-trimester vitamin D levels ≤19 predicted adverse maternal outcomes with 73.2% sensitivity and 49.2% specificity (AUC=0.630, P=0.006) (Fig. 1).

DISCUSSION

Vitamin D deficiency has become a major health problem worldwide, especially among pregnant women. Current literature provides increasing data on the relationship between adverse perinatal outcomes and vitamin D deficiency [1, 2].

A systematic review in 2013 showed that vitamin D levels <20 ng/mL was associated with an increased risk of GDM, preeclampsia, small for gestational age, and preterm birth [6]. Contrary to this study, first-trimester vitamin D was not found to be associated with adverse perinatal outcomes in Malaysian women in 2020 [15]. This conflicting results may be due to the heterogenity of the study populations and different cut-off points for vitamin D deficiency. In the current study, maternal adverse outcomes were found to be

Table 2. Comparison of maternal outcomes between groups

	P_{1-2}	P_{1-3}	P_{2-3}
Gestational diabetes	**0.024**	0.073	1.000
Preeclampsia	0.055	**0.044**	1.000
Preterm birth	0.130	**0.008**	0.216
Maternal adverse outcome	0.076	**0.023**	0.511

Table 3. Sociodemographic features and maternal outcomes of severe and mild vitamin D deficiency groups

	Severe Vitamin D Deficiency (n=72)	Mild Vitamin D Deficiency (n=55)	P value
Age (years)	27 (18-42)	30 (18-40)	0.216
Parity (n)	1 (0-5)	1 (0-4)	0.449
Gestational week (week)	10 (5-14)	10 (6-14)	0.746
Body mass index (kg/m^2)	26.5 (21-36)	27 (23-36)	0.448
Presence of insurance, n (%)	64 (88.9)	51 (92.7)	0.464
Economic status, n (%)			0.195
Low	35 (48.6)	32.7)	
Middle	34 (47.2)	34 (61.8)	
High	3 (4.2)	3 (5.5)	
Season, n (%)			0.706
Spring	17 (23.6)	13 (23.6)	
Summer	34 (47.2)	27 (49.1)	
Autumn	13 (18.1)	12 (21.8)	
Winter	8 (11.1)	3 (5.5)	
Educational status, n (%)			0.608
Illiterate	18 (25)	10 (18.2)	
Literate	54 (75)	45 (81.8)	
Gestational diabetes, n (%)	10 (13.9)	5 (9.1)	0.406
Preeclampsia, n (%)	11 (15.3)	5 (9.1)	0.298
Preterm birth, n (%)	15 (20.8)	7 (12.7)	0.232
IUGR, n (%)	4 (5.6)	3 (5.5)	1.000
Adverse maternal outcome, n (%)	20 (27.8)	10 (18.2)	0.207
Vitamin D level (ng/mL)	9 (4-11)	18 (13-19)	**<0.001**

Data are shown as median (minimum-maximum) or n (%). IUGR=Intrauterine growth restriction.

higher in vitamin D deficient group as compared to vitamin D sufficient group. However, interestingly, no significant difference was detected between mild and severe vitamin D deficiency. Moreover, first-trimester vitamin D levels ≤19 ng/mL predicted adverse outcomes with 73.2% sensitivity and 49.2% specificity. Considering these results, it would be more logical to compare the outcomes for each adverse outcome than compositely.

The underlying mechanisms of the relationship between preeclampsia and vitamin D deficiency are not well recognized. Possible mechanisms include susceptibility to proinflammatory responses in the absence of vitamin D, oxidative stress, and endothelial dysfunction [16, 17]. Moreover, vitamin D receptors are present in fetal tissues and the placenta which plays role in the regulation of fetal growth and placental implantation [18]. In the literature, seasonal variations were shown in preeclampsia. This can be related to the vitamin D synthesis from sunlight [19, 20]. Bodnar *et al*. [21] reported that first-trimester vitamin D levels were significantly lower in patients who subsequently developed preeclampsia. There was a confusing point in the literature on the subject, which was

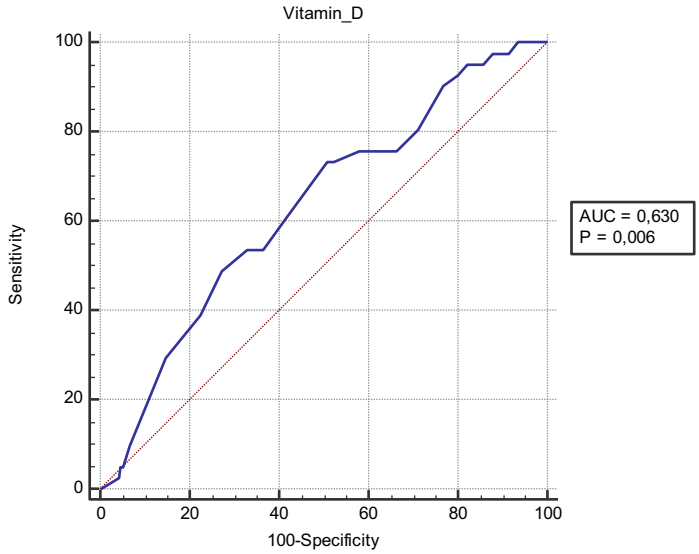

Fig. 1. The ROC analysis evaluating the predictive role of vitamin D for adverse maternal outcomes.

the role of vitamin D supplementation. A study from Norway has shown that taking vitamin D supplements reduces the risk of preeclampsia [22]. In our study, we first showed that the risk of preeclampsia is increased in vitamin D deficiency, independent of the severity of deficiency and supplementation. In contrast to these studies, Al-Shaikh *et al*. [23] revealed no association between low vitamin D levels and pregnancy-induced hypertension. This difference may be explained with confounding factors such as vitamin D sampling time, race, season, and diet. In addition to inflammation, oxidative stress and endothelial dysfunction; vitamin D receptor modulate pancreatic beta cell functions, stimulates insulin receptors, increase insulin sensitivity [24]. Consequently, vitamin D deficiency comes across with GDM in clinical practice. In a meta-analysis searching 22000 pregnant women, vitamin D deficiency was associated with increased GDM rates [25]. Similarly, a meta-analysis searching 16515 patients revealed that GDM risk increase by 45% in vitamin D deficiency [26]. Two meta-analyses searching the effects of supplementation claimed that supplementation improves pregnancy outcomes in women with GDM [27, 28]. In contrast to these studies, in a study of Abd Aziz *et al*. [15], no significant relationship was reported between vitamin D deficiency and GDM. Similarly, Ni *et al*. [13] found no correlation between vitamin D and GDM in unadjusted and adjusted models. In the present study, we found no difference between vitamin D deficiency and sufficient group while GDM was more prevalent in the vitamin D deficient group as compared to vitamin D insufficiency in pregnant women who do not take any supplementation. Similar to preeclampsia, the severity of the deficiency did not affect GDM rates.

Vitamin D regulates the production of inflammatory cytokines and reduces the response to pathogen organisms that cause bacterial vaginosis. It also plays a role in uterine relaxation via calcium metabolism. Impairment of these mechanisms results in preterm birth [29]. The association between vitamin D and preterm birth has conflicting results. A meta-analysis found that low vitamin D levels were associated with an increased rate of preterm birth [30]. A cohort study evaluating 2327 patients demonstrated that vitamin D levels <50 nmol/L significantly increase preterm birth [31]. Perez-Ferre *et al*. [32] showed that a cut-off value of 35 nmol/L predicted preterm birth with 66.7% sensitivity and 71% specificity. Similarly, in a study of Tahsin *et al*. [1] defining vitamin D deficiency as being below 30.25 nmol/L, low second-trimester vitamin D levels were related to increased preterm birth risk. The latest meta-analysis in 2024 showed that low vitamin D levels increase the risk of preterm birth [33]. Consistent with these findings, we found that vitamin D deficiency increases the risk of preterm birth. Con-

trary to this, other studies have not claimed this relationship [34-38]. A study searching 3465 pregnant women reported that there was no relation between vitamin D deficiency and preterm birth [39]. These conflicting results can be related to the different study populations having different nutrition habits, race, sunlight exposure, and vitamin D supplementation.

Limitations

The current study has several limitations. First, it has a retrospective design and a small sample size. Second, confounding factors such as nutritional status were not taken into account. Third, second, and third-trimester vitamin D levels were absent. Finally, neonatal and offspring outcomes were lacking.

CONCLUSION

Vitamin D deficiency is a common condition in pregnancy. Although the effect of supplementation has conflicting results, considering the high incidences of adverse maternal outcomes in vitamin D deficiency, it could be an appropriate strategy to screen vitamin D levels at first trimester and give supplementation for our population.

Authors' Contribution

Study Conception: BD, GÖ, LÖ; Study Design: BD, GÖ; Supervision: BD; Funding: LÖ; Materials: LÖ; Data Collection and/or Processing: BD, LÖ; Statistical Analysis and/or Data Interpretation: BD; Literature Review: GÖ, BD; Manuscript Preparation: BD, GÖ, LÖ and Critical Review: BD, GÖ.

Ethical Statement

This study was approved by local ethics committee of University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital (date: 12.06.2024 and number: 2024-TBEK 2024/06-10).

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2024;10(5):482-489 Original Article

DOI: https://doi.org/10.18621/eurj.1476645 *Pediatrics*

Effect of oxidative stress on cognitive functions in children with obesity

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ABSTRACT

Objectives: This study aims to evaluate the relationship between the oxidative stress induced by obesity and metabolic changes in the cognitive functions of obese children.

Methods: Thirty-three obese children and adolescents (age: 8-18); and 33 healthy children similar in terms of age and gender were enrolled. Children were diagnosed with obesity according to the Turkish children's body mass index (BMI) curves. Patients over the 95th percentile in terms of Turkish children's BMI curves considering their genders and age were called obese children. Obese children were excluded whose obesity was related to any syndrome or disease. Neurocognitive functions including the Visual Memory Test, Finger Tapping Test, Memory Test, Symbol Digit Coding, Stroop Test, Continuous Performance Test, and Shifting Attention Test were evaluated with the battery tests of Central Nervous System Vital Signs (CNSVS) via computer. Malondialdehyde (MDA) and protein carbonyl (PC) were analyzed to determine the oxidative stress. After 10 hours overnight fast, blood samples were collected to determine Fasting glucose, total cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein, liver enzymes aspartate aminotransferase and alanine aminotransferase by using enzymatic methods.

Results: MDA and PC levels in obese children were founs significantly higher (0.78±0.16 µmol/L;198.30±84.45 nmol/mL) than the controls (0.5±0.10 µmol/L; 125.35±43.52 nmol/mL) (P<0.001). All of the cognitive performance domains were statistically significantly different between the study and control groups. A statistically significant correlation was found between neurocognitive indexes and MDA and PC levels.

Conclusions: Obese children's cognitive functions must be evaluated. Elevated oxidative stress may be the reason for the bad cognitive performance in children with obesity. However, this cognitive performance study in obese children should be supported with large study groups.

Keywords: Oxidative stress, cognition, obesity, children

Childhood obesity is a remarkable clinical situation among children with devastating health consequences. It is linked with adult obesity and other complications such as diabetes mellitus, hypertension, hypercholesterolemia, and metabolic syndrome [1, 2]. Now it is understood that obesity is more than a weight management problem. Reduced cognitive functioning has also been reported to be a promi-

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How to cite this article: Özer S, Bütün İ, Bozkurt H. Effect of oxidative stress on cognitive functions in children with obesity. Eur Res J. 2024;10(5):482-489. doi: 10.18621/eurj.1476645

Received: May 1, 2024
Accepted: July 29, 2024
Published Online: August 8, 2024

nent complication in children and adolescents with obesity in recent years [3, 4].

Oxidative stress emerges from the imbalance between the prooxidant state and the antioxidant defense system. Free radicals are continuously produced during daily metabolism [5]. They are highly reactive and may diminish cell function by damaging lipids, proteins, and other macromolecules of the cell [6]. Lipids are the most targeted molecules by oxidative stress through all other classes of biomolecules. Malondialdehyde (MDA) is the end product of lipid peroxidation and a major marker of oxidative stress. It interacts with certain proteins in the cell and also nucleic acid bases to form different adducts [7].

Fat tissue is an important source of reactive oxygen products. During the process of excess free fatty acids in mitochondria uncoupling may occur and electron transfer to oxygen may not be completed [8]. Fat accumulation has been shown to be associated with oxidative stress, and a study by Furukawa *et al* found that levels of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) were reduced in obese individuals [9]. Substances modified by oxidation have also been found to accumulate in adults with obesity [10]. Studies related to oxidative stress in childhood obesity suggest similar findings. In this context, Codoner-Franch *et al*. [11] have proposed a risk of metabolic syndrome in children with obesity because of fat tissue-induced oxidative stress.

The brain is highly susceptible to oxidative damage, and lipid peroxidation is abundant in white and gray matter. Increased oxidative stress in the brain tissue is likely to play a role in cognitive impairment. Some antioxidants have been also shown to attenuate cognitive impairment in experimental studies [12, 13]. As evidenced above, there is a relationship between obesity and oxidative stress but the effect of oxidative stress on cognitive functions in obese children is less clearly lightened. The study goal is to understand oxidative stress' effects on the cognitive status of children and adolescents with obesity in this study.

METHODS

Participants

We included two groups of obese children and healthy controls in this study. Obese children were evaluated in the pediatric department of a university hospital inner northern of Türkiye. Thirty-three children with obesity were selected age between 8 to 17 years. Children with any health problems or syndrome were not included in the obese children group. A healthy control children group was conducted with similar ages and genders from healthy children policlinic of pediatrics department. The control group was conducted from thirty-three children with normal BMI. Informed consent was obtained from all participants and/or their parents. This research study was approved by Gaziosmanpasa University (Tokat, Türkiye) Medical Research Ethics Committee (Date: 30.03.2016 and number: 16-KAEK-043/2016-04)

Measurements

Anthropometric Measurements

Measurements were made using a measurement system (Seca Corp, Chino, CA, USA) while the patients were barefoot and wearing light clothes. Height was measured using an internal stadiometer (Seca). Body Mass Index (BMI) was calculated by dividing weight in kg by the square of height in square meters (kg/m²). Obesity was identified according to Turkish children's BMI percentiles and if the percentile was >95th percentile considered obesity [14].

Laboratory measurements

Fasting glucose (FG), total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by using an autoanalyzer (COBAS 6000; Roche Diagnostics, Indianapolis, IN, USA). C-reactive protein (CRP) and ferritin were detected by electrochemiluminescence immunoassay (COBAS C-501&E-601; Roche Diagnostics).

Determination of MDA and PC levels

The position of serum thiobarbituric acid reactive substance (TBARS) was determined by a system predicated on a response with thiobarbituric acid (TBA) at 90-100°C that was previously described by Esterbauer and Cheeseman [15]. In the TBA test response, MDA or MDA- suchlike substances and TBA reply to produce a pink color with an absorption outdoors at 532 nm. The response was performed at pH 2-3 and 90°C

for 15 min. The sample was mixed with a double volume of cold 10 (w/v) trichloroacetic acid to precipitate the protein. The precipitate was rolled by centrifugation (1,500 × g for 10 min) and an aliquot of the supernatant was replied with an equal volume of 0.67 (w/v) TBA in a scorching water bath for 10 min. Following cooling, the absorbance at 532 nm was measured (GBC Cintra 10e UV/ VIS Spectrophotometer, Victoria, Australia). Results are expressed in ng/ml, according to the graphic standard prepared from measures with a standard result (tetramethoxypropane). The carbonyl contents were determined spectrophotometrically (Shimadzu UV-160 A, Tokyo, Japan) by a system predicated on response of carbonyl group with 2, 4-dinitrophenylhydrazine to form 2, 4-dinitrophenylhydrazone. 2, 4- dinitrophenylhydrazine was the reagent originally used for proteins vanquished to substance-catalyzed oxidation [16].

Central Nervous System Vital Signs (CNSVS)

A neurocognitive test method using with computer The CNSVS is used in this clinical research. The manner of the neuropsychological tests and the psychometric characteristics of this test battery are very similar and the validity and reliability have been demonstrated [17]. This CNSVS test can be used after 7 years of life. Cohen's d sample ranged from d = 0.44 to d = 1.19 for repeat-retest reliability in children and adolescents [18]. This test only can be used with a computer and the total time to complete the test is approximately 30 to 40 minutes. Seven common neuropsychological measures were evaluated in this test: Visual Memory Test, Symbol Digit Coding (SDC), Verbal Memory Test, Finger Tapping Test (FTT), Stroop Test (ST), Continuous Performance Test (CPT), and Shifting Attention Test (SAT). To calculate seven domain scores (Memory, Psychomotor speed, Processing speed, Reaction time, Complex attention, Executive Function, and Cognitive flexibility) and a summary score (Neurocognition Index-NCI) test creates 15 primary scores.

Procedure

Children were diagnosed with obesity according to the Turkish children's BMI curves. Patients over the 95th percentile in terms of Turkish BMI curves considering their genders and age were called obese children. Obese and healthy children completed 2 visits. In the first visit, participants completed the CNSVS test via computer in a silent room near with one of the parents. One week later blood samples were collected from participants in the second visit.

Statistical Analysis

Test scores were compared by the SPSS package program (IBM SPSS Statistics 18). Values are presented as n (%) or mean±SD. Independent samples t-tests were used for continuous variables compared between two groups. Pearson correlation coefficient was used to correlate variables. P values less than 0.05 were considered significant.

RESULTS

The obese children group consisted of 33 patients (18 females, 15 males) with a mean age of 11.58±2.22 years. The control group was conducted with 33 children. The gender and age of healthy participants are similar to the obese group. There is no difference between the two groups considering number, gender, and age (P>0.05).

Table 1 shows the demographic, anthropometric characteristics, and biochemical test results for the group differences. BMI-(standard deviation score) SDS was higher in children and adolescents with obesity (P<0.05). LDL of obese children group was significantly higher and HDL was significantly lower than the healthy control group (P<0.05). Mean CRP was 6.86±5.35 mg/dL and 3.45±2.32 mg/dL in the obese study and healthy control groups, respectively (P<0.05).

The mean MDA and PC levels for the obesity group were 0.78±0.16 µmol/L and 198.30±84.45 nmol/mL, respectively, while the mean MDA and PC levels for the control group were 0.5±0.10 µmol/L and 125.35±43.52, respectively (P<0.001). There was a statistically significant difference in terms of the mean MDA and PC levels between the obesity and control groups (P<0.001). The characteristic scores of the different groups are shown in Table 2.

Eight index scores of the CNSVS were evaluated in two groups and groups were compared. It was found that there was a statistically significant difference between obese children and healthy groups on all cog-

Table 1. Demographic, anthropometric characteristics and laboratory measurements

	Obesity (n=33)	Control (n=33)
Genders (Male/Female)	15/18	15/18
Age (years)	11.58±2.22	11.89±2.79
BMI-SDS	2.43±0.29	1.21±0.44*
Fasting glucose (mg/dL)	87.48±10.73	87.22±10.27
ALT (U/L)	21.91±9.09	22.5±12.95
AST (U/L)	24.39±5.24	23.08±8.39
Total cholesterol (mg/dL)	161.48±29.43	159.49±36.11
LDL-cholesterol (mg/dL)	103.83±29.07	87.30±29.42*
HDL-cholesterol (mg/dL)	45.49±10.72	52.46±14.26*
Trygliceride (mg/dL)	113.42±46.16	111.72±41.64
CRP (mg/dL)	6.86±5.35	3.45±2.32*
Ferritin (ng/mL)	41.33±19.51	39.3±11.2

Data are shown as mean±standard deviation or frequency (n). BMI=body mass index, SDS=standard deviation score, ALT=alanine aminotransferase, AST=aspartat aminotransferase, LDL=low density lipoprotein, HDL=high density lipoprotein-cholesterol, CRP=C-reactive protein

nitive domains. The mean NCI score belonging to obese children was 83.27±9.74 compared to 96.64±4.59 for healthy children. It is seen that obese children's NCI scores were statically lower than healthy children (P<0.001). Also, the other components of cognitive functions measured with this test

Table 2. Distributions of characteristics according to patient and control groups

	Obesity (n=33)	Control (n=33)	Bonferroni 95% CI	
			Lower	Upper
MDA (µmol/L)	0.78±0.16	0.5±0.10*	-	-
PC (nmol/mL)	198.30±84.45	125.35±43.52*	-	-
Neurocognition index	83.27±9.74	96.64±4.59*	7.91	18.82
Composite memory	87.7±10.69	98.88±8.45*	4.28	18.08
Verbal memory	89.58±12.45	101.33±11.10*	3.32	20.20
Visual memory	88.36±10.61	97.91±7.17*	3.06	16.03
Processing speed	86.61±10.43	98.82±5.75*	6.19	18.24
Executive function	82.88±14.67	99.36±6.75*	8.31	24.66
Psychomotor speed	86.67±10.10	96.88±6.83*	4.04	16.38
Reaction time	75.12±13.96	97.15±8.54*	13.75	30.32
Complex attention	84.58±17.49	100.52±6.09*	6.57	25.31
Cognitive flexibility	82.03±16.69	98.21±7.43*	6.94	25.43

Data are shown as mean±standard deviation. MDA=malondialdehyde, PC=protein carbonyl
Hotelling's T2 test was used for Bonferroni 95% confidence intervals. *P<0.05

Table 3. Correlations of oxidative and metabolic parameters with cognition indexes

		PC (n=66)	MDA (n=66)	CRP (n=66)	LDL (n=66)	HDL (n=66)
Neurocognition index	r	-0.118	-0.475	-0.115	-0.279	0.291
	P value	0.355	**<0.001**	0.374	**0.023**	**0.020**
Composite memory	r	-0.257	-0.348	-0.106	0.027	0.161
	P value	**0.042**	**0.004**	0.414	0.829	0.205
Verbal memory	r	-0.288	-0.396	-0.056	0.023	0.080
	P value	**0.022**	**0.001**	0.665	0.856	0.532
Visual memory	r	-0.133	-0.291	-0.136	0.006	0.278
	P value	0.299	**0.018**	0.291	0.962	**0.026**
Processing speed	r	-0.071	-0.436	-0.248	-0.159	0.177
	P value	0.580	**<0.001**	0.052	0.202	0.163
Executive function	r	-0.112	-0.391	0.039	-0.320	0.228
	P value	0.382	**0.001**	0.763	**0.009**	0.070
Psychomotor speed	r	-0.104	-0.389	-0.208	-0.094	0.032
	P value	0.418	**0.001**	0.104	0.453	0.804
Reaction time	r	-0.250	-0.460	-0.230	-0.366	0.325
	P value	**0.048**	**<0.001**	0.073	**0.003**	**0.009**
Complex attention	r	-0.092	-0.403	-0.057	-0.247	0.225
	P value	0.472	**0.001**	0.661	**0.046**	0.074
Cognitive flexibility	r	-0.106	-0.347	0.055	-0.294	0.237
	P value	0.408	**0.004**	0.670	**0.017**	0.060

MDA=malondialdehyde, PC=protein carbonyl, CRP=C Reactive protein, LDL=low density lipoprotein-cholesterol, HDL=high density lipoprotein-cholesterol

were statically lower in children with obesity than in healthy controls (P<0.001).

MDA levels were correlated inversely with all cognitive indexes (P<0.05). Composite memory, verbal memory, and reaction time were correlated negatively with PC values (P<0.05). No significance was detected between CRP levels and any of the cognitive domains although it was inversely correlated with almost all cognitive indexes. LDL negatively and HDL positively were correlated with NCI score (P<0.05). Besides, LDL was negatively correlated with, complex attention, executive function, reaction time, and cognitive flexibility (P<0.05). It is found that there is a positive correlation between HDL and visual memory, and reaction time (P<0.05). Correlation values are shown in Table 3.

DISCUSSION

In the literature, the numbers of studies on cognitive functions in obese children show that bad cognitive performance is linked to obesity. Many of the studies using different assessment methods demonstrate a big association between obesity and bad cognitive performance.

Regarding the role of obesity on cognitive functions in young women, Bove *et al*. [19] found a significant negative association between the visceral adipose tissue and the cognitive domains of verbal learning and memory. Low cognitive scores of 37,414 young Danish men were found to be associated with obesity [20]. There are several studies investigating the relationship between obesity and cognitive impair-

ment in the elderly, however, the results are conflicting [21-24]. On the other hand, cognitive functioning in childhood obesity has been examined in a limited number of studies. These studies prove that a tight conjuctionn between obesity and cognitive disfunction. Mean reaction time was associated with BMI and about 30% of the genetic variance between reaction time performance and BMI were shared in a study comprising 1,312 twins aged 7-10 [25]. Preadolescent children with high BMI were found to have poorer academic scores. Adiposity and school performance were inversely related [26]. Impairment in visual-spatial organization and general mental abilities in children has also been associated with an increase in body weight [27]. Studies demonstrated a poor relationship between neurocognition and obesity in the preschool period [28]. This study examined the effects of oxidative stress on cognitive functions in obese children. The results show that obese children and adolescents performed worse than healthy controls on all cognitive measures. We found a correlation between the reduced neurocognitive functions and the increased oxidative stress in the obesity group.

Given this poor functioning in individuals with obesity, studies investigated the associated factors between obesity and reduced cognition. However, the effect of oxidative stress on reduced cognitive functions in obese children and adolescents is less clearly established. We found a significant increase in lipid oxidation and protein oxidation in the obesity group in this context. High MDA and PC levels were correlated with reduced cognitive domains; MDA for all domains and PC for the memory and reaction time domains.

Increased adipose tissue in obesity is a rich source of cytokines, hormones, and other similar molecules released by the fat tissue. Such diseases like diabetes mellitus and metabolic syndrome are likely to develop by a process triggered by these molecules in children with obesity. The central nervous system (CNS) is more sensitive to changes in childhood since this period is critical for brain development. Therefore, CNS-related problems caused by obesity should be illuminated.

Regarding lipid peroxidation, children and adolescents with obesity had higher levels of MDA compared to the healthy controls in this study. MDA is one of the most studied lipid electrophiles generated from lipid peroxidation [29]. Brain disorders have been usually evaluated in terms of lipid peroxidation because the axonal membranes and myelin sheaths of the brain are rich sources of lipids. Moreover, The central nervous system is susceptible to oxidative stress due to high oxygen consumption. Lower antioxidant status has been associated with impaired cognition in the institutionalized elderly [22]. In addition, circulating adipose tissue cytokines are associated with oxidant-antioxidant status. Carbonyl proteins have been used to present oxidative damage to proteins. This enhances thermodynamic instability inducing tertiary structural changes. Thus, protein aggregation or inactivation emerges. It was reported that restoring PC formation with antioxidant agents could improve cognitive function [30]. PC levels were significantly associated with cognitive impairment in a postmortem study examining the frontal cortex of individuals [30]. In agreement with these findings, we found that PC levels of obese children and adolescents were higher than the healthy controls and there was a negative correlation between PC levels and cognitive functions, particularly memory and reaction time.

We evaluated several biochemical parameters and found that CRP, LDL, and HDL cholesterol levels were significantly different in the obesity group. LDL levels were statically higher while the HDL levels were statically lower in the obesity group. LDL is the principal lipoprotein particle for the transport of cholesterol to the peripheral tissues. LDL plays an important role in the atherosclerotic process and the pathogenesis of Alzheimer's disease [31, 32]. It is suggested that elevated levels of LDL lead to memory deficits. HDL possesses important functions such as anti-oxidation, anti-inflammation, pro-endothelial function, anti-thrombosis, and modulation of immune function. Low HDL was associated with poorer cognitive performance in very old adults. Another study found better executive function in 60-year-old individuals with higher HDL levels [33-35]. Supporting these data from the elderly, we found a negative correlation for LDL and a positive correlation for HDL regarding the cognitive parameters of children and adolescents with obesity. Executive function, reaction time, complex attention, and cognitive flexibility were found to be negatively correlated with LDL while visual memory and reaction time were positively correlated with HDL. To our knowledge, this is the first study suggesting the association between LDL-HDL cholesterol

levels and cognition in pediatric obesity.

Chronic low-grade inflammation has been reported in obesity. Inflammation has also been shown to impact cognition in several psychiatric disorders like anxiety disorders, major depressive disorder, and bipolar disorder [36]. However, no study has been conducted on the inflammation-related cognitive decline in obesity. So we also investigated the association between CRP levels and cognitive functioning in the obesity group. We found elevated CRP levels in the obesity group but we did not find a correlation between the CRP levels and the cognitive domains.

Limitations

Our study has several limitations that should be addressed. We could only evaluate the CRP levels of the children and adolescents to indicate inflammation. Other inflammatory markers or proinflammatory cytokines may be analyzed in future studies. A larger study group would be better for estimating regression analysis. Despite these limitations, we reported cognitive decline along with elevated oxidative stress and an inverse relationship between MDA and all cognitive domains in obese children in this study.

CONCLUSION

Oxidative stress should be evaluated when assessing the complex mechanism of this decline in the neurocognitive functioning of obese children and adolescents. Data regarding the effect of lipid peroxidation and protein oxidation on cognitive functioning may lead to new future intervention strategies for cognitive impairment in youth with obesity.

Authors' Contribution

Study Conception: SÖ; Study Design: SÖ, İB; Supervision: SÖ, HB; Funding: N/A; Materials: SÖ, İB, HB; Data Collection and/or Processing: SÖ, İB, HB; Statistical Analysis and/or Data Interpretation: SÖ, HB; Literature Review: SÖ, İB, HB; Manuscript Preparation: SÖ and Critical Review: SÖ, İB, HB.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

Acknowledgements

This study was presented as a poster at the 41st FEBS Congress, Molecular and Systems Biology for a Better Life, Ephesus/Kuşadasi, Turkey, September 3-8, 2016. It was published in The FEBS Journal Special Issue for the 41st FEBS Congress, indexed in CPCI.

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The European Research Journal 2024;10(5):490-500 Original Article

DOI: https://doi.org/10.18621/eurj.1513218 *Chest Diseases*

Ultrasonographic evaluation of post-COVID long-term lung changes and relation to prolonged COVID symptoms: a prospective cross-sectional study

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ABSTRACT

Objectives: This study aims to evaluate lung findings using lung ultrasonography (USG) in patients with PCR-positive COVID-19 pneumonia treated in the ward or intensive care unit and to explore the relationship with prolonged COVID-19 symptoms.

Methods: This prospective cross-sectional study was conducted at the University Medical Faculty Hospital outpatient clinic from December 2022 to April 2023. It involved 54 adult patients with PCR-positive COVID-19 pneumonia, treated and discharged from the ward or intensive care unit (ICU). Clinical and demographic data, lung ultrasonography results, and prolonged COVID-19 symptoms were recorded.

Results: Among the 54 patients, the ICU group had a mean age of 52.84±12.30 years, while the ward group had a mean age of 58.80±11.36 years (P=0.085). Shortness of breath was the most common prolonged symptom in both groups. The ICU group had significantly more right lung B lines than the ward group (P=0.002). Myalgia was more frequent in the ward group (P=0.024). No significant differences were observed in other variables (P>0.05). Prolonged COVID-19 symptoms were interrelated (P<0.05), and the total number of B lines was significantly associated with dyspnea (P=0.023).

Conclusions: Our study demonstrated that lung USG is a valuable tool in the assessment of lung injury after COVID-19 and in the management of long-term COVID-19 symptoms. B lines detected by lung USG are significantly associated with the long-term COVID symptom of shortness of breath, and the number of B lines may be an important biomarker in the management of shortness of breath.

Keywords: Lung ultrasonography, B lines, COVID-19, prolonged COVID, dyspnea

Since the beginning of the COVID-19 pandemic (13/09/2023), 770,563,467 confirmed cases, including 6,957,216 deaths, have been reported to the World Health Organization (WHO) [1]. In our country, it has infected more than 17,000,000 people; more than 100,000 of them have died [1]. COVID-19 and its strains have repeatedly caused and continue to cause disease in humans. Some of those who have had the disease have reported symptoms such as persistent or subsequent dyspnea, cough, muscle pain, fatigue,

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How to cite this article: Tan Tabakoğlu N, Hancı Yılmaztürk P, Yılmam İ, Süt N, Hatipoğlu ON. Ultrasonographic evaluation of post-COVID long-term lung changes and relation to prolonged COVID symptoms: a prospective cross-sectional study. Eur Res J. 2024;10(5):490-500. doi: 10.18621/eurj.1513218

Received: July 9, 2024
Accepted: August 3, 2024
Published Online: August 16, 2024

palpitations, extreme forgetfulness, and difficulty concentrating, which reduce the quality of life of patients and cannot be explained by other diagnoses. The term 'prolonged COVID' has been used for patients with these symptoms associated with COVID-19 for more than 12 weeks [2-4].

COVID-19 infection has shown involvement of many organs, including the heart and central nervous system, but it was lung involvement that determined mortality and morbidity. Again, the most common symptoms in patients with prolonged COVID-19 are those related to the respiratory system [5].

Lung USG is widely used as a non-invasive, portable, and reproducible imaging method, especially in intensive care units [6]. It is also precious in evaluating pleura and parenchyma [7]. Artifacts known as (B-lines) in lung USG provide essential clues in diagnosing interstitial or alveolar pathologies [8].

Recent studies have reported that lung ultrasonography (USG) is mainly similar to the results of computed tomography (CT), which is known to be diagnostic in lung diseases [9, 10].

Our study aimed to determine the changes in the lungs of patients with a positive COVID-19 Polymerase Chain Reaction (PCR) test, who were hospitalized in the ward or intensive care unit with a diagnosis of pneumonia and who were discharged after recovery by USG; also to question the prolonged COVID symptoms in these patients and to determine the relationship of common symptoms with lung USG findings.

METHODS

Study Design and Participants

The study was prospective-cross-sectional conducted at the Health Research and Development Centre of the University Faculty of Medicine from December 2022 to April 2023. Patients with a positive COVID-19 PCR test between April 2020 and June 2021 who were diagnosed with pneumonia and discharged after inpatient treatment in the Chest Diseases Service or Respiratory Intensive Care Unit were included in the study.

In December 2022, patients who met the criteria for participation in the study and who had been discharged for at least 18 months after receiving inpatient treatment in our hospital's chest diseases service or intensive care unit were identified from our hospital's automation records, and the study was started on the same date. 73 of these patients were contacted by telephone. Sixty patients who agreed to participate in the study were invited to the University Hospital Health Research and Development Center outpatient clinic for the study. Among the patients who agreed to participate in the study, one patient receiving hemodialysis treatment, two patients receiving chemotherapy treatment, two patients who were pregnant, and one patient with a history of radiotherapy to the thorax region in previous years were excluded from the study.

In a total of 54 patients who agreed to participate in the study and met the inclusion criteria, anamnesis was taken, physical examinations were performed, prolonged COVID symptoms were questioned, thoracic USG was performed, and all findings were recorded. In the anamnesis and physical examination of the patients, symptoms, gender, age, and physical examination findings were recorded on pre-prepared forms. Modified Medical Research Council (mMRC) dyspnea scores were calculated and recorded. All data were entered into the SPSS database and statistically evaluated.

Inclusion criteria include: (1) To be at least 18 years old, (2) to have been diagnosed with pneumonia with a positive COVID-19 PCR test during hospitalization at the University Health Application and Research Center, (3) to have received inpatient treatment due to COVID-19 in the ward or intensive care unit, (4) to have been discharged at least 18 months after discharge, (5) not to be pregnant or suspected of pregnancy during the study, and (6) to sign the consent form by agreeing to participate in the study.

Exclusion criteria iinclude: (1) Being under the age of 18; (2) being pregnant or suspecting pregnancy during the study; (3) having received or currently receiving chemotherapy or radiotherapy before or during the study; (4) having a diagnosis of chronic kidney failure; (5) having a diagnosis of heart failure, (6) having a diagnosis of tuberculosis, chronic obstructive pulmonary disease (COPD), asthma, or malignancy, fibromyalgia, rheumatoid arthritis; and (7) inability to be positioned for ultrasound examination due to general health status. Patients' previous diagnoses and health conditions were verified through the e-Nabız information system and hospital records [11, 12].

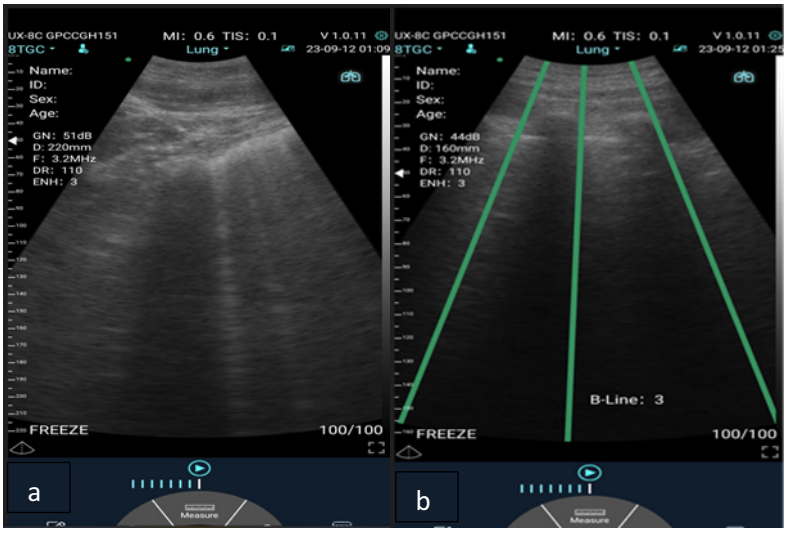

Fig. 1. B lines detected in the lung ultrasonography of participants (a) ET and (b) CB).

Lung Ultrasonography

The evaluation of the lungs was carried out using a wireless ultrasonography device that had a dual probe with a linear and convex tip. The examination was conducted in a standardized manner by dividing each lung into three regions - anterior, lateral (axillary region), and posterior. Each section was further divided into upper and lower regions, and the findings in all six regions were recorded for each hemithorax. Consolidation, air trapping (reverberation artifact), pneumothorax, pleural effusion, pleural thickening, and interstitial syndrome (B-line) findings in each USG region were noted on the form (Fig. 1). USG findings were evaluated based on the International Evidence-Based Recommendations for Bedside Lung Ultrasonography report [10].

Practitioner Information

All participants' ultrasound examinations were conducted by Specialist Dr. NTT, who holds a certificate in 'Emergency and Basic Ultrasound,' under the supervision of Prof. Dr. ONH, who has extensive experience in lung ultrasonography.

Ethical Approval

Ethics committee approval for this study was obtained from the Trakya University Faculty of Medicine Ethics Committee on 14/06/2021, with decision number:13/35 and protocol code: TUTF-BAEK-2021/292. The Declaration of Helsinki performed all procedures in this study. Written informed consent was taken from the patients.

Statistical Analysis

Numerical results were expressed as mean ± standard deviation and categorical data as number (%). The suitability of the normal distribution of quantitative data was tested using the Shapiro-Wilks test. Mann-Whitney U test was used to compare quantitative data between intensive care unit and ward groups. Fisher exact test was used to compare categorical data between groups. The relationship between prolonged Covid symptoms was analyzed using Spearman's test. Logistic regression analysis was performed to determine the factors affecting dyspnea. The appropriateness of the regression model was evaluated by the Omnibus test, Cox & Snell R2, Nagelkerke R2, and Hosmer-Lemeshow test. Activity-induced dyspnea was considered significant dyspnea and was used in the logistic regression analysis with a mMRC scale of ≥2 [13]. A value of $P<0.05$ was accepted as the limit of statistical significance. SPSS 20.0 (SPSS Inc. Chicago, II, USA) statistical package program was used for data analysis.

RESULTS

The study was completed with 54 patients. Of the 54 patients included in the study, 9 of 13 patients dis-

charged from the intensive care unit were male (69.2%), and 4 were female (30.8%), 30 of 41 patients discharged from the ward were male (73.2%), and 11 were female (26.8%).

In our study, demographic and clinical characteristics were compared between the ward and intensive care unit groups (Table 1).

The mean age was 52.84±12.30 for the intensive care unit group and 58.80±11.36 for the ward group. There was no significant difference between the groups (P=0.085) in terms of age. Gender distribution was similar in both groups (P=1.000). When compared in terms of symptoms, myalgia was significantly higher in the ward group (53.7% vs 15.4%, P=0.024). There was no statistically significant difference between the groups in terms of cough, dyspnea, fatigue, and sweating (For all P>0.05). Although higher grades of dyspnea severity were observed in the intensive care group, this difference did not reach statistical significance (P=0.167). In lung ultrasonography findings, the total number of B lines was significantly higher in the intensive care group (14.1±15.1 vs 4.24±5.88, =0.004). Especially the number of B lines in the right lung was significantly higher in the intensive care group (9.8±10.4 vs 2.2±3.0, P=0.002). There was no significant difference between the groups in the number of B lines in the left lung (P=0.055).

To better understand these relationships, the correlations of prolonged COVID symptoms with each other were analyzed with Spearman's test (Table 2).

In our analysis, we found significant correlations between dyspnea and other symptoms. Dyspnea was positively correlated with myalgia (r=0.271, P=0.047) and sweating (r=0.315, P=0.020), indicating an association with these symptoms. However, dyspnea showed no significant correlation with cough (r=0.008, P=0.954) or fatigue (r=0.209, P=0.130). Additionally, myalgia was significantly correlated with fatigue (r=0.725, P<0.001) and sweating (r=0.361, P=0.007). These findings revealed interrelated symptoms such as dyspnea, fatigue, myalgia, and sweating. Considering that shortness of breath is a symptom that

Table 1. Clinical and demographic characteristics of the ward and intensive care groups

	Ward (n=41)	ICU (n=13)	P value
Age (years)	58.80±11.36	52.84±12.30	0.085¥
Gender, male	30 (73.2)	9 (69.2)	1.000*
Cough, yes	22 (53.7)	5 (38.5)	0.526*
Dyspnea,			
mMRC Grade 0	26 (63.4)	6 (46.2)	
mMRC Grade 1	9 (22)	2 (15.4)	0.167*
mMRC Grade 2	6 (14.6)	4 (30.8)	
mMRC Grade 3	0(0)	0 (0)	
mMRC Grade 4	0 (0)	1 (7.7)	
Fatigue, yes	25 (61)	5 (38.5)	0.206*
Myalgia, yes	22 (53.7)	2 (15.4)	0.024*
Sweating, yes	6 (14.6)	2 (15.4)	1.000*
Total B Lines, total	4.24±5.88	14.1±15.1	**0.004¥**
Right lung total	2.2±3.0	9.8±10.4	**0.002¥**
Left lung total	2.0±3.6	4.4±5.6	0.055¥

Data are shown as mean±standard deviation or n (%). ICU=Intensive Care Unit; mMRC=Modified Medical Research Council, Grade 0=Only during heavy exercise, Grade 1=Walking fast on a flat road or going up a slight slope, Grade 2=Due to shortness of breath, I walk more slowly on a flat road or rest from time to time, Grade 3=After walking 100 meters or after walking for a few minutes I have to stop, Grade 4=I can't leave the house because of shortness of breath.

*Fisher exact test, ¥Mann Whitney U test

Table 2. Results of Spearman correlation analysis among prolonged COVID symptoms

		Cough	Dyspnea	Fatigue	Myalgia	Sweating
Cough	r	1.000	0.008	0.224	0.149	0.104
	P value		0.954	0.104	0.282	0.453
Dyspnea	r	0.008	1.000	0.209	0.271	0.315
	P value	0.954	.	0.130	**0.047**	**0.020**
Fatigue	r	0.224	0.209	1.000	0.725	0.373
	P value	0.104	0.130	.	**<0.001**	**0.005**
Myalgia	r	0.149	0.271	0.725	1.000	0.361
	P value	0.282	**0.047**	**<0.001**	.	**0.007**
Sweating	r	0.104	0.315	0.373	0.361	1.000
	P value	0.453	**0.020**	**0.005**	**0.007**	.

r=Spearman's correlation coefficient. Values range between +1 and -1, +1 indicates a perfect positive correlation, -1 indicates a perfect negative correlation, and 0 indicates no correlation, P=Statistical significance value. P<0.05 indicates that the correlation is significant at the 95% confidence level, P<0.01 indicates significance at the 99% confidence level, n=Sample size, which is 54 in this study.

most affects other prolonged COVID-19 symptoms and significantly reduces the quality of life of patients, logistic regression analysis was performed to examine the relationship between independent variables (age, group, gender, total B lines, and other prolonged COVID-19 symptoms) affecting shortness of breath. Since significant dyspnea is considered as mMRC ≥ 2 in clinical practice, significant dyspnea was taken as the dependent variable, and the relationship between this variable and other independent variable was examined by logistic regression analysis.

Omnibus tests were performed to assess the significance of the model before proceeding to logistic regression analysis to examine the independent factors affecting dyspnea (Table 3). The results showed that the model was statistically significant (Table 3, χ^2= 20.883, P=0.007). Statistical analyses were conducted to assess the performance and fit of the model (Table 4).

According to the logistic regression model summary, the -2 Log Likelihood value is 36.325, the Cox & Snell R^2 value is 0.321, and the Nagelkerke R^2 value is 0.491. These values indicate that the model fits the data well and explains a moderate to high portion of the variance in the dependent variable. These findings suggest that the model is effective in predicting the dependent variable and performs well.

Furthermore, the Hosmer and Lemeshow test results show that the model's Chi-square value is 3.429, with 8 degrees of freedom and a p-value of 0.905. These results indicate that the model is valid and reliable.

Logistic regression analysis was conducted to examine the effects of independent variables (age, group, gender, cough, fatigue, myalgia, sweating, and total B lines) on the dependent variable (dyspnea) (Table 5).

According to the analysis's results, as the total B-line score increases, the likelihood of experiencing dyspnea also increases. This relationship is statistically significant (B=0.154, P=0.023). The odds ratio is 1.167, and the confidence interval (1.022 - 1.333) supports that this relationship is positive and statistically

Table 3. Omnibus tests of model coefficients

		Chi-square	P value
Step 1	Step	20.883	**0.007**
	Block	20.883	**0.007**
	Model	20.883	**0.007**

Table 4. Logistic regression model summary

Step 1	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
	36.325[a]	.321	.491

[a]Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.

significant. Other variables were not found to be statistically significant (for all P>0.05).

There was no sign of consolidation, pneumothorax, air trapping, or pleural effusion in the lung USG of the participants. This may be because patients who agreed to participate in the study had no active disease.

Fig. 1 shows the B-lines detected by ultrasound in the lung radiographs of study participants E.T. and C.B.

DISCUSSION

In this study, we investigated prolonged COVID symptoms by performing lung ultrasonography of COVID-19 patients who were discharged after inpatient treatment in the ward or intensive care unit and who had been hospitalized for at least 18 months. Our study revealed that patients hospitalized for PCR-positive COVID-19-induced pneumonia continued to have prolonged COVID symptoms even 18 months after discharge and that the symptoms were interrelated among themselves. It also showed that shortness of breath, a prolonged COVID symptom, significantly increased with an increasing total number of B lines detected on lung ultrasonography.

The onset of the COVID-19 pandemic was characterized by SARS-CoV-2, a rapidly spreading respiratory infection agent detected in China in the last months of 2019. With the World Health Organization (WHO) declaring a pandemic on March 11, 2020, the global impact of this new viral threat was officially recognized [14]. Although it has been over 4 years since the first case was detected, many survivors are still experiencing unexpected symptoms. Because although lung involvement determines hospitalization and mortality in COVID-19, many different organs or systems, such as the heart, central nervous system, kidney, musculoskeletal system, and gastrointestinal system, have been affected by this disease [15].

The occurrence of these unusual symptoms in the patient for more than 12 weeks due to the involvement of many different organs is referred to in the literature as 'Prolonged COVID'. It is thought that the SARS-CoV-2 virus, the causative agent of COVID-19, enters cells by binding to Angiotensin 2 (ACE2) receptors present in many organs, causes severe damage in the organs it infiltrates, and being a neurotropic virus, plays a role in the emergence and persistence of pro-

Table 5. Logistic regression analysis of factors affecting dyspnea

		Beta	P value	Odds Ratio (95% Confidence Interval)
Step 1[a]	Group	1.175	0.364	3.237 (0.256-40.990)
	Gender	-0.854	0.448	0.426 (0.047-3.871)
	Age	0.052	0.362	1.053 (0.942-1.176)
	Cough	0.181	0.843	1.199 (0.199-7.218)
	Fatigue	2.465	0.169	11.765 (.352-393.494)
	Myalgia	-3.197	0.066	0.041 (0.001-1.236)
	Sweating	-1.978	0.125	0.138 (0.011-1.736)
	Total B Lines	0.154	**0.023**	1.167 (1.022-1.333)
	Constant	-3.267	0.322	0.038

[a]Variable(s) entered on step 1: Group, Gender, Age, Cough, Fatigue, Myalgia, Sweating, Total B Lines

longed COVID symptoms [3].

Table 1 shows the clinical and demographic characteristics of the patients who participated in the study. There was no statistically significant difference in age and gender between the intensive care unit and ward patient groups (P=0.085 and P=1.000, respectively).

More than 50 symptoms of prolonged COVID-19 have been identified in studies. The symptoms of prolonged COVID-19 questioned in our study and their frequency of occurrence are shown in Table 1. The most common symptoms are dyspnea, myalgia, cough, and weakness, and the least common symptom is sweating. The TURCOVID study involving 504 patients and 13 different centers reported that the most common symptoms were dyspnea and fatigue [5]. In another study in which 7139 patients participated and prolonged COVID symptoms were questioned, shortness of breath, cough, fatigue, and muscle pain were found among the most common symptoms [16]. In another study conducted in Italy, 143 patients who were discharged after inpatient treatment due to COVID-19 were questioned about their prolonged symptoms after 60 days, and it was reported that at least 1 symptom persisted in 87.4% of the patients and at least 3 symptoms persisted in 55% of the patients and the 3 most common symptoms were fatigue-weakness, shortness of breath and joint pain, respectively [17]. The results of our study were found to be consistent with these studies. Sweating is not included in the common prolonged COVID symptoms and was the least common symptom in our study. The results of our study were consistent with these studies. Sweating is not among the common symptoms of prolonged COVID-19 and was the least common symptom in our study.

In our study, the number of B lines detected in the right lung was significantly higher in patients treated in the intensive care unit than in patients treated in the ward. The right lung is larger than the left lung. Therefore, the area affected by COVID-19 pneumonia is more. After the trachea, the right main bronchus is separated from the left main bronchus at a narrower angle. Therefore, aspiration and aspiration pneumonia are more common in the right lung. In intubated patients, if the tracheal tube is placed further forward than it should be, it enters the right main bronchus more. In this case, the volume or pressure that should be given to both lungs is applied to the right lung; volume and barotrauma complications occur. For these reasons, right lung pneumonia and lung damage due to mechanical ventilation may leave longer-lasting or permanent damage in the right lung [18]. The higher incidence of B lines in this region can be explained in this way, which is consistent with the results of our study and is shown in Table 1.

In our study, the relationship between prolonged COVID symptoms was analyzed using Spearman's test, and significant correlations were found between dyspnea and other symptoms. Dyspnea was positively correlated with myalgia (r=0.271, P=0.047) and sweating (r=0.315, P=0.020). These findings suggest that patients who experience shortness of breath are also likely to experience muscle pain and sweating. Furthermore, myalgia showed significant correlations with both fatigue (r=0.725, P<0.001) and sweating (r=0.361, P=0.007) (Table 2). This analysis suggests that symptoms are interrelated, especially dyspnea, which is the symptom that most affects patients' quality of life, and that myalgia may trigger other symptoms. In an article published in 2023 involving 76 patients, dyspnea scores were measured at 3, 6, and 12 months after discharge, and it was reported that dyspnea persisted in 46% of patients at 12 months, worsened in 24%, and 20% had new onset dyspnea. The same article showed that the clinical scores of the patients during intensive care hospitalization did not affect this situation and found that dyspnea was also associated with other prolonged COVID symptoms [19]. This is consistent with the results of our study. In our study, it was shown that receiving treatment in the intensive care unit and in the ward was not statistically significant in terms of dyspnea (Table 1, P=0.167), and dyspnea was associated with other symptoms of prolonged COVID (Table 2). Many articles on quality of life months after COVID have shown that prolonged COVID symptoms, especially dyspnea, impair physical and mental performances and reduce the quality of life of patients [19, 20]. For these reasons, we investigated the relationship between dyspnea and other prolonged COVID symptoms we questioned in our study, age, gender, place of hospitalization, and total B lines in the lungs. We tested the validity, performance, and fit of the model we built to perform logistic regression analysis (Table 3, Table 4). The results showed that the model was valid, reliable, and significant (Table 3, Omnibus test, P=0.007; Table 4, -2 Log Likelihood=36.325, Cox &

Snell R^2=0.321, Nagelkerke R^2=0.491; Hosmer and Lemeshow test, P=0.905). Logistic regression analysis showed that the total number of B lines was significantly associated with dyspnea risk (Table 5; P=0.023, OR=1.167, 95% CI: 1.022-1.333). This finding suggests that each unit increase in the number of B lines increases the risk of dyspnea by approximately 16.7%. This result suggests that B lines detected on lung ultrasonography may be a potential biomarker for predicting dyspnea symptoms. The effects of being treated in the ward or intensive care unit, as well as gender, age, cough, fatigue, myalgia, and sweating, were not statistically significant (Table 5).

B lines are hyperechoic, vertical lines seen when the ultrasound probe is placed on the surface of the pleura and usually extend vertically into the lung parenchyma [21]. The detection of B lines is associated with alveolar-interstitial conditions. B lines are seen in diseases characterized by fluid accumulation or fibrosis in alveolar and interstitial tissues, especially acute pulmonary edema, pneumonia, acute respiratory distress syndrome, and interstitial lung diseases. Therefore, to evaluate only COVID-induced B lines, patients with disease diagnoses in which B lines are likely to be seen were excluded from our study. A 2006 study pointed out that lung USG in alveolar-interstitial syndromes is rapid, reproducible, and sensitive, without radiation exposure, and more advantageous to be used instead of chest radiography and CT, which are traditional methods for monitoring disease progression or response to treatment [22]. In the same study, some disadvantages of lung USG were also mentioned. Among these disadvantages, it was stated that deep tissues could not be visualized, and the method was user-dependent. User dependency and the inability to visualize deep tissues make it difficult to detect lesions, especially in deeper parts of the lung or in more central areas such as mediastinal structures [22].

The detection of B lines on lung USG in the post-COVID-19 period has been accepted as an essential indicator of interstitial damage in these patients. In a study, lung USG was performed in patients diagnosed with COVID-19 within 6 weeks following the diagnosis, and B lines were detected in all patients. A correlation was found between the frequency of B lines and dyspnea, one of the symptoms of prolonged COVID [23]. This result is compatible with our study. However, the same study reported that the detection of B lines decreased to 31% after 3 months and to 6% after 12 months. The reason for this was that lung USG findings changed over time, but gas exchange disorders detected by the Diffusing Capacity of the Lungs for Carbon Monoxide (DLCO) continued, indicating that lung USG after COVID may reflect gas exchange disorders, especially in the early stages of the disease. This result contradicts our study. Because, we performed lung USG 18 months after COVID-19 and found a significant positive correlation between the total number of B lines and dyspnea. This difference may be since the long-term effects of COVID-19 on the lungs have yet to be discovered. Because the study by Kimmel *et al*. covered 12 months after the diagnosis of COVID [23]. However, our study examined patients who were at least 18 months post-COVID. The results of a follow-up study in which dyspnea and lung function were evaluated for 1 year in recovered COVID-19 patients support this contradictory situation in favor of our study. In this study, 45% of patients complained of new-onset dyspnea, and lung function tests, especially DLCO, tended to improve over time; however, persistent deterioration was observed in some patients. In these patients, regional ventilation distribution was evaluated by electrical impedance tomography, and ventilation disorders were detected in some regions [24]. These studies show that the effects of COVID-19 on the lungs change with time.

Recent literature shows that myalgia after COVID-19 is one of the common symptoms of prolonged COVID-19, just like in our study [25]. One of the studies suggests that the SARS-CoV-2 virus infects skeletal muscle cells by binding to the ACE2 receptor [26]. Another is that the virus acts by binding to skeletal muscle cells through TMPRSS2 receptors independent of the ACE2 receptor [27]. During symptomatic COVID-19, cytokines such as interleukin-6, interleukin-1β, interleukin-8, interferon-gamma, interferon-gamma inducible protein 10, and tumor necrosis factor alpha released due to the cytokine storm have been suggested to cause symptoms of chronic fatigue and myalgia by causing proteolysis in muscle fibers and reducing protein synthesis and disrupting the myogenic process [28-30]. However, since the patients included in the studies were mostly inpatients, it should be considered that prolonged immobilization may cause musculoskeletal symptoms.

This suggests that the musculoskeletal symptoms observed may be due not only to COVID-19 infection but also to immobilization and related complications of prolonged hospitalization.

Previous studies have found that myalgia as a symptom of prolonged COVID is more frequent and severe in patients treated in the intensive care unit [31, 32]. This may be related to both the disease itself and factors such as prolonged hospitalization and immobility in the intensive care unit. However, in our study, myalgia, one of the symptoms of prolonged COVID, was found to be more common in patients treated in the ward compared to patients treated in the ICU. This finding suggests that patients treated in the ward reported myalgia symptoms more commonly. The reason for this is that the number of patients receiving treatment in the intensive care unit in our study was lower than in the ward group.

In the follow-up of patients presenting with prolonged COVID symptoms, it is recommended to perform a complete blood count, biochemical tests, pulmonary function tests, and Thoracic CT and chest radiography [33]. However, CT cannot be easily applied due to its disadvantages, such as additional radiation exposure, inability to use in pregnant women or those who are likely to become pregnant, transferring the patient to the department where CT will be performed, and the need to position the patient. Similar disadvantages apply to chest radiography. The position of the patient is significant for a good chest radiograph. Lung USG is almost equivalent to CT in detecting parenchymal tissue and pleural pathologies [9]. In addition, it is more advantageous than both CT and chest radiography because it is non-invasive, does not emit X-rays, is easily applicable, reproducible and cost-effective, and is portable and wireless. In a study of 212 patients diagnosed with COVID-19, lung USG, and chest radiography were compared, and it was shown that lung USG was more successful than chest radiography in detecting early pulmonary findings [34]. For all these reasons, the use of lung USG in the follow-up or triage of patients is becoming increasingly common.

It is known that lung USG was used effectively during the COVID-19 pandemic, reducing the number of chest radiographs and CT scans. Studies have shown that the use of wireless or bedside lung USG is an effective way to determine the prevalence and severity of lung involvement and to be used in the daily follow-up of patients [35-38].

Limitations

Our study's limitations include its single-center design and the relatively small number of patients in the intensive care unit.

CONCLUSION

This study examined the effectiveness of lung USG in evaluating structural changes in the lungs after COVID-19 and the relationship between these structural changes and prolonged COVID symptoms. The results revealed that B lines detected on lung USG had a significant association with dyspnea, one of the prolonged COVID symptoms. In addition, symptoms such as dyspnea, myalgia, and sweating were found to be associated with each other.

Considering that long-term lung damage continues after COVID-19 and causes permanent respiratory problems in some patients, patients recovering from COVID-19 should receive long-term follow-up and support. Our study shows that lung USG can make essential contributions to the follow-up of post-COVID patients as a non-invasive, reproducible, and portable method in the post-COVID period. In addition, the number of B lines may be an essential biomarker in managing dyspnea, a prolonged COVID symptom. Monitoring B lines with lung USG can be a valuable tool for monitoring treatment efficacy and long-term management of patients.

Authors' Contribution

Study Conception: NTT; Study Design: NTT; Supervision: ONH; Funding: N/A; Materials: NTT; Data Collection and/or Processing: NTT; Statistical Analysis and/or Data Interpretation: NS; Literature Review: İY; Manuscript Preparation: NTT and Critical Review: PH.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

This study was carried out with the support of the

Trakya University Scientific Research Project, numbered 2022/131.

Acknowledgment

We would like to thank patients E. T. and C. B., who gave written informed consent to participate in this study and gave permission for lung ultrasonography to be performed and published.

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The European Research Journal 2024;10(5):501-512 Original Article

DOI: https://doi.org/10.18621/eurj.1527408 *Medical Biology*

Tonsil mesenchymal stem cells-derived exosomes differentially effect the cell proliferation depending on different temperature conditions

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ABSTRACT

Objectives: Exosomes are suggested as cellular components with therapeutic and prognostic potential in various disease therapies and exhibit different secretion profiles under cellular stress. Mesenchymal stem cells (MSCs), which play an important role in regenerative medicine, are particularly rich in exosome release compared to other cell types. In this context, the effect of exosomes obtained from palatine tonsil tissue derived MSCs (T-MSC) under different temperature conditions on cell proliferation were investigated *in vitro* on cancer and healthy cells.

Methods: Exosomes were isolated from MSCs under normal temperature conditions and heat stress, and their concentrations and size distribution were determined by nanoparticle tracking analysis. The effects of exosomes obtained under different conditions on cell proliferation in healthy and cancer cell lines were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, while their effects on cell migration were evaluated by wound healing assay.

Results: Exosomes obtained under normal temperature conditions and heat stress did not exhibit very different properties in terms of size distribution, but particles were obtained at approximately 2 times higher concentrations under normal temperature conditions. They showed different effects in terms of cell proliferation. Exosomes obtained under normal conditions significantly increased cell proliferation in keratinocyte cells depending on the concentration. However, exosomes obtained in cancer cells, especially after heat stress, significantly inhibited cell proliferation.

Conclusions: While exosomes obtained under heat stress come to the forefront in inhibiting cell proliferation in cancer cells, exosomes obtained under normal conditions stand out as effective in wound healing by stimulating increased normal cell proliferation.

Keywords: Tonsil derived mesenchymal stem cell, heat stress, exosomes, cell proliferation

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How to cite this article: Yüce M, Şişli İ, Çiftcioğlu E, et al. Tonsil mesenchymal stem cells-derived exosomes differentially effect the cell proliferation depending on different temperature conditions. Eur Res J. 2024;10(5):501-512. doi: 10.18621/eurj.1527408

Received: August 8, 2024
Accepted: August 23, 2024
Published Online: August 25, 2024

Exosomes, defined as small membrane microvesicles of endosomal origin, have prospered in the latest years as potential therapeutic tools and prognostic biomarkers. Exosomes hold many roles in cellular executions such as intercellular communication via transportation of proteins, lipids, miRNA, mRNA, and DNA; tissue regeneration; immunity responses; signal transduction; antigen presentation. It has been shown with these properties that exosomes hold prognostic and therapeutic potentials regarding chronic inflammation, cardiovascular and renal diseases, neurodegenerative diseases, cancer etc. [1-4]. Exosomes, rich in annexins, tetraspanins (CD63, CD81, CD9) and heat shock proteins (Hsp60, Hsp70), contain low amounts of phosphatidylserine and cell type-specific proteins [5] and exhibit different secretion profile under cellular stress [6]. Oxidative stress alters the RNA contents of exosomes secreted from mast cells of a mouse. The subjection of hypoxia and TNF-a to endothelium cells has caused secreted exosomes to have modulated contents of both mRNA and protein, although high concentrations of glucose or mannose subjected to endothelium cells have no effect on the exosomal profiles of protein or mRNA. These results show the exosomal communication occurs via both protein transfer and RNA. Exosomes are also held responsible for the secretion of heat shock proteins (HSP's) in various cell types including tumor cells, B lymphocytes, astrocytes and endothelium cells. HSP's are found on the surface of exosomes and they hold an important role in the modulation of immunologic responses. Heat shock protein 70 (HSP70) concentration in exosomes derived from peripheral blood mononuclear cells (PBMC) is significantly high [5, 7, 8]. HSP expression in normal cellular conditions increases greatly under the additional conditions of heat shock, toxin exposure, oxidative stress, glucose deficiency, and various other cellular stress factors [9-11].

Mesenchymal stem cells (MSCs) are multipotent cells that hold a crucial role in tissue healing and regenerative medicine due to their ability to differentiate into various cell types and self-renewal. In addition to bone marrow originated MSCs, they can be commercially obtained from various adult and fetal tissues such as fat, muscle, umbilical cord blood, peripheral blood, liver, placenta, skin, amniotic fluid, breast milk, synovial membranes, and dental pulp [12, 13]. MSCs have a significant place in regenerative medicine due to their secretive ability regarding signaling molecules that support tissue regeneration and their low immunogenicity. The therapeutic potentials of MSCs are associated with the paracrine factors they release. They are known to be richer in exosome secretions compared to other cell types. The therapeutic potential of stem cell-derived exosomes has been shown for various disease therapies, can repairing damaged tissues [14]. Exosomes purified from MCSs have been reported to reduce infarct size and mediate cardioprotective paracrine effects in a mouse model of myocardial ischemia/reperfusion injury [15]. It has also been shown that MSC exosomes have positive effects on acute kidney injury [16], induce the proliferation of human and rat hepatocytes *in vitro* [17], and can protect heart tissue from ischemic damage [18].

Although MSC derived exosomes are morphologically and marker expressionally same as other exosomes, they differ with their contents of RNA and proteins. According to various studies, different exosomes of different cellular origins have varying effects of function and generally, MSC exosomes have the potential to alter the capacity of MSC's differentiation and regeneration [1, 19].

It is known that stress conditions and heat stress affect exosome behavior and cause changes in the content of released exosomes. MSC-exosomes obtained from different sources also exhibit different regenerative potential. In this context, the effect of exosomes obtained from palatine tonsil tissue derived MSCs (T-MSC) under normal conditions and heat stress on cell proliferation was investigated *in vitro* on cancer and healthy cells.

METHODS

The tissues used in the study were obtained from patients (age, <15 years; patient signed a consent form) who underwent tonsillectomy at the Samsun Health Sciences University, Training and Research Hospital, the Department of Ear, Nose and Throat Diseases, after being approved by the Ondokuz Mayıs University, Clinical Research Ethics Committee (Ethics committee number: OMÜ KAEK 2022/282; 2023/120).

Enzymatic Isolation of Tonsil Derived Mesenchymal Stem Cells

T-MSCs were obtained by enzymatic isolation with 0.075% collagenase type 1 at 37°C for 30 minutes. Cells were incubated in a humidified incubator at 37°C with 5% CO_2 in high-glucose Dulbecco's Modified Eagle Medium (H-DMEM) supplemented with 10% Fetal Bovine Serum (FBS) as well as 1% antibiotic/antimycotic. After the initial incubation, cells that were unable to adhere to the surface of the culture dish were washed away with PBS, and the adherent cells were cultured under the same conditions as above. When the cell density reached 80-90%, the cells were subcultured by trypsinization with 0.25% trypsin containing 0.02% EDTA. The characterization of T-MSCs was performed at the third passage. T-MSCs at the fifth passage were used for exosome isolation. Approximately 120-150 mL of MSC-conditioned medium was collected for the experiments [20, 21].

Determining of Tonsil Derived Mesenchymal Stem Cells Surface Antigens by Flow Cytometer Analysis

The characterization of third passage T-MSCs by their specific surface antigens have been done with flow cytometer analysis (BD FACSCalibur Cell Analyzer). Following trypsinization, 1×10^5 cells were suspended in 100 µL PBS supplemented with 3% FBS and stained with CD90, CD105, CD73, CD34, and C45 monoclonal antibodies and 4°C for 30 minutes. The cells were then washed with PBS, resuspended in 200 µL of PBS, and analyzed using a flow cytometer [22, 23].

Determination of Tonsil Derived Mesenchymal Stem Cells *in vitro* Adipogenic and Osteogenic Differentiation Potentials

Cells on the third passage were used for determining adipogenic and osteogenic differentiation potentials of T-MSCs. For adipogenic differentiation, a specific media was cultured by adding 10% FBS, 1 µL dexamethasone, 100 µg/mL 3-isobutyl-1 methyxanthine, 5 µg/mL insulin, and 60 µM indomethacin to a basal DMEM growth medium for 3 weeks. Osteogenic differentiation was made possible by the preparation of an osteogenic media made from a basal DMEM growth medium supplemented with 10% FBS 0.1 mM dexamethasone, 10 µM ß-glycerophosphate, and 50 µg/mL ascorbic acid for the duration of three weeks. Culture mediums were changed once every three days. At the end of the third week, T-MSC's were anchored in 4% paraformaldehyde at room temperature for 30 minutes. Following that, T-MSCs were washed with PBS twice; for adipogenic differentiation they were stained with 2% Oil Red O solution in room temperature for 1 hour, and for osteogenic differentiation they were stained with 2% Alizarin Red S solution in room temperature for 15 minutes. The cells were observed under an inverted microscope again after the staining procedure [24, 25].

Obtaining Conditioned Media from Tonsil-Derived Mesenchymal Stem Cells under Normal and Heat Stress Conditions for Exosome Isolation

Media were acquired under normal and heat stress conditions from the cells at their 5th passage. Under normal temperature circumstances, the cells were incubated in a 5% CO_2 environment at 37 oC for 48 hours in order to obtain growth media. After the incubation, the conditioned media was collected and then centrifuged at 1500 rpm for 10 minutes to remove cell debris, followed by filtration through a 0.22 µm sized filter. The cells were washed once with PBS and once with serum-free culture media after the cell concentration reached 80-90% confluence for the obtainment of conditioned media from the heat-stressed T-MSCs. In pursuit of this process, serum-free culture media was added and the cells were then incubated at 43°C for 1 hour in a 5% CO_2 environment for 48 hours. Cell debris was removed after the process of this incubation via centrifugation, and the media was filtered through a 0.22 µm filter. The conditioned media were stored at -80°C until the exosome isolation could proceed.

Isolation of Exosomes of Normal and Heat-Stress Conditions by Sucrose Cushion Method from Conditioned Media

For the isolation of exosomes from T-MSCs media conditioned under normal heat conditions and 43°C temperature have been filtered and centrifuged at 12000 ×g in 4°C for 10 minutes. The media was then put through a 0.22 µm sized filter after collecting the supernatant. The exosomes were released from inside the cells into the media. To purify the mentioned exosomes, sucrose cushion method was used. The collected media was centrifuged at 1000 ×g for 5 minutes

to remove the cell debris. Supernatant was removed again, and the remaining solution was centrifuged at 18000×g for 30 minutes to repel the microvesicle pollution. The 10mL of supernatant was then added into a 1M 1.5 mL sucrose solution with a pasteur pipette to create a phase. The prepared samples were centrifuged at 100000 ×g at 4°C for 1 hour with ultracenrifuge (Beckman CoulterOptima XE100, SW-41 Rotor, USA). After the centrifugation the 1mL of exosome-bearing sucrose solution was pipetted out from the bottom of the test tubes. In order to expel the sucrose molecules from the remaining solution, 100 kDa ultrafiltration colons were used. 13 mL of deionized water was added to a 1 mL of exosome solution for dilution and re-concentration purposes. At each washing process, colons were opened and centrifuged in 3500×g for 10 minutes in rotored centrifuges for re-concentration purposes. After the third process of washing, 200 µL of pure exosome solution was acquired and then kept at -80°C to be used in the later analyses [26, 27].

Nanoparticle Tracking Analysis of Exosomes

Exosomes obtained from T-MSCs cells under normal and heat stress conditions were quantified by nanoparticle tracking analysis. Accordingly, a wavelength of 488 nm laser module from the device Nanosight NS300 was used to minimize the effects of several contaminants such as protein aggregates. Quantification of exosomes was done on 10 video clips each 30 seconds long. Depending on these analyses, both the concentrations and the sizes of exosomes obtained from media were identified [26].

Cell Culture

The cells were cultured with HG-DMEM supplemented with 10% FBS, 2 mM L-glutamine, 1% antibiotic/antimycotic. The cell cultures were incubated at 37°C in a 5% CO_2 atmosphere. After the density of the cells reached 80-90%, they were trypsinized with 0.25% trypsin containing 0.02% EDTA and subcultured for use in subsequent experiments.

Determination of Proliferation of Exosomes of Different Conditions on MIA PaCa-2 and HaCaT Cells via MTT Analysis

After the trypsinization process, 100 µL of growth media that contained 1×10^4 of cell concentration were added to each well of a 96 well culture plate. After 24 hours of incubation, after the cells adhered to the surface, exosomes obtained under different conditions at concentrations of 10^6 particles/mL, 5×10^6 particles/mL, 5×10^7 particles/ml were applied for 24 and 48 hours. The cells were treated with their own culture media as negative control and culture media containing PBS in which the exosomes were suspended was used as vehicle control. At the end of the incubation period, 10 µl MTT solution was added and incubated for 4 hours. At the end of incubation, 100 µL of solvent solution containing 10% SDS in 0.01 M HCl was added to each well and optical density was measured 16 hours later using a microplate reader at 570 nm. Experiments were performed three times for each group. Each concentration was compared against its vehicle control [28].

Determination of the Effects of Exosomes Obtained Under Different Conditions on Cell Migration Using *in vitro* Wound Healing Assay

The wound healing assay is one of the oldest methods developed to study cell migration *in vitro*. It is particularly suitable for studies on the effects of cell matrix and cell-cell interactions on cell migration. This method mimics cell migration. Accordingly, MiaPaCa and HaCaT cells were seeded into a 6 well culture plate at a density of 10×10^5 cells per well, and once they reached 100% confluence, two straight lines were drawn across the well using a 100 µL pipette tip, from 12 o'clock to 6 o'clock and from 9 o'clock to 3 o'clock. Subsequently, the wells were washed several times with PBS to remove cell debris. MSC exosomes were applied at the optimum concentration determined by the MTT assay. After particle application, the cells were incubated for 24 hours in H-DMEM containing 10% FBS. Images of cell migration into the scratch area were captured at 0, 24, and 48-hour intervals using an inverted microscope for each sample. The obtained images were analyzed using ImageJ [29, 30].

Statistical Analysis

The statistical analysis was performed using GraphPad Prism software. The experiments were performed in triplicate and analysis of variance was used to analyse the variance among groups. “2-tailed Student's t-test” was used to determine the significance level. The results were considered statistically significant if the values were $P<0.05$.

RESULTS

Stem Cell Properties of Tonsil Tissue Derived Mesenchymal Stem Cells

Stem cells which were attained from enzymatic isolation from tonsil tissue showed morphologically adherent and fibroblastic properties (Fig. 1a). In order to investigate the multipotent differentiation lineages of T-MSCs, adipogenic and osteogenic differentiation potentials were studied. The formation of lipid droplets and calcium aggregates in T-MSCs was determined by Oil Red O and Alizarin Red S staining after a 3-week induction period. As observed in Figs. 1b and 1c, the cells exhibited a positive staining profile characteristic of MSCs. To evaluate the cell surface antigen phenotypes of T-MSCs, an additional flow cytometry analysis was performed, and it was observed that more than 95% of the cells were positive for the expected MSC markers CD90 and CD73. For the hematopoietic cell surface antigens CD34 and CD45, less than 1% expression was detected (Fig. 1d).

Nanoparticle Tracking Analysis of Exosomes Obtained from T-MSCs under Normal and Heat Stress

Nanoparticle tracking analysis (NTA) was performed to determine the size distribution and average

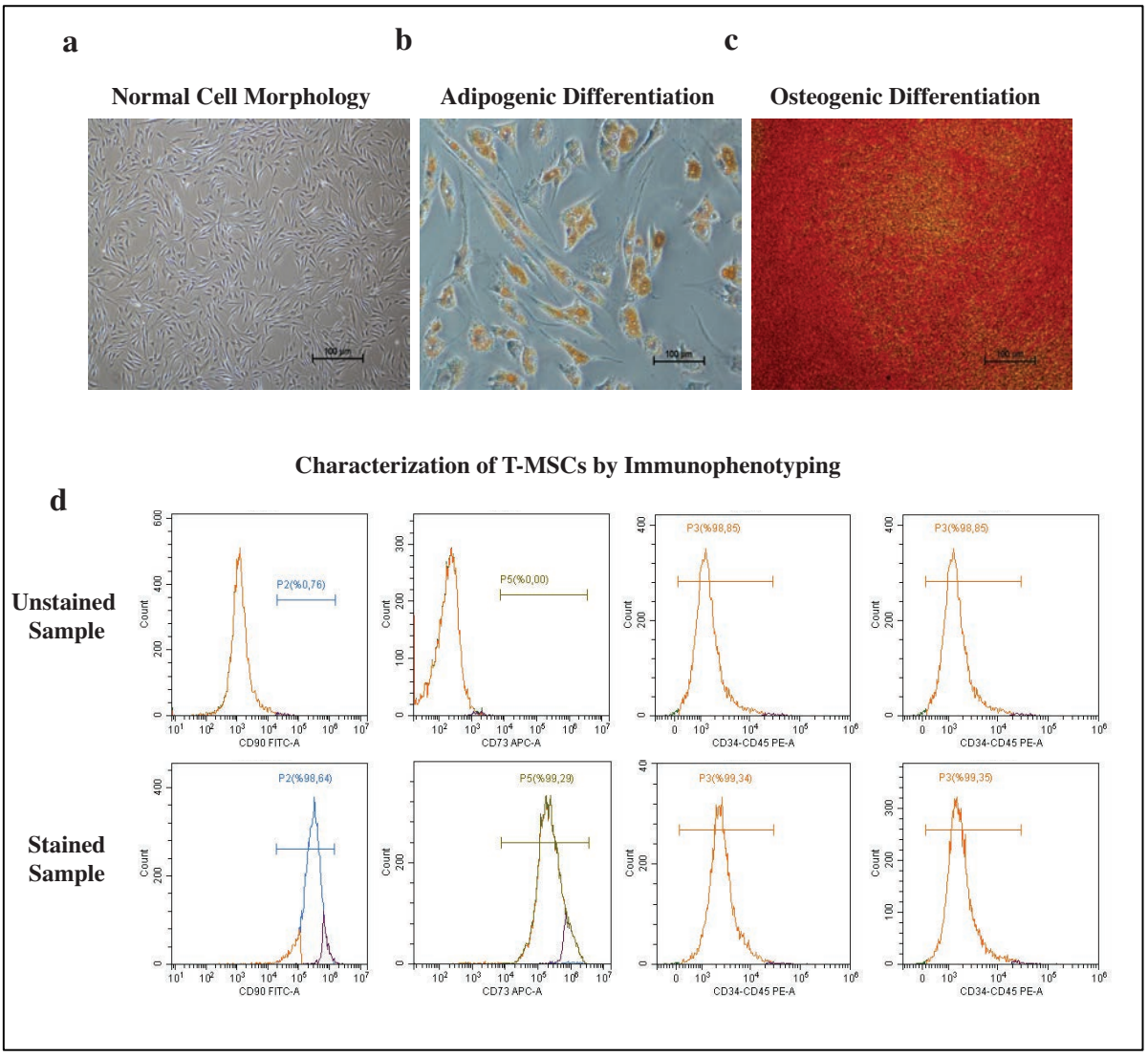

Fig. 1. Characteristic properties of tonsil-derived MSCs. (a) morphology, (b) microscopic images of lipid droplets of adipogenic differentiation potential after staining with Oil Red O, (c) microscopic images of lipid droplets of osteogenic differention potential after staining with Alizarin Red S, and (d) expression of cell surface antigens. PE=Phycoerythrin, APC=Allophycocyanin, FITC=Fluorescein isothiocyanate. Scale bar 100 µm.

size of exosomes obtained from T-MSCs under normal and heat stress. As a result of these analyses, the concentrations and size distributions of exosomes obtained from the medium were determined. Accordingly, the particle concentration of T-MSC exosomes isolated under normal temperature conditions was calculated as approximately 4.28×10^9. This concentration was approximately 2-fold higher compared to exosomes obtained after heat stress. As observed in Fig. 2a, two distinct peaks were observed in the graph, indicating that it contained highly concentrated groups of particles with two different sizes in general, but mostly around 72 nm in size. The peak with the highest concentration has a range around the ~72 nm region. The particle concentration of T-MSC exosomes isolated under heat stress was calculated as $1.98\text{x}10^9$. As observed in Fig. 2b, several peaks were observed in the graph, suggesting that it contains concentrated groups of particles that share more similar particle sizes to each other. The peak with the highest concentration has a range around the ~76 nm region. Afterwards, concentrated exosome groups with sizes of 120 and 162 nm are observed.

Exosomes Obtained under Different Temperature Conditions Induce Different Proliferative Effects in Healthy and Cancer Cells

The effects of T-MSC exosomes obtained under normal conditions and heat stress on cell proliferation were investigated on cancer and healthy cells. The effects of exosomes on cell proliferation on pancreatic cancer cell lines MiaPaCa-2 and immortal keratinocyte cell lines obtained from adult human skin, HaCaT cells, were determined by MTT analysis for

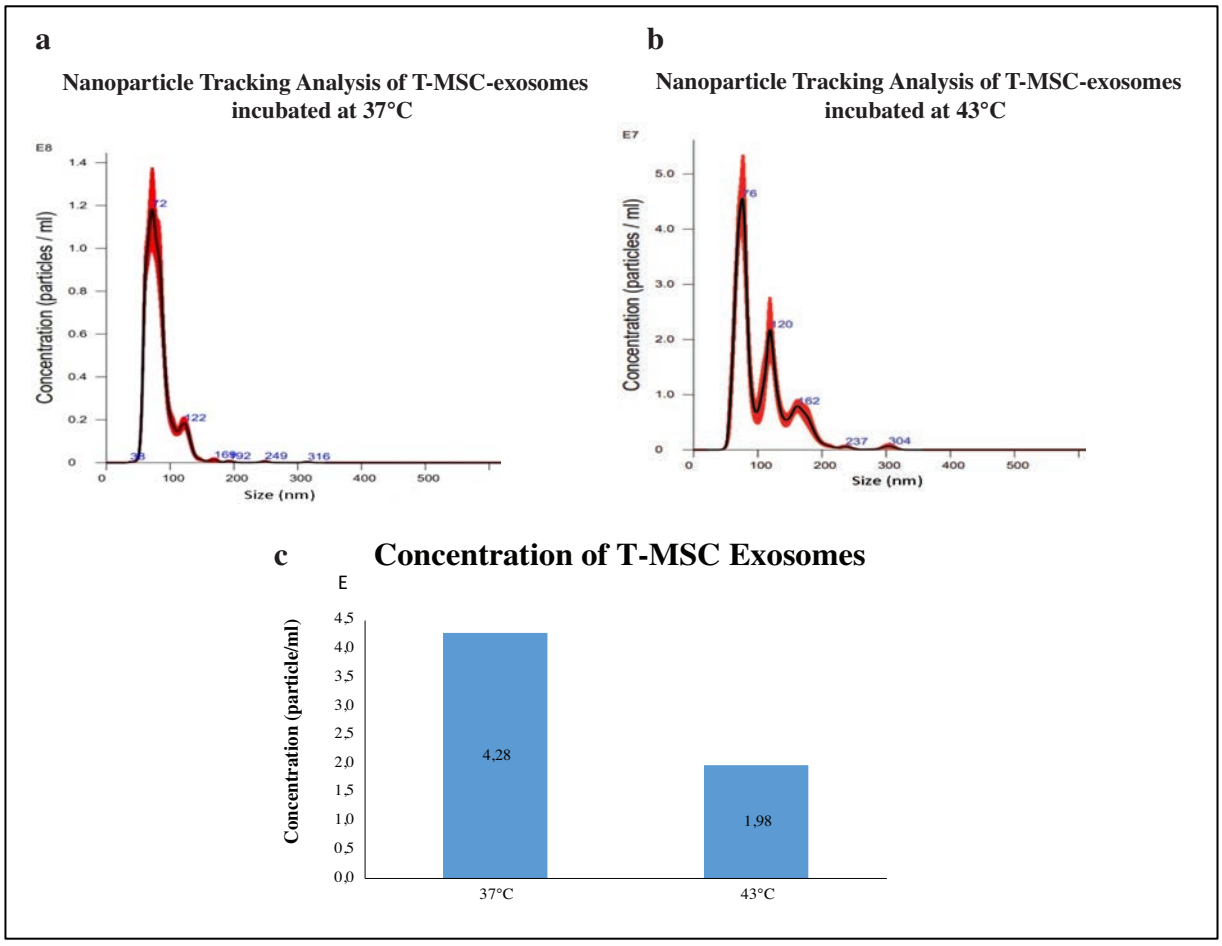

Fig. 2. Nanoparticle tracking analysis profile showing the size distribution of MSC exosomes isolated from tonsil tissue. (a) Nanoparticle tracking analysis graph of exosomes obtained under 37°C temperature, (b) Nanoparticle tracking analysis graph of exosomes obtained under 43°C temperature, and (c) The concentration of T-MSC exosomes obtained under different temperature conditions were also presented.

24 and 48 hours. It was determined that exosomes obtained under different conditions exhibited different proliferative effects on cells. The exosomes obtained at a concentration of 5×10^7 particles/ml under 37°C from the exosomes obtained under two different conditions at concentrations of 106, 5×106 and 5×10^7 particles/ml were found to induce significant cell proliferation in HaCaT cells at the end of 24 and 48 hours of application (27% and 30%, respectively) (***P< 0.001). However, exosomes obtained at the same concentration applied at 43°C stimulated 21% cell proliferation after 48 hours of incubation, but this induction was lower than exosomes obtained at normal temperature conditions (**P<0.01) (Fig. 3a).

The proliferative/non-proliferative effects of exosomes applied at the determined concentrations on MiaPaCa cancer cells showed a different profile than HaCaT cells. It was observed that exosomes obtained under heat stress (43°C) on cancer cells inhibited cell viability by 10% (**P<0.01) (Fig. 3b), but exosomes which were obtained at 37°C and applied at a concentration of 5×10^6 particles/ml for 48 hours caused a decrease in the proliferation of cancer cells by approximately 7% (*P<0.05).

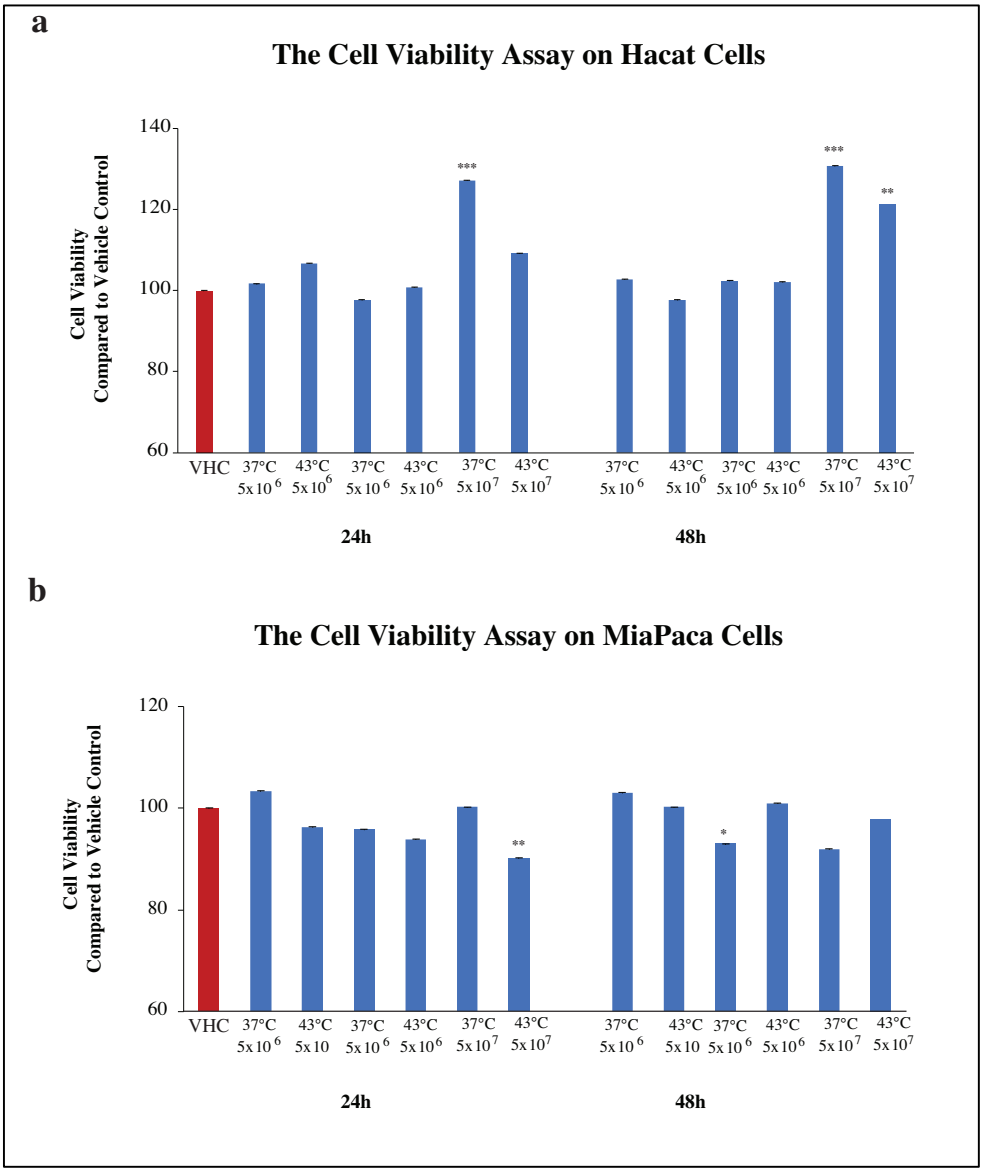

Fig. 3. Effects of T-MSC exosomes obtained under normal conditions and heat stress on healthy (a) and cancer (b) cell proliferation. Each concentration was compared with its own vehicle control. n=3, *P<0.05, **P<0.01, and ***P<0.001.

Exosomes Obtained under Different Conditions Show Different Effects on Cell Migration

After determining the effective concentrations of exosomes obtained under normal and heat stress conditions by MTT assay (5×10^7 particles/mL obtained at 37°C for HaCaT cells and 5×10^7 particles/mL obtained at 43°C for MiaPaCa cells), their effects on cell migration of HaCaT and MiaPaCa cells were determined by wound healing assay. The scratch closure percentages of the groups 24 and 48 hours after treatment were analyzed using ImageJ software. Consistent with the MTT assay, 100% closure was observed in exosomes obtained under normal temperature conditions for HaCaT cells 24 h after treatment (***P<0.001) (Fig. 4a). In cancer cells, exosomes applied at the same concentration obtained under heat stress caused approximately 80% of the scratch closure after 24 hours and 97% at the end of 48 hours (***P<0.001) (Fig. 4b).

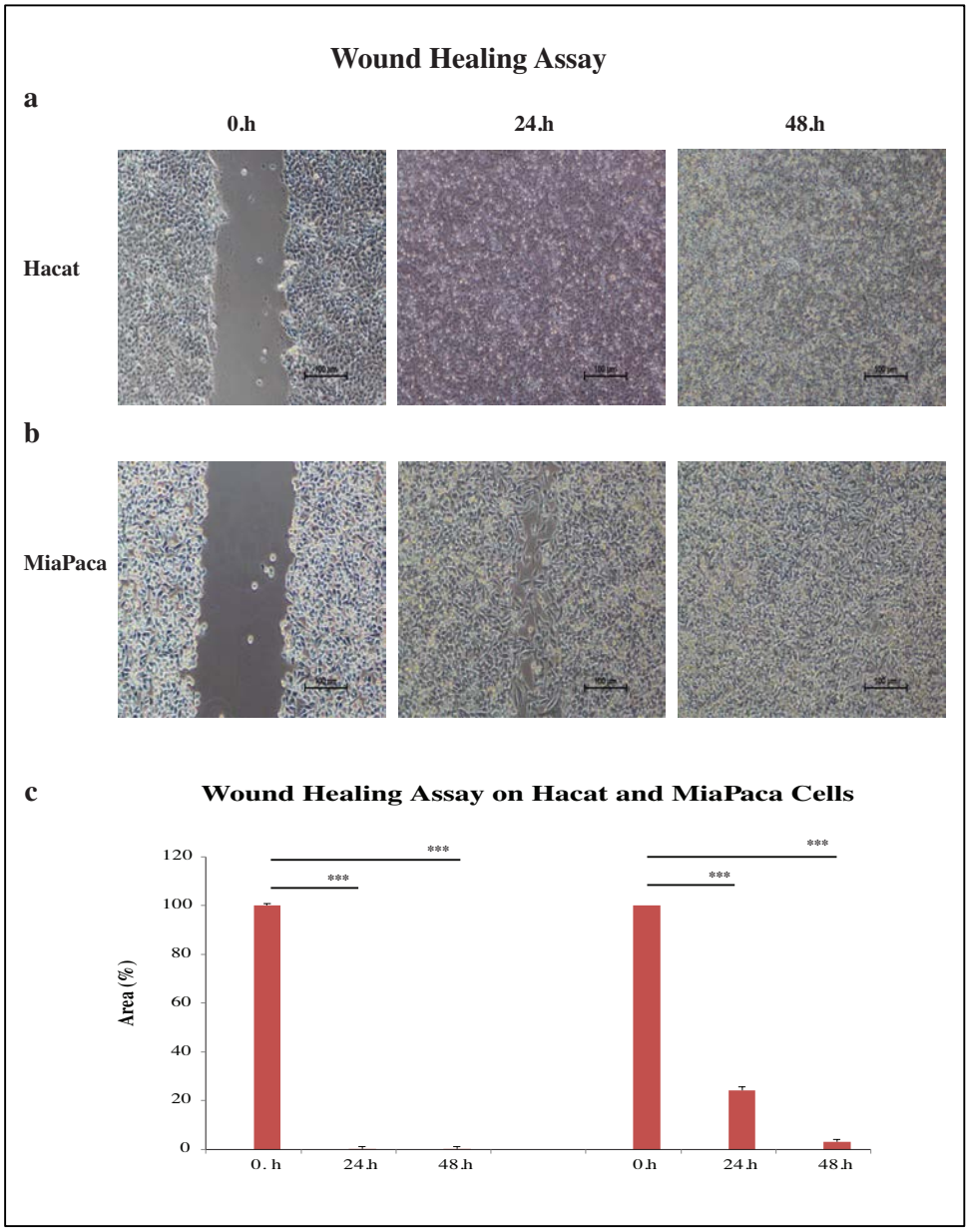

Fig. 4. Effects of T-MSC exosomes obtained under normal conditions on wound healing. (a) Scratch closure micrographs of HaCaT cells at 0, 24 and 48 hours after exosome treatment, (b) Scratch closure micrographs of MiaPaCa cells at 0, 24 and 48 hours after exosome treatment, and (c) Scratch closure rates as %. Scale bar 100 µm. n=3, *P<0.05, **P<0.01, and ***P<0.001.

DISCUSSION

The increasing data of the recent years have highlighted the therapeutic potential of exosomes, which essentially act as mediators of cellular communication [31, 32]. Exosomes derived from stem cells have been reported to have a wide range of therapeutic effects, from healing liver damage [33, 34], to inhibiting cancer cells [35]. The mechanisms by which mesenchymal stem cell exosomes affect wound healing have been explored so far, particularly through the activation of various signaling pathways and the expression of growth factors [29]. The studies have also shown that exosomes can inhibit cancer cells in various types of cancer, depending on different factors [36, 37]. Conversely, some studies suggest that exosomes from different sources can induce the progression of cancerous cells and promote metastasis [38, 39].

It is known that mesenchymal stem cells showcase different secretion profiles depending on the source of the cells which exosomes are derived from [19] and the different stress conditions that they are exposed to [6]. Additionally, the effects of exosomes on cell proliferation and migration can vary depending on the exosomes' source, target cell population, various cellular processes and concentrations [40]. In this context, our study aimed to research the effects of exosomes obtained under different heat conditions on cell proliferation and migration in healthy cells and cancerous cells, and to determine the impact of heat stress on exosome behavior compared to exosomes acquired under normal circumstances. According to our results, exosomes released from tonsil-derived MSCs under normal temperature conditions significantly induced cell proliferation in healthy keratinocyte cells in a concentration-dependent manner. These findings are generally consistent with the literature's information. For instance, bone marrow-derived MSC exosomes were reported to stimulate the proliferation of keratinocyte cell lines and dermal fibroblasts by 54-80% in a concentration-dependent manner after a 3-day application period, with a more pronounced effect in keratinocyte cells [41]. In our study, the cell proliferation was increased by 27-30% in keratinocyte cells. The percentage difference might be related to the duration of application and the different stem cell sources from which the exosomes were obtained. Another study investigating the effects of MSC-derived exosomes from different sources on dermal fibroblast and keratinocyte cell proliferation also reported that exosomes stimulated fibroblast proliferation depending on the MSC source and applied concentration [40]. Interestingly, in our study, exosomes obtained under normal temperature circumstances did not significantly alter the cell proliferation in cancerous cells. The studies have reported both stimulatory and inhibitory effects of exosomes on cancerous cells [36, 38]. In our study, although exosomes at a concentration of 5×10^6 particles/mL had shown some inhibitory profile after 24 hours, it was not statistically significant; but showed a statistically significant inhibition after 48 hours. Higher concentrations of exosomes also inhibited cancer cell proliferation after 48 hours, but this decrease was not statistically significant.

The main focus of our study was to determine the effect of heat stress on exosome behavior. Exosomes obtained after exposure to heat stress did not significantly induce cell proliferation in HaCaT keratinocyte cells compared to exosomes obtained under normal circumstances. But at the highest dose applied, there was a statistically significant increase in cell proliferation after 48 hours of treatment. Importantly, in pancreatic cancer cells (MiaPaCa), exosomes obtained after heat stress inhibited cell proliferation compared to exosomes not subjected to heat stress. Particularly at a concentration of 5×10^7 particles/mL, there was a statistically significant decrease in cell viability after 24 hours of application. The rate of decrease in cell viability slowed as the application duration increased. There are limited studies on the behavior of exosomes derived under heat stress. It has been shown that exosomes released from cancer cells after heat stress inhibit tumor growth by inducing the formation of T helper type 17 (Th17) cells through Heat Shock Protein 70 (HSP70) [42]. Heat shock proteins are known to be crucial in maintaining cellular homeostasis and protecting cells against heat stress. Although hyperthermia does not change the amount of exosomes released from peripheral blood mononuclear cells, it significantly increases the HSP70 content of the exosomes [8]. HSPs are excessively expressed in damaged tissues following injury under the healing process, and delays in their expression can lead to delayed healing in various clinical conditions [8, 43]. HSPs also exhibit anti-apoptotic properties and play a role in protecting tumor cells and maintaining their

survival during malignant progression [44]. In our study, we did not investigate the content of exosomes after heat stress. However, the concentration and size distribution of exosomes were determined using nanoparticle tracking analysis (NTA). Under normal temperature circumstances, approximately 4.28×10^9 particles per mL of exosomes were calculated. The exosomes with the highest concentration had a size range of approximately ~72 nm, followed by the presence of exosomes with a size range of ~122 nm at a much lower concentration. After the application of heat stress, the exosomes with the highest concentration had a size range of approximately ~76 nm, followed by exosomes with size ranges of ~120 and ~162 nm. Approximately 1.98×10^9 particles per mL of exosomes were obtained. The studies have shown that the condition of hyperthermia suppresses the proliferation of cancer cells and also supports exosome secretion [45]. Unlike our study, it was shown that exosomes obtained after hyperthermia in cancer cells were in higher concentration compared to normal heat application [45]. The reason for this difference is thought to be related to the fact that the cells used in our study were not cancer cells but MSCs obtained from tonsil tissue. Indeed, it has also been reported that cellular stress does not significantly affect vesicle size or concentration in healthy endothelial cells [7].

Along with cell proliferation the effect of exosomes obtained under different circumstances on cell migration was also investigated. Consistent with our cell viability results, exosomes obtained under normal conditions rapidly closed the scratch in keratinocyte cells within 24 hours. However, exosomes obtained after heat stress did not cause complete closure of the scratch in pancreatic cancer cells after 24 hours. It is thought that the inhibition of cell proliferation observed in cancer cells after heat stress may be related to the heat shock proteins secreted. To support these findings, it is necessary to comprehensively investigate the biological content of exosomes obtained from stem cells under different heat circumstances especially focusing on heat shock proteins.

Limitations

In the study, determining the effect of exosomes obtained under different temperature conditions on cell proliferation has made an important contribution to the literature. However, there are some limitations of the study, it is important to determine apoptosis-related assays and changes in the gene level, which are indicators of cell death, to support the obtained data more comprehensively. In addition, the study data should be supported by in vivo models.

CONCLUSION

Exosomes obtained under heat stress circumstances are more prominent in inhibiting cell proliferation in cancer cells, while exosomes obtained under normal circumstances are important for stimulating cell proliferation and promoting wound healing. It is necessary to investigate the content of exosomes in more detail according to heat conditions to uncover the underlying mechanisms. The results of this study highlight the importance of the circumstances to which exosomes are exposed in their therapeutic potential. However, these findings need to be supported by further detailed analyses.

Authors' Contribution

Study Conception: MY; Study Design: MY; Supervision: MY, EA; Funding: İŞ, EÇ, MY; Materials: İŞ, EÇ, MY, OKK, PNT, DÖ; Data Collection and/or Processing: MY, EA, OKK; Statistical Analysis and/or Data Interpretation: MY, EA; Literature Review: MY, İŞ; Manuscript Preparation: MY, İŞ, EA, EC, OKK, PNT and Critical Review: MY, EA, PNT, FŞ.

Ethics Approval

Ondokuz Mayıs University, Clinical Research Ethics Committee (Ethics committee number: OMÜ KAEK 2022/282; 2023/120).

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

This work was supported by TUBITAK 2209-A Research Project Support Programme.

Acknowledgement

This work was supported by the scientific and technological research council of Turkey (TUBİTAK) under 2209-A Research Project Support

Programme (Project number; 1919B012112778 and 1919B012217125).

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The European Research Journal 2024;10(5):513-521 Original Article

DOI: https://doi.org/10.18621/eurj.1524168 *Emergency Medicine*

Investigation of warfarin overdose and related factors in the emergency department

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ABSTRACT

Objectives: This study aims to analyze the clinical features, symptoms, laboratory findings, and treatment approaches of patients presenting to the emergency department with elevated INR due to warfarin overdose.

Methods: The study was conducted retrospectively from August 1, 2023, to June 1, 2024, in the emergency department of a tertiary hospital in a city with a population of 5 million. Patients aged 18 and over with an INR value of 3.5 or above were included. Data were obtained from electronic health records and patient files. Statistical analyses were performed using IBM SPSS Statistics.

Results: A total of 121 patients were included in the study. The mean age was 71.85±12.28 years, with 53.7% female and 46.3% male. The most common diagnoses were atrial fibrillation (33.1%) and valve replacement (31.4%). The main reasons for emergency admission included general condition disorder (22.3%) and abdominal pain (16.5%). The bleeding rate was 47.9%, with the gastrointestinal system being the most common bleeding site (49.2%). The mean INR value was 9.27±5.45. Vitamin K was administered to 47.1% of patients and fresh frozen plasma to 28.1%. The discharge rate was 38.8%, and the mortality rate was 2.5%.

Conclusions: Patients presenting to the emergency department with warfarin overdose are at significant risk of severe bleeding, requiring careful management. Close monitoring and accurate dose adjustments are essential, especially in elderly and comorbid patients. Antidotes such as vitamin K, fresh frozen plasma, and prothrombin complex are effective in managing bleeding complications. Future studies should aim to standardize and enhance the effectiveness of these treatment protocols.

Keywords: Warfarin, overdose, emergency department

Warfarin is an anticoagulant drug commonly used to prevent thromboembolic events and inhibit blood clotting [1]. It is frequently preferred in the treatment of cardiovascular diseases such as heart disease, atrial fibrillation, mechanical heart valves, deep vein thrombosis, and stroke prophylaxis [2]. The pharmacological activity of warfarin is achieved by inhibiting the synthesis of vitamin K-dependent clotting factors through the inhibition of the vitamin K epoxide reductase enzyme [3]. However, it has a narrow therapeutic index, making it difficult to effectively determine the correct dose in patients and control blood clots [4].

The International Normalized Ratio (INR) is es-

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How to cite this article: Çınaroğlu OS, Şengül M, Çınaroğlu D, Coşkun SM, Bora ES. Investigation of warfarin overdose and related factors in the emergency department. Eur Res J. 2024;10(5):513-521. doi: 10.18621/eurj.1524168

Received: July 29, 2024
Accepted: August 27, 2024
Published Online: August 27, 2024

sential in controlling the treatment of patients using oral anticoagulants and is used to monitor the appropriate dose [5].

Warfarin treatment is warfarin deficiency, which occurs as a result of incorrect dosing or drug interactions [6]. Overdose can lead to excessive dilution of blood and undesirable bleeding complications [7].

Warfarin admission to the emergency department in the literature Comprehensive studies on the analysis of overdosed patients appear to be limited [8]. Such analyses are of great importance to understanding the clinical characteristics, symptoms, laboratory findings, and treatment approaches of patients presenting to the emergency department. Additionally, such studies can serve as an important reference source for future clinical applications, with the aim of providing patients with more effective and safe treatment.

This study aims to add new data to the literature by presenting the analysis of patients with elevated INR due to warfarin overdose who presented to the emergency department and to provide medical personnel in emergency departments with a better understanding of how to deal with such emergencies. The study aims to provide important information about the etiology, clinical course, complications, and treatment approaches of overdose in patients receiving warfarin treatment. The results indicate that emergency department medical personnel are aware of warfarin. It may contribute to decision-making processes in providing more effective and safe treatment for overdose patients.

METHODS

This retrospective study was conducted in the emergency department of a tertiary hospital in a city with a population of 5 million. The non-invasive research ethics committee of the university with which the hospital is affiliated approved the research on 20.07.2023 and numbered 0389.

Study Population

The research covers August 1, 2023, and June 1, 2024.

Inclusion Criteria

Patients aged 18 years and over whose INR value was determined to be 3.5 or above due to warfarin overdose in the emergency department were included in the study.

Exclusion Criteria

Pregnant women, Patients under 18 years of age were excluded due to developmental differences and the presence of factors that may affect INR values in this age group. We also excluded patients with comorbidities such as severe liver diseases, malignancies, coagulopathy and other bleeding tendencies that may cause elevated INR values, in order to be able to attribute the increase in INR to warfarin use alone.

We also included in the exclusion criteria patients taking medications such as antiplatelet or NSAIDs that may alter INR levels in combination with warfarin. Finally, we excluded patients with missing demographic and laboratory data to maintain the accuracy and reliability of the analyses.

Although the upper limit value of INR is 25, higher values are labelled as ‘High’ in the result section. Therefore, we considered patients with INR values above 25 as missing data and excluded them from the study for the sake of clear statistical analysis.

Data Collection

Data were collected through electronic hospital health records and patient files. The data collected includes sociodemographic characteristics of the patients, such as age, gender, and existing diseases. In addition, clinical data such as the patient's complaint at the emergency department, bleeding status, treatment given, and outcome were also noted. Warfarin Hemogram, coagulation tests, and biochemistry tests were used in the evaluation of patients with overdose. In addition, the patients were examined to determine whether they were given vitamin K, fresh frozen plasma (FFP), or prothrombin complex. Discharge, hospitalization, and mortality were also analyzed. The collected data were subjected to statistical analysis and warfarin. The etiology, clinical course, complications, and treatment approaches of overdose were examined.

Statistical Analysis

Data IBM SPSS Statistics standard It was evaluated in the statistical package program Concurrent User V 26 (IBM Corp., Armonk, New York, USA). Descriptive statistics were given as number of units

(n), percentage (%), mean ± standard deviation, median (M), minimum (min) and maximum (max) values. Normal distribution of data of numerical variables Shapiro The homogeneity of variances was evaluated with the Wilk normality test and the Levene test. The characteristics of the patients in the three groups were compared with One-Way Analysis of Variance when the data met the normal distribution conditions and with the Kruskal Wallis H test when the data did not meet the normal distribution conditions. When significant results were obtained in groups according to One-Way Analysis of Variance results, post hoc comparisons were evaluated with the Tukey test, and when significant results were obtained in groups according to Kruskal Wallis H results, post hoc comparisons were evaluated with the Bonferroni-Dunn test. Pearson and Fisher's exact tests were used to compare categorical variables with each other. In Cofact evaluations, INR values were evaluated using ROC analysis. A P-value of <0.05 was considered statistically significant.

RESULTS

The average age of the patients is 71.85±12.28 years; the median age is 72, the minimum is 23, and the maximum is 100. Regarding gender distribution, 53.7% are females and 46.3% are males. According to the diagnosis distribution, 33.1% of the patients presented with atrial fibrillation, 31.4% with valve replacement, 14.0% with coronary artery disease, 11.6% with cerebrovascular disease, and 9.9% with other diagnoses. (Table 1). Among the reasons for emergency admission: dyspnea at 11.6%, poor general condition at 22.3%, hematochezia at 7.4%, hematuria at 7.4%, INR control at 13.2%, abdominal pain at 16.5%, Melena is 9.1%, and other causes are 12.4%. While the rate of patients without bleeding detected in the emergency department was 52.1%, bleeding was detected in 47.9%. Bleeding sites were reported as a gastrointestinal system at 49.2%, genitourinary system at 16.9%, intracranial bleeding at 13.6%, and hematoma at 20.3% (Table 1).

The average hemoglobin value is 9.70±2.82 g/dL; the median value is 10.2 g dL, minimum 3.4, maximum 16.9. The mean hematocrit was 29.39±8.15%, the median value was 30.8%, the minimum was

Table 1. Descriptive characteristics of patients (n=121)

Variables	Data
Age (years)	71.85±12.28 72 (23-100)
Gender, n (%)	
Female	65 (53.7)
Male	56 (46.3)
Diagnosis, n (%)	
Atrial fibrillation	40 (33.1)
Valve replacement	38 (31.4)
Coronary artery disease	17 (14.0)
Cerebrovascular diseases	14 (11.6)
Other	12 (9.9)
Emergency application, n (%)	
Dyspnea	14 (11.6)
General condition disorder	27 (22.3)
Hematochezia	9 (7.4)
Hematuria	9 (7.4)
INR control	16 (13.2)
Stomach ache	20 (16.5)
Melena	11 (9.1)
Other	15 (12.4)
Bleeding in the emergency department, n (%)	
Not Detected	63 (52.1)
Detected	58 (47.9)
Bleeding site, n (%)	
Gastrointestinal system	29 (49.2)
Genitourinary system	10 (16.9)
Intracranial hemorrhage	8 (13.6)
Hematoma	12 (20.3)
Hemoglobin (g/dL)	9.70±2.82 10.2 (3.4-16.9)
Hematocrit (%)	29.39±8.15 30.8 (10.7-48.4)
Platelets ($\times 10^9$/L)	254.23±102.89 245 (16.3-575)
INR	9.27±5.45 7.62 (1.08-24.73)
Prothrombin time (seconds)	106.98±64.57 89.3 (12.7-299.1)
Vitamin K, n (%)	
Not given	64 (52.9)
Granted	57 (47.1)
Contact, n (%)	
Not given	97 (80.2)
Granted	24 (19.8)
TDP, n (%)	
Not given	87 (71.9)
Granted	34 (28.1)
Unit, n (%)	
Discharge	47 (38.8)
Service	37 (30.6)
Intensive care	37 (30.6)
Outcome, n (%)	
Alive	118 (97.5)
Ex	3 (2.5)

Data are shown as mean±standard deviation and median (minimum-maximum or n (%)

Table 2. Comparison of patient identifier values by groups

Variables	Group			Test Statistics	
	Discharge	Service	Intensive care	Test value	P value
Age (years)	72.34±12.34 74 (34-92)	70.08±9.66 70 (45-87)	73.0±13.44 74 (23-100)	H=2.311	0.315
Gender, n (%)					
Female	23 (48.9)	18 (48.6)	24 (64.9)	χ^2=2.664	0.264
Male	24 (51.1)	19 (51.4)	13 (35.1)		
Diagnosis, n (%)					
Atrial fibrillation	14 (29.8)	13 (35.1)	13 (35.1)	χ^2=12.071	0.137*
Valve replacement	19 (40.4)	9 (24.3)	10 (27.0)		
Coronary artery disease	3 (6.4)	4 (10.8)	10 (27.0)		
Cerebrovascular disease.	7 (14.9)	5 (13.5)	2 (5.4)		
Other	4 (8.5)	6 (16.2)	2 (5.4)		
Emergency application, n (%)					
Dyspnea	7 (14.9)	3 (8.1)	4 (10.8)	χ^2=21.579	0.072*
General condition is bad	5 (10.6)	10 (27.0)	12 (32.4)		
Hematochezia	2 (4,3)	2 (5.4)	5 (13.5)		
Hematuria	5 (10.6)	2 (5.4)	2 (5.4)		
INR control	11 (23.4)	3 (8.1)	2 (5.4)		
Stomach ache	8 (17.0)	9 (24.3)	3 (8.1)		
Melena	2 (4.3)	3 (8.1)	6 (16.2)		
Other	7 (14.9)	5 (13.5)	3 (8.1)		
Bleeding, n (%)					
Not Detected	36 (76.6)[a]	16 (43.2)[b]	11 (29.7)[b]	χ^2=19.882	**<0.001**
Detected	11 (23.4)[a]	21 (56.8)[b]	26 (70.3)[b]		
Bleeding site, n (%)					
Gastrointestinal system	2 (18,2)[a]	12 (54.5)[a]	15 (57.7)	χ^2=15.386	**0.017***
Genitourinary system	5 (45.5)[a]	3 (13, 6)[ab]	2 (7.7)[b]		
Intracranial hemorrhage	0 (0,0)[a]	2 (9.1)[a]	6 (23.1)[a]		
Hematoma	4 (36.4)[a]	5 (22.7)[a]	3 (11.5)[a]		
Hemoglobin (g/dL)	10.45± 2.37 [a] 10.8 (4.3-15.6)	10.14± 2.52 [a] 10.9 (5.3-16.2)	8.30± [3.14b] 7.4 (3.4-16.9)	F = 7.441	**<0.001**
Hematocrit (%)	31.50± 6.58[a] 32.1 (13.3-44.1)	30.71± 7.42[b] 32.3 (16.3-45.2)	25.38± 9.32[b] 22.6 (10.7-37.7)	F = 7.203	**0.001**
Neutrophil (×10^9/L)	8.25±6.88 7.08 (0.71-42.7)	9.07±5.25 7.61 (2.6-24.4)	9.54±5.48 8.41 (1.8-24.65)	H =2.759	0.252
Lymphocyte (×10^9/L)	1.30±0.71 1.14 (0.29-3.98)	1.45±0.71 1.38 (0.28-2.8)	1.37±0.65 1.3 (0.43-3.48)	H =1.328	0.515
Platelets (×10^9/L)	254.93±107.14 229 (45-548)	261.84±86.77 257 (16.3-494)	245.72±113.84 248 (34-575)	F =0.226	0.798
WBC (×10^9/L)	10.47±7.63 9.26 (2.0-50.19)	11.62±5.41 10.33 (5.2-27)	11.81±5.66 10.98 (3.7-27.6)	H =3.533	0.171
INR	7.97±5.18 7 (1.08-24.73)	9.39±5.15 8.23 (1.1-21.7)	10.81±5.79 9.5 (1.2-23.1)	H=5.921	0.052
Prothrombin time (seconds)	94.57±62.66 82.9 (12.8-299)	109.67±58.82 98.4 (13-227)	120.06±70.98 99.5 (14-252.8)	H=3.494	0.174
Vitamin K, n (%)					
Not given	25 (53.2)	19 (51.4)	20 (54.1)	χ^2=0.057	0.972
Granted	22 (46.8)	18 (48.6)	17 (45.9)		
Contact, n (%)					
Not given	41 (87.2)	29 (78.4)	27 (73.0)	χ^2=2.755	0.295
Granted	6 (12.8)	8 (21.6)	10 (27.0)		
TDP, n (%)					
Not given	38 (80.9)	29 (78.4)[E.U.]	20 (54.1)[b]	χ^2 =8.465	**0.015**
Granted	9 (19.1) [a]	8 (21.6) [ab]	17 (45, 9) [b]		
Outcome, n (%)					
Alive	47 (100.0)	37 (100.0)	34 (91.9)	χ^2=4.718	0.054*
Ex	0 (0.0)	0 (0.0)	3 (8.1)		

Data are shown as mean±standard deviation and median (minimum-maximum or n (%).
χ^2=Chi-square test, F=One-way analysis of variance, H=Kruskal Wallis test,
*Significance value obtained by Exact method

Table 3. ROC analysis by INR value cofact categories

	Area Under the Curve (AUC)	if	P value	Area Under the Curve (AUC) 95% Confidence Limits		Sensitivity	Selectivity	Limits
				lower limit	upper limit			
INR	0.632	0.071	**0.046**	0.539	0.718	41.67	86.60	>14.84

SE=standard error

10.7%, and the maximum was 48.4%. The mean neutrophil is 8.90±5.98 $\times 10^9$/L, the median value is 7.73 $\times 10^9$/L, minimum 0.71, maximum 42.67. The lymphocyte mean was 1.37±0.69 $\times 10^9$/L, and the median value was 1.28 $\times 10^9$/L, minimum 0.28, maximum 3.98. The mean platelet count is 254.23±102.89 $\times 10^9$/L, and the median value is 245 $\times 10^9$/L, minimum 16.3, maximum 575. White blood cell mean 11.23±6.41 $\times 10^9$/L, the median value is 10.15 $\times 10^9$/L, minimum 2, maximum 50.19. The mean INR is 9.27±5.45, the median value is 7.62, the minimum is 1.08, the maximum is 24.73. The mean prothrombin time was 106.98±64.57 seconds, the median value was 89.3 seconds, minimum 12.7, maximum 299.1. Vitamin K treatment was not applied in 52.9% and was applied in 47.1%. Cofact treatment was not applied to 80.2%, but was applied to 19.8%. Fresh frozen plasma was not applied to 71.9% but was applied to 28.1%. The discharge rate of patients was reported as 38.8%, ward admission as 30.6%, and intensive care admission as 30.6%. According to outcome status, 97.5% of the patients survived, and 2.5% died (Table 1).

Table 2 shows the comparison of patient identifier values by groups. The average age was 72.34±12.34 in the discharged group, 70.08±9.66 in the ward group, and 73.0±13.44 in the intensive care group (P=0.315).

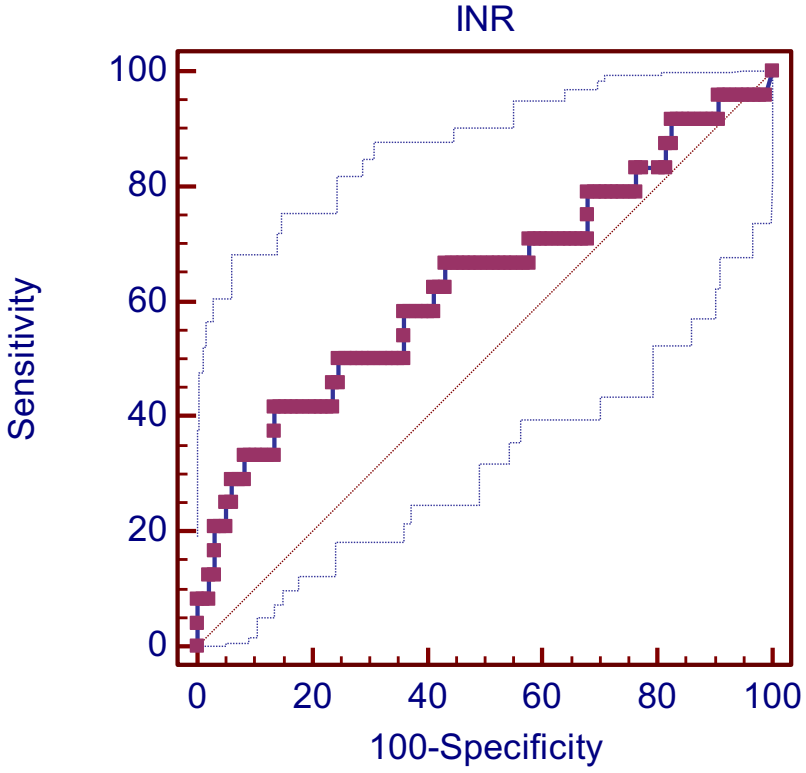

Fig. 1. ROC chart according to INR value cofact categories.

Gender distribution was 48.9% female and 51.1% male in the discharge group, 48.6% female and 51.4% male in the ward group, and 64.9% female and 35.1% male in the intensive care group (P=0.264). There is no significant difference in the diagnosis distribution (P=0.137), and the reasons for emergency admission do not show a similarly significant difference (P=0.072). The presence of bleeding was observed at 23.4% in the discharge group, 56.8% in the ward group, and 70.3% in the intensive care group (P<0.001). Regarding bleeding site distribution, gastrointestinal bleeding was seen as 18.2% in the discharge group, 54.5% in the ward group, and 57.7% in the intensive care group (P=0.017). The mean hemoglobin was 10.45±2.37 in the discharge group, 10.14±2.52 in the ward group, and 8.30±3.14 in the intensive care group (P<0.001). Hematocrit values were recorded as 31.50±6.58 in the discharge group, 30.71±7.42 in the ward group, and 25.38±9.32 in the intensive care group (P=0.001). Neutrophil, lymphocyte, platelet, white blood cell (WBC), INR, and Prothrombin times values do not show any significant difference between the groups. Vitamin K and Cofact administration rates are similar but do not show a significant difference. The FFP administration rate was 19.1% in the discharge group, 21.6% in the ward group, and 45.9% in the intensive care group (P=0.015). In the outcome data, no deaths were observed in the discharge and service groups, but a death rate of 8.1% was observed in the intensive care group (P=0.054).

The Cofact variable, the area under the curve value at INR value, is statistically significant (P=0.046) (0.632 (0.539-0.718)). The differential diagnosis value for INR is over 14.84. In the area under the curve analysis we conducted for Cofact, it was statistically determined that the INR value is a differential diagnosis. In addition, this value, being over 14.84, is an important indicator in the selection of patients. (Table 3, Fig. 1.)

DISCUSSION

This study aimed to examine the clinical features, symptoms, laboratory findings, and treatment approaches of patients with elevated INR due to warfarin overdose who presented to the emergency department.

The average age of the patients in the study was 71.85±12.28 years. This age group generally consists of individuals with chronic diseases requiring multidisciplinary treatment, which increases the difficulties and risks of complications of warfarin treatment [9]. Elderly patients are at higher risk of warfarin therapy, often due to polypharmacy and comorbidities [10].

Among the reasons for patients' admission to the emergency department, general condition disorder (22.3%) stands out. This finding suggests the possibility of the general deterioration of the sections due to warfarin overdose, and the possibility that patients who come to the emergency department with general condition disorder and who are on warfarin should also be evaluated in terms of overdose [11].

The reasons for admission of the patients included in the study included symptoms such as hematochezia (7.4%), hematuria (7.4%), melena (9.1%), and abdominal pain (16.5%), indicating that warfarin overdose may affect various organ systems. Moreover, it may present with different clinical presentations [12]. Similarly, in a study by Fihn *et al*. [13], warfarin administration in elderly patients is associated with a reduced likelihood of experiencing bleeding complications compared to younger patients, with no notable disparity in the severity of such complications between the two age groups.

The rate of patients with bleeding was 47.9%, and the gastrointestinal system (49.2%) has an essential place among the bleeding sites. Both the widespread use of NSAIDs in society and the frequent occurrence of gastroenterological disorders due to eating habits may have paved the way for this situation. In a study of Zapata *et al*. [14], concomitant use of warfarin and an NSAID or COX-2 inhibitor significantly augments the risk of bleeding, underscoring the importance of exercising caution when combining these drugs.

The fact that the average hemoglobin of the patients was 9.70±2.82 g/dL and the average hematocrit was 29.39±8.15% suggests severe bleeding that may develop due to warfarin overdose. These low values may indicate patients experiencing acute blood loss and developing anemia. This situation requires close monitoring and rapid intervention. Regular monitoring of hemoglobin and hematocrit levels may be necessary in these patients. Depending on the clinical course, blood transfusion and hematological support treatments may be applied in a study by Leonard *et al*.

[15]. Within the group of individuals receiving warfarin treatment, the utilization of fibrates raises the likelihood of being admitted to the hospital due to gastrointestinal bleeding or intracranial hemorrhage. On the other hand, there is no connection between the use of statins and these adverse events [16].

Antidotes such as vitamin K, fresh frozen plasma (TDP), and prothrombin complex (Cofact) are used to treat patients with warfarin overdose. In this study, 47.1% of patients were treated with vitamin K, 19.8% with COFACT (4% coagulation factor concentrate), and 28.1% with TDP. These treatment approaches are important for rapidly decreasing INR values and controlling bleeding complications. However, differences in the application rates of these treatment modalities may indicate a need for more standardization in clinical decision-making processes [17, 18].

Demographic, clinical, and laboratory characteristics of the patients participating in the study were compared according to different clinical outcome groups such as discharge, ward admission, and intensive care admission. The rate of patients who applied to the emergency department and were found to have bleeding was 23.4% in the discharge group, 56.8% in the service group, and 70.3% in the intensive care group (P<0.001). This finding highlights the significant impact of bleeding complications due to warfarin overdose on clinical outcomes, similar to the Güven *et al*. [18] study. The fact that most patients with bleeding require intensive care admission reveals the seriousness of this situation and the need for urgent intervention.

Gastrointestinal system (GIS) bleeding was observed at 18.2% in the discharge group, 54.5% in the service group, and 57.7% in the intensive care group. The rate of intracranial bleeding was 0% in the discharge group, 9.1% in the service group, and 23.1% in the intensive care group. These findings show that gastrointestinal and intracranial bleeding are essential factors that increase the need for intensive care and affect patient mortality [19].

The mean hemoglobin of patients in the discharge group was determined as 10.45±2.37 g/dL, 10.14±2.52 g/ dL in patients in the ward group, and 8.30±3.14 g/dL in patients in the intensive care group (P<0.001). These low hemoglobin levels reveal that patients in the intensive care group experienced profound blood loss. Anemia and severe blood loss indicate that these patients require further hematological support therapy and close monitoring. These results are in line with other studies [20].

FFP was given at 19.1% of patients in the discharge group, 21.6% in the ward group, and 45.9% in the intensive care group (P=0.015). This finding shows that patients in the intensive care group need more FFP in cases of severe bleeding. Of TDP, It is clear that warfarin plays a critical role in the management of bleeding complications due to overdose. On the other hand, no significant difference was observed in patients given PCC and vitamin K. The scope and sample size of the study prevented the emergence of a statistically significant difference in patients given prothrombin complex concentrates (PCC) and vitamin K. Studies that are larger and include different patient groups may provide more precise results about the effectiveness of these treatments [21, 22].

Mortality was observed in 8.1% of the patient group admitted to intensive care in the study. No mortality was observed in patients admitted to the ward (P=0.054). This finding indicates that severe complications due to warfarin overdose carry a high risk of mortality, especially in patients requiring intensive care. This highlights the need for careful management of warfarin therapy and tighter follow-up and monitoring to prevent overdose. These results are in line with the literature [23, 24].

Limitations

This study has a retrospective design. The fact that the study was conducted in a single center may limit the generalizability of the findings. Additionally, the limited sample size reduced statistical power in some subgroup analyses.

CONCLUSION

This study shows that patients presenting to the emergency department with warfarin overdose are at risk of severe bleeding and require careful management. It is essential to closely monitor warfarin treatment and make accurate dose adjustments, especially in elderly and comorbid patients. The use of vitamin K, fresh frozen plasma, and prothrombin complex offers a practical treatment approach in the management of complications related to warfarin overdose. Future

studies require more extensive studies to increase the standardization and effectiveness of these treatment protocols.

Authors' Contribution

Study Conception: OSÇ; Study Design: MŞ, SMC; Supervision: DÇ, ESB; Funding: DÇ, SMC; Materials: MŞ, ESB; Data Collection and/or Processing: OSÇ; Statistical Analysis and/or Data Interpretation: MŞ, MSC; Literature Review: ESB, DÇ; Manuscript Preparation: OSÇ and Critical Review: DÇ, MŞ.

Ethics Approval

This study was approved by the Izmir Katip Çelebi University Non-Interventional Clinical Research Ethics Committee (Decision no.: 0337, Date: 20.07.2023).

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2024;10(5):522-532 Original Article

DOI: https://doi.org/10.18621/eurj.1518743 *Bioengineering*

Impact of internal phase volume on the physical, morphological and mechanical characteristics of emulsion templated scaffolds

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ABSTRACT

Objectives: The high porosity of tissue engineering scaffolds is advantageous as they provide a high degree of infiltration of nutrients, enable cell penetration, and support vascularisation. However, the mechanical strength is also critical for providing structural support to the defect site throughout the regeneration process. In this study, we aimed to establish a relationship between internal phase volume and emulsion-templated scaffolds' physical, morphological and mechanical characteristics.

Methods: In this work, tetra methacrylate functionalised polycaprolactone (4PCLMA) polymers were synthesised via ring-opening polymerisation followed by methacrylation. 4PCLMA-based emulsion templated matrices with 60%, 75% and 82% internal phase volumes were fabricated (P60, P75, and P82). These scaffolds' densities, porosities, average pore and window sizes, degree of interconnectivity values, and mechanical properties were investigated.

Results: Increasing internal phase volume reduced the density of the foams by almost two-fold. No direct correlation was observed between average pore size and internal phase volume. Both the average window sizes and the degree of interconnectivity values increase with increasing internal phase volume. Compression modulus values are calculated as 0.46±0.04 MPa, 0.23±0.02 MPa and 0.14±0.01 MPa for P60, P75, and P82, respectively. Increasing internal phase volume from 60% to 82% caused a more than 2-fold reduction in the stiffness of the emulsion-templated matrices.

Conclusions: Accordingly, by reporting on this experimental framework, we established a relationship between internal phase volume and the physical, morphological and mechanical characteristics of 4PCMA-based scaffolds to precisely engineer these characteristics for specific tissue engineering applications.

Keywords: Biomaterials, emulsion templating, porosity, tissue engineering, mechanical characterisation

Polymer foams are cellular polymeric materials that have a wide range of applications, such as thermal energy storage [1], sound insulation [2], filtration and separation, packaging, battery applications [3], environmental applications [4-6] and biomedical applications [7-10]. In the biomedical field, one of the most popular applications of polymeric foams is their use as 3D cell culture platforms or tissue engineering scaffolds [11, 12]. Tissue engineering is a multidisciplinary field that combines the principles of

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How to cite this article: Aldemir Dikici B. Impact of internal phase volume on the physical, morphological and mechanical characteristics of emulsion templated scaffolds. Eur Res J. 2024;10(5):522-532. doi: 10.18621/eurj.1518743

Received: July 18, 2024
Accepted: August 23, 2024
Published Online: August 27, 2024

biology and engineering and looks for solutions for the regeneration of tissues [13]. In scaffold-based tissue engineering, 3D porous matrices (scaffolds) are implanted into the defect site and provide a surface for cell attachment, proliferation and mechanical support until the tissue is regenerated. There are various scaffold fabrication techniques, such as electrospinning, decellularisation [14], freeze-drying [15], porogen leaching [16, 17], and 3D printing [18].

Emulsion templating is another porous polymer fabrication route with a vast number of advantages [2, 19-22]. In this technique, an emulsion is created using two immiscible liquids where the droplets are defined as the internal phase, and the liquid that covers the internal phase is defined as the continuous phase. Polymers/polymer solutions form the continuous phase, and depending on the polymer properties, both water-in-oil (w/o) and oil-in-water (o/w) emulsions can be used. Surfactants [23-26] or Pickering particles [27] are used to provide emulsion stability by reducing the surface tension and providing a mechanical barrier between the two phases, respectively. Depending on the nature of the polymer, the continuous phase of the emulsion is polymerised either by thermal or photo polymerisation or solvent evaporation. Once the polymer is solidified, water droplets in the internal phase are removed, and the porous matrices are obtained (Fig. 1) [7].

If the volume of the internal phase in the emulsion is more than 74%, these emulsions are classified as High Internal Phase Emulsions (HIPE). Emulsions with an internal phase volume of 30-74% are defined as Medium Internal Phase Emulsions (MIPE). The matrices obtained with their polymerisation are named PolyHIPEs and PolyMIPEs, respectively [28]. Porous materials can have open cellular and closed cellular morphologies. Open cellular structures are characterised by the presence of windows that connect neighbouring pores to each other (Fig. 2). PolyHIPEs and PolyMIPEs generally exhibit open-cellular structures due to the high packing density of the water droplets. Polymer film between droplets gets thin and ruptures during polymerisation, resulting in the formation of windows [19].

In the design of tissue engineering scaffolds, high porosity with high interconnectivity (open cellular architecture) is desired to provide a high degree of infiltration of nutrients, enable cell penetration, and support vascularisation. However, the mechanical strength of a material is also critical for providing structural support to the defect site throughout the regeneration process [29].

One other advantage of emulsion-templated matrices is the high tunability of their morphology and, correspondingly, their mechanical properties [7, 24, 30]. Both emulsion formulation and the process parameters, such as temperature and stirring speed [31], have a direct impact on the morphological characteristics of the scaffolds. Oil and water phase components [32, 33], their amounts, and the relative ratios of their sub-components [23] have a direct impact on architectural features and mechanical properties. As the internal phase volume defines the porosity of the emulsion-templated matrices, scaffolds with a high degree of porosity could be easily obtained simply by increasing the water content of the emulsion.

This study aims to investigate the impact of internal phase volume on the physical, morphological, and mechanical characteristics of photocurable polycaprolactone (PCL) based emulsion templated scaffolds.

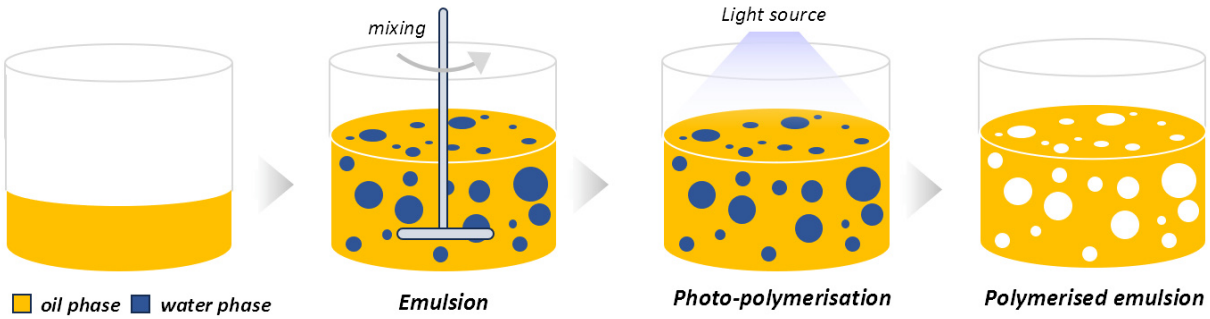

Fig. 1. Fabrication steps of emulsion templated matrices.

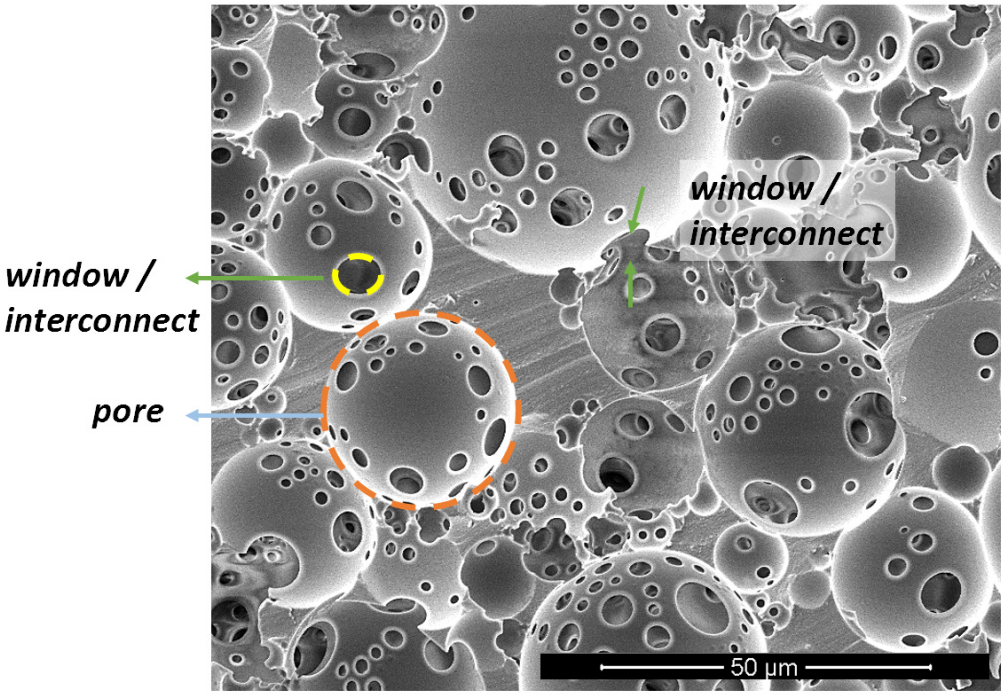

Fig. 2. Scanning electron microscope micrograph of a scaffold fabricated via emulsion templating (an orange circle and blue arrow indicate pores, and a yellow circle or green arrow indicates windows/interconnects).

PCL is a biocompatible and bioresorbable polymer and one of the most advantageous biomaterials for use in the biomedical field [34]. Accordingly, in this work, tetra methacrylate functionalised polycaprolactone (4PCLMA) polymers were synthesised via ring-opening polymerisation followed by methacrylation. 4PCLMA-based emulsion templated matrices with internal phase volumes of 60%, 75% and 82% were fabricated. Densities, porosities, morphologies, average pore and window sizes, pore and window size distributions, degree of interconnectivity values and mechanical properties of these scaffolds were investigated, and the changes in these parameters in relation to internal phase volume were discussed.

METHODS

Polyglycerol polyricinoleate (PGPR) was used as a surfactant in the emulsion composition and was kindly donated by Paalsgard (Juelsminde, Denmark). Ɛ-caprolactone, pentaerythritol, tin (II) 2-ethylhexanoate, dichloromethane (DCM), and triethylamine (TEA), methacrylic anhydride (MAAn), hydrochloric acid (HCl), photoinitiator (2,4,6-trimethylbenzoyl phosphine oxide/2-hydroxy-2-methylpropiophenone blend) and dichloroethane (DCE) were purchased from Sigma Aldrich and used without further modifications unless otherwise stated.

Polymer Synthesis

A detailed protocol for the synthesis of photocurable PCL has been described elsewhere (Fig. 3) [35, 36]. Briefly, Ɛ-caprolactone and pentaerythritol were mixed at 160°C in a flask until pentaerythritol was dissolved. Then, tin (II) 2-ethylhexanoate was added as a catalyst, and the system was left for the reaction overnight. At the end of the synthesis of the hydroxyl-terminated polymer, the system was cooled down to room temperature. The obtained 4PCL was dissolved in DCM, and TEA was added. Then, the flask was transferred to an ice bath. In a separate beaker, MAAn was dissolved in DCM, the solution was added via a dropping funnel and all the setup was kept for the reaction at room temperature for 68 hours. Then, 4PCLMA was washed with hydrochloric acid (HCl) solution and deionised water, respectively, and the solvent was removed using a rotary evaporator. Finally,

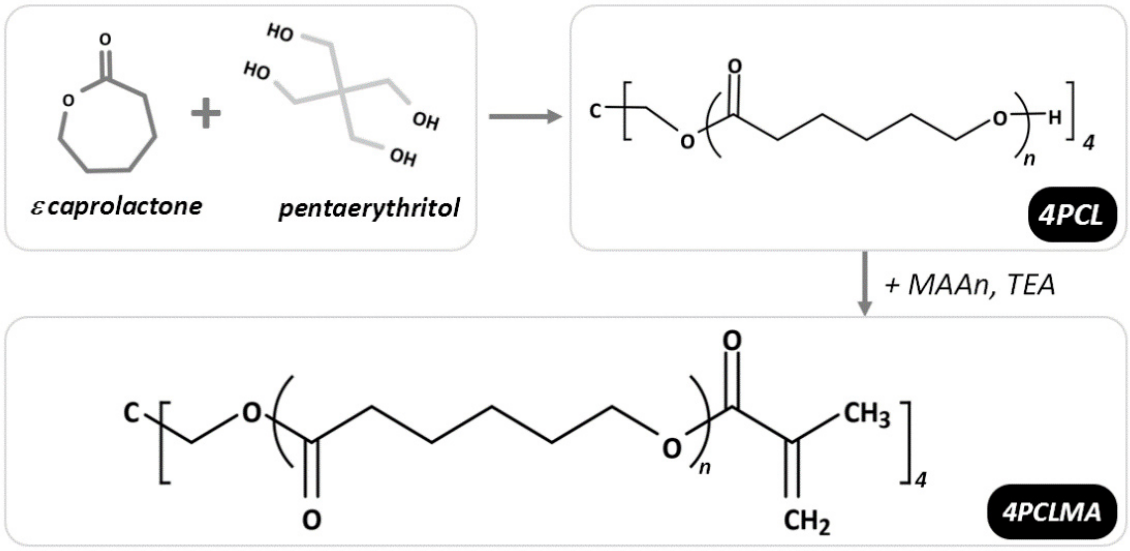

Fig. 3. **Synthesis and methacrylation of 4-arm PCL.**

the polymer was washed with methanol, and the solvent was removed. The resulting 4PCLMA was stored for further use.

Synthesis of MIPEs and HIPEs and Their Polymerization

Three compositions of emulsions with varying internal phase volumes were prepared: 60%, 75% and 82% for P60, P75 and P82, respectively. The amounts of the ingredients of each component of the emulsion are given in Table 1. Briefly, 4PCLMA, surfactant (PGPR), photoinitiator and solvent (DCE) were mixed using a magnetic stirrer at 370 rpm in a glass container for 1 minute to create a homogeneous blend. Then, water as an internal phase was added dropwise using a Pasteur pipette to the mixture. Lastly, the emulsion was mixed for 2 minutes, transferred into circular silicone moulds with a diameter of 6 mm and height of 4 mm, respectively, and photocured for 2 minutes on each side. All emulsion compositions were cured immediately (within 10 s) once prepared. The obtained MIPEs and HIPEs were washed with methanol to remove any remaining contaminants of uncured polymer, surfactant, or solvent, and the scaffolds were freeze-dried.

Densities of Emulsion Templated Matrices

The densities of polymerised emulsions were calculated by measuring the mass (m) and volume (V) values of the cylindrical samples (Equation 1) [37].

$$density = \frac{m}{V} = \frac{m}{\pi r^2 h} \quad (1)$$

Porosities of Emulsion Templated Matrices

Equation 2 was used to calculate the porosities of the polymerised emulsions [35, 36].

$$\%\ Porosity = (1 - \frac{\rho_{PolyHIPE}}{\rho_{wall}}) \times 100 \quad (2)$$

$\rho_{PolyHIPE}$ and ρ_{wall} state the densities of PolyHIPE and the wall of the PolyHIPE, respectively. The wall density is the measured density of the bulk polymer.

Morphological Characterization

Scanning electron microscopy (SEM) was used to investigate the morphology of the fabricated scaffolds. Cross-sectional samples were prepared from dried polymerised emulsions, and all samples were placed on aluminium pins coated with carbon stickers. Then, samples were gold coated and visualised using SEM (FEI QUANTA 250 FEG, United States).

Pore and Window Size Quantification

100 pores and 150 windows were selected randomly from three different regions of the samples, and measurements were taken using Image J [38]. A sta-

Table 1. Ingredients of the emulsions and characteristics of the polymerised emulsions

	P60	P75	P82
4PCLMA (g)	0.2	0.2	0.2
PGPR (g)	0.03	0.03	0.03
DCE (g)	0.53	0.53	0.53
PI (g)	0.03	0.03	0.03
Water (mL)	1	2	3
Internal phase (%)	60	75	82
Density (g/cm^3)	0.395	0.258	0.208
Porosity (%)	65±1	77±1	81±2
Pore size (D, µm)	45±21	38±17	50±26
Window size (d, µm)	4±4	5±3	9±4
Degree of interconnectivity (d/D)	0.089	0.132	0.180
Compression modulus (MPa)	0.46±0.04	0.23±0.02	0.14±0.01

4PCLMA= tetra methacrylate functionalized polycaprolactone, PGPR=Polyglycerol polyricinoleate, DCE=dichloroethane, PI=photoinitiator, MPa= Megapascal

tistical correction factor (2/√3) was applied for the measured pore values to correct the underestimation of the measurements due to uneven sectioning [7, 26]. Pore and window histograms were created using Microsoft Excel. The degree of interconnectivity values were calculated by dividing the average window diameter by the average pore diameter (d/D) [7].

Compression Test

Mechanical characterisation of 4PCLMA-based emulsion templated matrices was conducted using a compact tabletop universal tester with a 100 N load cell (EZ-TEST EZ-S, Shimadzu, Japan). Cylindrical discs with approximately 5.5 mm diameter and 3.5 mm height were used. The exact dimensions of the samples were measured using a calipper, and recorded. Compression samples were placed on the bottom part of the compression plate, the upper place was lowered, and the compressive tests were performed at the rate of 1 mm/min. Samples were compressed up to 90 N or until ~70% strain was achieved. The compression modulus values were calculated from the linear region of the stress-strain curve of each sample [39].

Statistical Analysis

GraphPad Prism 6 was used to perform statistical analyses. One-way analysis of variance (ANOVA) was applied for mechanical testing, and the graph was plotted as mean ± SD. A difference was deemed statistically significant if the P-value was less than 0.05, and the statistical differences are denoted in the figures. The total number of replicates (n) is stated in the figure legends, where relevant.

RESULTS

Fabrication of PCL PolyMIPEs and PolyHIPEs

Following polymer synthesis, all three groups of emulsion-templated scaffolds were successfully fabricated. There was no observable phase separation during and right after emulsification until the photopolymerisation. 60%, 75% and 82% water were incorporated into the emulsion composition as an internal phase for groups P60, P75 and P82, respectively.

At the end of the emulsification and mixing process, all three groups formed opaque and white emulsions but with different observable viscosities. Whilst P60 has the lowest observable viscosity among these three groups, and the emulsion was transferred into the moulds by pouring, P75 was comparably more viscous. Accordingly, increasing the internal phase volume to 82% increased the observable viscosity of the emulsion, and P82 has a mayonnaise-like consistency and could not be transferred into the mould by pouring; it was transferred using a spatula. Following the poly-

merisation of the emulsions, they were fully solidified, and no visible uncured region was detected when samples were cross-sectioned using a surgical blade.

Impact of Internal Phase Volume on the Physical and Morphological Characteristics of Emulsion Templated Scaffolds

Densities of emulsion-templated scaffolds were measured as 0.395, 0.258 and 0.208 g/cm3 for P60, P75 and P82, respectively. Increasing internal phase volume reduced the density of the foams. Porosities were measured as 65%, 77% and 81% for emulsions, with internal phase volumes of 60%, 75%, and 82%.

Following polymerisation and post-washing processes, scaffolds were freeze-dried to prevent any potential collapse, which was morphologically investigated by SEM. Fig. 4 shows the morphologies of P60, P75 and P82 at three different magnifications. At this point, it is critical to distinguish the difference between the polydisperse distribution of the pores and heterogeneous morphology. The polydisperse distribution of the pores indicates the broad distribution of the pore sizes. However, if this polydisperse distribution is in a similar trend throughout the sample, it can be concluded that these samples have homogenous morphology. Capturing multiple images from different regions of the samples and having a zoom-out image that shows the morphology of the full thickness of the sample can give an idea of these two characteristics. As shown in the first column (Figs. 4A, 4D and 4G), polydisperse pores are homogeneously dispersed throughout the scaffolds in all three groups of scaffolds. There is no heterogeneous region different from the overall morphology. Also, no separated full layer of non-porous polymer phase was detected.

The average pore sizes were measured as 45±21 µm, 38±17 µm, and 50±26 µm for P60, P75, and P82, respectively. When the internal phase volume was increased to 82%, water droplets incorporated into the emulsion composition took a longer time to melt in the emulsion during the emulsification process. It is an indication that 82% is around the maximum internal phase volume that could be incorporated into the emulsion composition. Thus, although the frequency

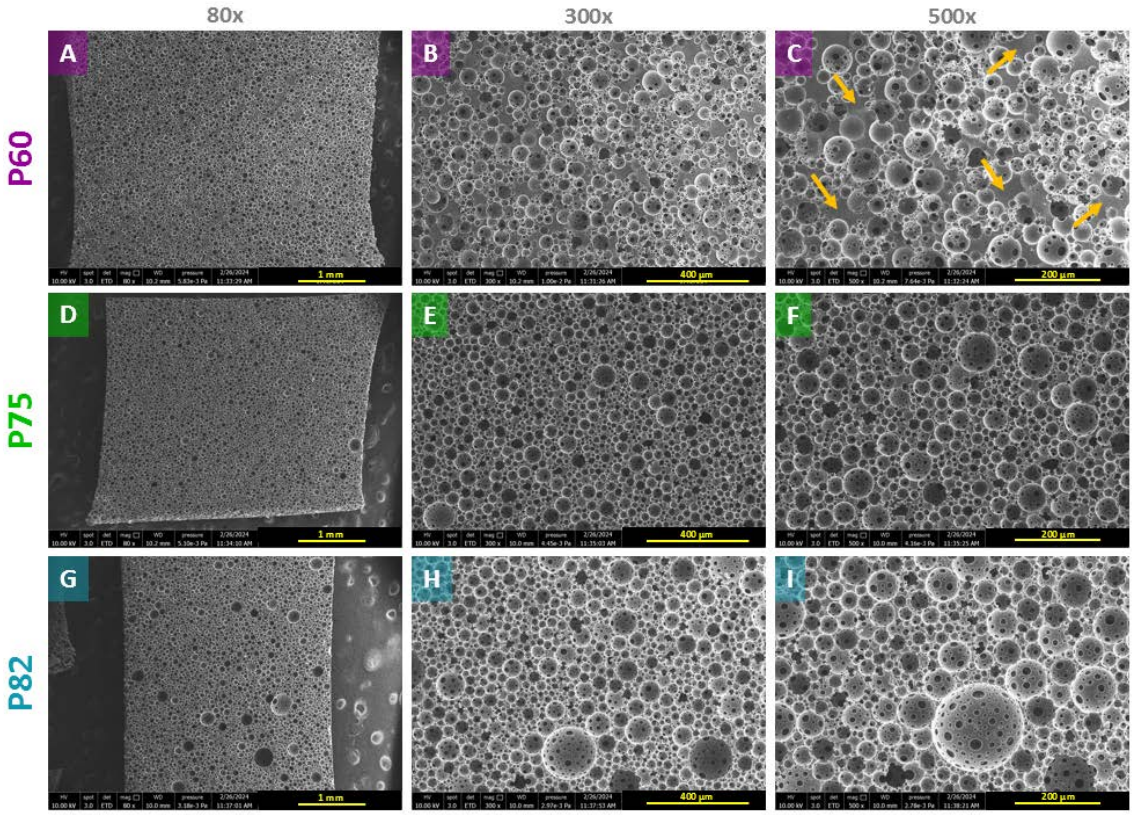

Fig. 4. Scanning electron microscope images of polymerised emulsions prepared with varying internal phase volumes. (A-C) P60, (D-F) P75, and (G-I) P82. Orange arrows indicate non-porous regions.

of pores with pore sizes between 30-40 µm is around 20-50% in both P75 and P82, the frequency of pores with pore sizes larger than 70 µm is 11% and 27%, respectively (Figs. 5B and 5C). Pore and window size distributions become broader in P82 when compared with P60 and P75 (Figs. 5A-5F).

The average window sizes were measured as 4±4 µm, 5±3 µm, and 9±4 µm, and the degree of interconnectivity values were calculated as 0.089, 0.132, and 0.180 for P60, P75, and P82, respectively. Both the average window sizes and the degree of interconnectivity values increased with increasing internal phase volume.

Impact of Internal Phase Volume on the Mechanical Characteristics of Emulsion Templated Scaffolds

Cylindrical samples were prepared, and the compression test was conducted to investigate the impact of internal phase volume on the mechanical characteristics of emulsion-templated scaffolds. None of the samples undergo failure under this condition. Compressive stress−strain curves of polymerised emulsions and their compressive modulus calculated from the linear region of the stress-strain curve are given in Figs. 6A and 6B, respectively.

A significant difference can be seen in stress-strain curves of emulsion-templated matrices fabricated with varying internal phase volumes (Fig. 6A). At low strain, where stiffness values were calculated, stress-strain values were linear, and dramatic increases in stress were observed with increasing strain. Compression modulus values are calculated as 0.46±0.04 MPa, 0.23±0.02 MPa and 0.14±0.01 MPa for P60, P75, and P82, respectively.

DISCUSSION

The densities of the foams were observed to reduce when internal phase volume increased, as increasing water content resulted in a reduction in the amount of solid materials per unit volume [7, 40].

Porosity values were calculated close to but not the same as internal phase volumes. Although the internal phase is the ingredient responsible for the formation of the pores and, consequently, the porosity, the difference between internal phase volume and the porosity of the emulsion-templated matrices has been reported before [35]. Some of the potential reasons be-

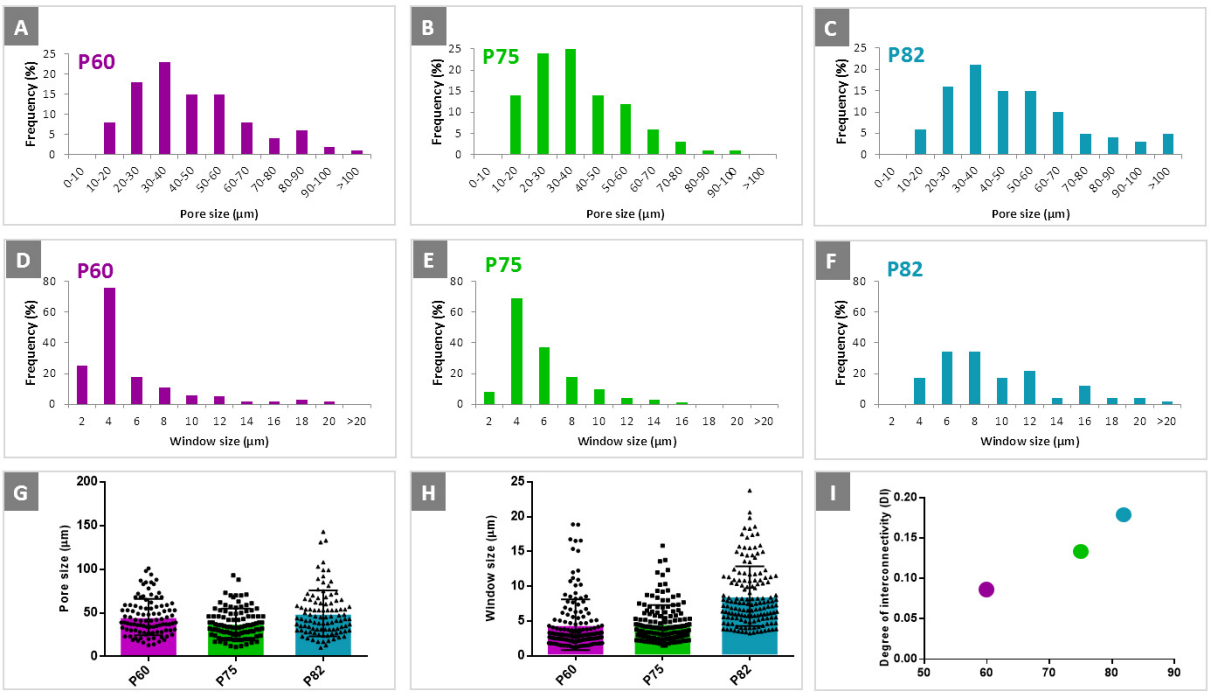

Fig. 5. Morphological characterisation of polymerised emulsions: (A-C) pore size distribution histograms, (D-F) window size distribution histograms, (G) average pore size, (H) average window size and (I) degree of interconnectivity graphs of polymerised emulsions (P60, P75 and P82).

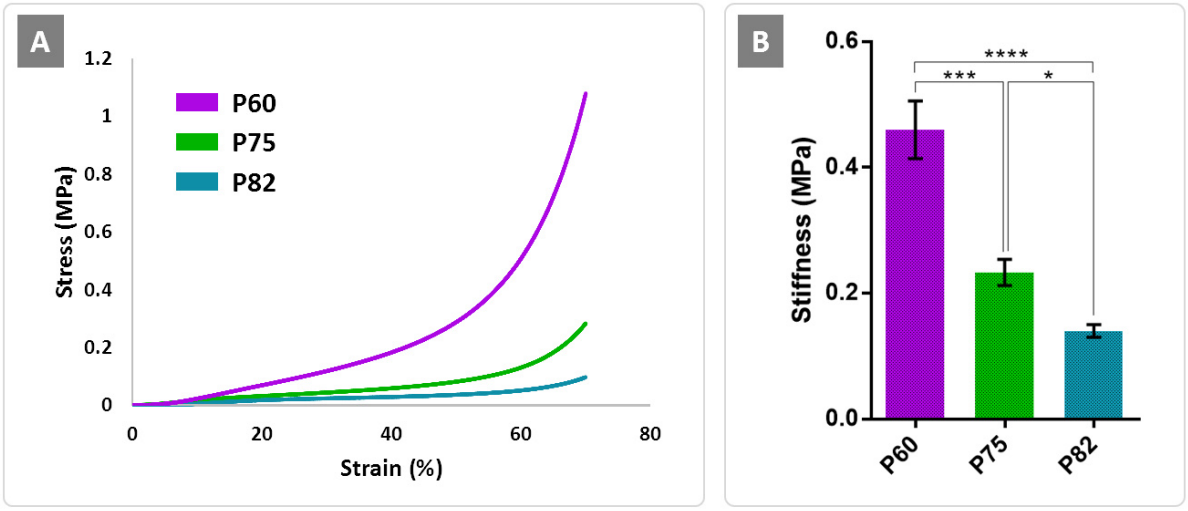

Fig. 6. Compression test results. (A) Stress-strain curve and (B) stiffness of the polymerised emulsions prepared with varying internal phase volumes (n=3, ****P<0.0001, ***P<0.001, and *P<0.05).

hind this result are likely to be the shrinkage of the polymer during polymerisation and drying and the variation of the degree of shrinkage depending on the thickness of the struts between neighbouring pores. Figs. 4A, 4D, and 4G show the zoom-out images of the full-thickness samples. No separated, non-porous polymer layer of any heterogeneous region was detected on the SEM images. In our previous study, we have shown that a full layer of non-porous polymer region appears in the SEM images of the polymerised unstable emulsions. This phase occurs on the bottom layer of the samples due to the density difference between the water and the oil phases [23, 35]. Accordingly, in this study, we can verify the stability of emulsions until the polymerisation point in light of SEM images.

No direct correlation between average pore size and internal phase volume was observed. In the SEM images of the P60 group, it can clearly be seen that water droplets are not crowdedly packed; there are distinguishable non-porous regions (shown with orange arrows). Increasing internal phase volume from 60% to 75% is likely to force water droplets to be more tightly packed with smaller-sized pores. Pore and window size distributions become broader in P82 when compared with P60 and P75 (Figs. 5A-5F).

There can be various potential reasons behind these observations. Firstly, as the internal phase is incorporated into the system dropwise, water droplets incorporated at the early stages are exposed to a longer duration of mechanical force to be divided and form smaller droplets, while the last portion of the internal phase is exposed to a shorter time of mixing and form larger droplets. Secondly, as internal phase volume increases, it leads to an increase in the emulsion viscosity, which may also have an adverse effect on the mixing efficiency and end up with broader pore size distribution. Thirdly, as there is enough continuous phase at the beginning, there is enough interfacial film that can form around the water droplets and let them increase their surface area by reducing their sizes [41]. Lastly, at the beginning of the emulsification, there is enough surfactant in the composition that can be placed around the water droplets to reduce the interfacial tension and eventually cause smaller pore size and increased emulsion stability. However, as the internal phase volume and the interfacial area increase, the surfactant amount falls short, the emulsion eventually results in larger pores, and the emulsion system does not accept a further increase in the internal phase volume. Barbetta *et al*. reported an increase in both pore and window diameters with increasing internal phase volume [41].

Both the average window sizes and the degree of interconnectivity values increase with increasing internal phase volume. This is potentially due to the enhanced packing density of the water droplets. As a result, the thickness of the interfacial polymer film be-

tween the pores is likely to reduce and more likely to rupture during polymerisation, resulting in the formation of interconnects [19].

In the stress-strain graph obtained from the compression test, it can be clearly seen that, at low strain, where stiffness values were calculated, stress-strain values were linear, and dramatic increases in stress were observed with increasing strain. In the stress-strain curve of porous polymers, as the compression load increases, the walls separating the pores are crushed, the porous material densifies, and the stiffness increases [29, 42]. Increasing internal phase volume from 60% to 82% caused a more than 2-fold reduction in the stiffness of the emulsion-templated matrices. If the pore and window sizes were the same for all three groups, this difference could be interpreted only because of the increasing porosity of the scaffolds. However, by increasing the internal phase volume, % porosity, pore size, window size and degree of interconnectivity values of the matrices are also changed. Thus, it can be concluded that the difference between the mechanical characteristics of these matrices is because of the cumulative effect of all of these changes. For instance, both window size and degree of interconnectivity values increase with increasing internal phase volume, which means the densities of the walls are less in scaffolds prepared with higher internal phase volumes, and this would cause a reduction in the stiffness of the designs.

Our group characterised the mechanical properties of a low degree of methacrylation (~50%) 4CPLMA-based PolyHIPEs prepared with different surfactant and solvent compositions previously [35, 43, 44]. Tensile modulus values were reported to be around ~0.4 MPa in these studies. Jackson *et al*. recently reported the compression modulus of 4PCLMA-based PolyHIPEs with a molecular weight of 20331 g/mol, methacrylation degree of 95%, and an average pore size of ~50 µm as ~1 MPa [39]. However, the compression behaviour of low molecular weight (with molecular weight ~2500 g/mol) 4PCLMA (with a high degree of methacrylation) PolyHIPEs are characterised for the first time in this study.

CONCLUSION

There is a wide range of characteristics of tissue engineering scaffolds that need to be engineered for specific biomedical applications. Mechanical characteristics and porosity are two of the important ones, alongside the pore size, window size, and interconnectivity. Although scaffolds with high porosity are desired to boost mass transfer and cell penetration throughout the scaffold, the stiffness of a scaffold is inversely proportional to porosity, as increased porosity simply reduces the solid portion of the scaffolds. In this study, we observed that average pore size and average window diameters can be increased by 30% and almost two-fold, respectively, simply by increasing the internal phase volume from 75% to 82%. However, the stiffness of the scaffolds also reduces almost twofold within these groups. To conclude, in this study, we established a relationship between internal phase volume and the physical, morphological and mechanical characteristics of 4PCMA-based emulsion templated scaffolds to be able to tune these characteristics for specific tissue engineering applications.

Authors' Contribution

Study Conception: BAD; Study Design: BAD; Supervision: BAD; Funding: BAD; Materials: BAD; Data Collection and/or Processing: BAD; Statistical Analysis and/or Data Interpretation: BAD; Literature Review: BAD; Manuscript Preparation: BAD and Critical Review: BAD.

Ethics Approval

There are no ethical issues with the publication of this manuscript.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

Funding

This study was supported by the Department of Scientific Research Projects of Izmir Institute of Technology (IZTECH-BAP, 2021-IYTE-1-0110 and 2022-IYTE-2-0025), Health Institutes of Turkey (TUSEB-2022B02-22517).

Acknowledgement

The author also acknowledges IzTech (Izmir Institute of Technology) Integrated Research Centers (IzTech IRC), the Center for Materials Research.

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The European Research Journal 2024;10(5):533-543 Review

DOI: https://doi.org/10.18621/eurj.1467569 *Obstetrics and Gynecology*

Management and complications of tubo-ovarian abscesses: a brief literature review

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ABSTRACT

Tubo-ovarian abscesses (TOAs) are identified as a severe and complex form of inflammatory disorder, marking an advanced progression of pelvic inflammatory disease (PID), mainly resulting from the escalation of polymicrobial infections within the female genital tract. This literature review is dedicated to elucidating the contemporary management strategies and associated complications of TOAs, amalgamating the current scholarly discourse while spotlighting the emerging therapeutic trends. The inherent complexity of TOAs, manifesting through a broad array of clinical presentations from mild symptoms to acute pelvic discomfort and fever, necessitates a comprehensive, multidisciplinary approach to achieve effective management. Traditional management has predominantly focused on antibiotic therapy, the cornerstone of initial treatment modalities. Nonetheless, this review expands on the increasing acknowledgment of minimally invasive surgical interventions, like ultrasound-guided drainage, especially in scenarios where medical therapy falters or in the presence of sizable abscesses, underscoring scenarios where antibiotic treatment may be insufficient. The discourse further explores the pivotal decision-making concerning surgical interventions, juxtaposing the benefits of abscess resolution against potential risks and complications, such as damage to adjacent structures and implications for future fertility. The review emphasizes the criticality of addressing PID's risk factors and root causes to avert TOA development. It also ventures into the ramifications of the emergence of antibiotic-resistant bacterial strains for empirical antibiotic therapy selection, highlighting the imperative for continuous research and the adaptation of therapeutic guidelines. The complications associated with TOAs, including sepsis, infertility, and chronic pelvic pain, are meticulously examined to underscore the potential for significant long-term morbidity. Advocating for prompt diagnosis and encompassing management strategies to curtail these adverse outcomes, the review ultimately calls for intensified, quality research to refine TOA management further, particularly against the backdrop of evolving microbial resistance and the advancements in minimally invasive surgical technologies.

Keywords: Tubo-ovarian abscess, pelvic inflammatory disease, minimally invasive surgical intervention, infertility

Tubo-Ovarian Abscess (TOA) is an inflammatory disease of the uterine tubes, ovaries, and surrounding adnexal tissues [1]. TOAs result from recurrent infections of the adnexal organs. As the infection progresses, involvement can extend to the intestines, uterus, omentum, and bladder. A Douglas ab-

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How to cite this article: Akıncı GE, Bornaun T, Güven HZ. Management and complications of tubo-ovarian abscesses: a brief literature review. Eur Res J. 2024;10(5):533-543. doi: 10.18621/eurj.1467569

Received: April 11, 2024
Accepted: June 24, 2024
Published Online: July 15, 2024

scess can also occur due to the spread from a TOA through contiguous structures [2]. TOAs may also arise secondary to the use of intrauterine devices, granulomatous infections, pelvic or intrauterine surgeries, and perforations of other intraabdominal organs [3].

Clinical presentations can vary. Tubo-ovarian abscesses typically emerge as complications following acute or chronic pelvic inflammatory disease (PID), which is more commonly observed in women of reproductive age. Annually, 1 million women receive treatment for PID, and approximately 200,000 patients require hospitalization [4]. Symptoms of TOA include pelvic and abdominal pain, nausea, vomiting, high fever, and tachycardia. A detailed pelvic examination may be challenging due to generalized abdominal defense. During a bimanual examination, an adnexal mass can often be palpated [5]. Ultrasound (USG), Computed Tomography (CT), and Magnetic Resonance Imaging (MRI) are imaging methods used to monitor treatment response and complications in patients [6].

In recent years, the mortality associated with TOAs has decreased. However, morbidity, including infertility, ectopic pregnancy, chronic pelvic pain, ovarian vein thrombosis, and pelvic thrombophlebitis, remains significant. Although antibiotic therapy is widely accepted for treatment, outcomes are often suboptimal due to the difficulty of antibiotic penetration through the abscess wall. Following antibiotic therapy, surgical intervention is required in 25% of cases [7]. Therefore, removal of the abscess or adnexa via laparotomy or laparoscopy is recommended [8]. For young patients, fertility-preserving surgery is prioritized, making laparoscopy the gold standard [9].
Untreated TOA can rupture, leading to life-threatening peritonitis and sepsis. Emergency surgery is indicated if TOA rupture is suspected. Untreated TOA poses a significant health problem in the long term due to its detrimental effects on chronic pain and fertility [10].

Based on the presented review of TOA management, the outlined introduction offers a comprehensive overview of TOA's etiology, clinical presentation, diagnostic approaches, and current treatment paradigms. This underscores the complex nature of TOAs, the diversity in clinical manifestations, and the criticality of a nuanced, multidisciplinary management strategy to mitigate the associated morbidity and improve patient outcomes. However, despite advances in diagnostic modalities and treatment options, significant gaps remain in our understanding of optimal management strategies, particularly in balancing antibiotic therapy with the need for surgical intervention, and in tailoring treatment to preserve fertility without compromising the efficacy of abscess resolution. The primary objective of this research is to further elucidate the optimal management strategies for TOAs, focusing on the efficacy and outcomes of different treatment modalities, including the role of minimally invasive surgical techniques and the impact of novel antibiotic regimens. Additionally, this study aims to explore the long-term outcomes of TOA treatment, particularly regarding fertility preservation and the prevention of recurrent infections, which represent critical concerns for affected individuals. The significance of this research lies in its potential to contribute valuable insights to the existing body of literature on TOAs, addressing critical gaps in knowledge and practice. By providing evidence-based recommendations for TOA management, this study seeks to enhance clinical decision-making, improve patient outcomes, and reduce the morbidity associated with this condition. Given the implications of TOAs on reproductive health and the potential for life-threatening complications, advancing our understanding of their management is of paramount importance. This research endeavors to pave the way for future studies, fostering a deeper comprehension of TOA treatment strategies that can significantly impact women's health worldwide.

TUBO-OVARIAN ABSCESS (TOA)

Tubo-ovarian abscesses (TOAs) are characterized as inflammatory masses involving the fallopian tubes, ovaries, and sometimes extending to other pelvic organs such as the bladder and intestines. Arising predominantly from infections within the upper genital tract, these abscesses are notably prevalent among women of reproductive age, frequently emerging as a severe complication of pelvic inflammatory disease (PID]. The condition necessitates urgent medical and sometimes surgical intervention due to its potential to progress to sepsis. Historically, before the advent of broad-spectrum antibiotics and advanced surgical techniques, TOA-associated mortality rates were reported to exceed 50% [11, 12].

The therapeutic approach to managing TOAs encompasses a spectrum of strategies, including the administration of broad-spectrum antibiotics, minimally invasive drainage procedures, and more invasive surgical interventions, with some cases requiring a combination of these modalities. Notably, for a majority of TOAs, particularly those of smaller to medium size (<9 cm), antibiotic therapy alone is deemed sufficient [12].

EPIDEMIOLOGY AND RISK FACTORS

Limited epidemiological studies have been conducted on TOAs. It has been reported that, in the United States, approximately 200,000 women were hospitalized due to PID from 1983 to 2000. It's imperative to acknowledge that not all TOAs are directly related to PID. Nonetheless, about 33% of patients presenting with PID are found to have TOAs, suggesting an estimation of around 66,000 cases annually in the U.S. A study in Norway highlighted a decrease in PID diagnoses from 1990-1992 to 2000-2002, with TOAs constituting a growing majority of these cases, indicating an increase from 26% to 43%. This surge could potentially be attributed to variations in PID management practices, which are predominantly outpatient for current PID treatments, reserving hospitalization for severe cases, including TOAs [13-16].

Although the majority of TOA cases are observed in women aged between 15-40, age does not exclude the diagnosis. The risk factors for TOA closely mirror those associated with PID, including: Age between 15-25 years, History of intrauterine surgical procedures, Intrauterine device (IUD) use, Multiple sexual partners, Previous pelvic surgery, Intraabdominal surgeries Immunosuppression. Undergoing IVF treatment While modern IUDs have been associated with a slight increase in PID risk, their use has been linked to an increased risk of TOAs (typically unilateral), though such associations have yet to be firmly supported by well-conducted studies [17, 18]. IUDs have been in existence since the late 1990s and play a minor role in PID development. The risk of PID is notably higher in the first three weeks following IUD insertion. Additionally, some research indicates that women with HIV may have a higher risk of developing TOAs [19-21].

PATHOGENESIS

The pathogenesis of TOA development remains not fully elucidated. Most commonly resulting from PID, TOAs typically originate from infections in the upper genital tract. They can also develop secondary to infections of the intestines and appendix, post-adnexal surgery, or through hematogenous spread. It's important to note that pelvic abscesses, which have a different etiology and management approach from TOAs, are not included in the tubo-ovarian complex. In rare cases, TOAs can be observed in women who have undergone a hysterectomy, arising from non-gynecological organs through hematogenous or local spread.
In instances stemming from the lower genital tract, sexually transmitted pathogens or the endogenous flora of women are usually implicated. The infection ascends to the fallopian tubes, damaging the endothelium, secretory, and ciliary cells, leading to edema. Previous infections disrupting tubal structure also predispose individuals to TOA formation.

The progressively invasive tubal infections result in increasing tissue edema and the production of purulent exudate. Occasionally, tubal structures may adhere to ovarian ligaments, forming a complex wherein necrosis develops, leading to one or more abscess cavities and facilitating the proliferation of a multitude of endogenous pelvic flora bacteria.

TOAs are predominantly polymicrobial in nature. The bacterial groups identified in TOAs and uncomplicated PID cases are generally the same. Occasionally, causative agents of bacterial vaginosis can also proliferate in women with TOAs, although a direct link between TOA and bacterial vaginosis has not been established [22-24].

Cultures from TOA patients can yield growth of aerobic, facultative anaerobic, and anaerobic bacteria, commonly including species such as Bacteroides Fragilis, Prevotella, Escherichia Coli, Group B Streptococcus, and Peptostreptococcus. Less frequently, Candida, Pasteurella Multocida, Salmonella, and Streptococcus Pneumoniae can be isolated. In immunosuppressed patients, Mycobacterium Tuberculosis often causes TOAs. For long-term IUD users, Actinomyces Israelii represents a specific anaerobic pathogen responsible for TOAs. *N. gonorrhoeae* and *C. trachomatis* are rarely cultured from TOA abscess

cavities, typically being associated with PID and cervicitis. In a series involving 232 women, N. gonorrhoeae was isolated from the endocervix in 33% of cases, yet less than 4% were linked to TOAs. Some data suggest N. gonorrhoeae's progression from the lower to upper genital tract may contribute to invasive infection [25, 26].

CT is preferred in cases where it's necessary to exclude pathologies related to the Gastrointestinal System (GIS), such as appendicitis, phlegmon associated with inflammatory bowel disease, abscess, etc. Several studies have demonstrated that CT possesses a higher sensitivity compared to USG. However, CT is significantly more expensive than USG. Additionally, for CT to achieve its high sensitivity, the administration of both IV and oral contrast is required, which may not be feasible for some patients due to various reasons [27-30].

The specific CT appearance of a TOA is that of a thick-walled adnexal mass, contrasting with the thin-walled appearance typical of non-inflammatory masses. Inflammatory masses are often multilocular and usually contain increased fluid in the form of purulent exudate [31]. In some patients with TOA, CT imaging may reveal thickened tubes (pyosalpinx), regional bowel thickening, and mesenteric nodes. Additionally, CT (and sometimes USG) can typically display signs of abscess ruptures, such as free fluid in the abdomen due to pus accumulation and abscess foci on the pelvic wall. Other imaging methods for diagnosing TOA include MRI and radionuclide imaging, though their clinical utility is limited and not preferred for routine use. Surgical evaluation in cases of TOA is indicated in the following scenarios [31-33]:

- Suspected abscess ruptures presenting with signs of acute abdomen or sepsis
- In postmenopausal women, where there is a TOA that could be confused with gynecological malignancy.

DIAGNOSIS

The diagnosis of TOA is established through the classic diagnostic criteria for PID (lower abdominal tenderness, adnexal tenderness, tenderness on cervical motion during bimanual examination, fever >38.3°C, abnormal vaginal discharge, elevated erythrocyte sedimentation rate, increased CRP, presence of N. gonorrhoeae and C. Trachomatis in vaginal cultures) combined with imaging revealing an adnexal mass. Drainage accompanied by imaging may be necessary for pelvic masses containing purulent material, as anatomical localization may not always be accurately determined. Sometimes, a definitive diagnosis of TOA requires direct observation during invasive surgery (L/T: Laparotomy, L/S: Laparoscopy] [26, 27].

Differential Diagnosis

A broad range of conditions that can cause lower quadrant abdominal and pelvic pain resembling PID includes [34-37]:

Reproductive system: ovarian mass, ruptured ovarian cyst, ovarian torsion, degenerated uterine fibroid, dysmenorrhea, ectopic pregnancy, septic abortion GIS system: appendicitis, AGE (acute gastroenteritis), inflammatory bowel, irritable bowel, diverticulitis, constipation, cholecystitis Urinary system: cystitis, pyelonephritis, nephrolithiasis, urethritis It's crucial to note; the absence of fever does not rule out TOA. Women with TOA may have or develop sepsis. Vital signs, physical examination, and laboratory tests can collectively exclude sepsis.

Drug choice for TOA; the outcome of blood culture and tubo-ovarian abscess culture is beneficial in selecting the treatment. If a blood culture is to be taken, it should be done before antibiotic therapy.

MANAGEMENT AND COMPLICATIONS OF TUBO-OVARIAN ABSCESSES

Choosing the Treatment Method

Depending on the character of the abscess and the patient's condition, treatment can be with antibiotic therapy alone or combined with drainage – surgical treatment. In some cases, emergency surgery may be necessary.

Emergency surgical indications include; suspected intraabdominal TOA ruptures that are life-threatening, necessitating urgent surgery [13]. Clinical signs such as hypotension, tachycardia, tachypnea, acute peritonitis, or acidosis suggest an intraabdominal rupture. Surgery or medical treatment is recommended for women with large abscesses or signs of sepsis, suspected of abscess rupture, but treatment often leans towards sur-

gery in acute onset cases. Antibiotics should be initiated as swiftly as possible during or before surgery in women undergoing surgical treatment. Crucially, in unstable patients, surgery should be swiftly planned without waiting for antibiotic treatment [17].

Who Should Receive Only Medical Treatment?

In most cases, antibiotic therapy is sufficient without the need for surgical treatment. Based on numerous studies, antimicrobial agents have been observed to be effective in approximately 70% of cases.

Only antibiotic therapy is recommended in the following situations: patients without signs of toa rupture (acute abdomen, sepsis) whose hemodynamics are stable, abscesses smaller than 9 cm, those who respond adequately to antibiotics and premenopausal women, signs of sepsis and USG follow-ups should be conducted approximately every 3 days or less frequently if clinical improvement is observed [18, 20].

Minimal invasive drainage is employed in patients who do not worsen but do not respond distinctly to antibiotic therapy. No studies compare minimal invasive drainage or surgical intervention in such cases. Surgery should be preferred if minimal invasive drainage is not possible (multiloculated mass, poor abscess localization, or lack of experience) [34].

Antibiotic therapy is the cornerstone of TOA treatment. Antibiotics must be administered before or after drainage and surgery. If the patient with TOA has a sexually transmitted disease, the partner must be identified and treated [33, 34].

Treatment Management

Antibiotic therapy is fundamental in TOA treatment. In some women, antibiotics should be combined with minimal invasive drainage and surgery. The excellent antimicrobial activity of broad-spectrum antibiotics, due to their penetration into the abscess cavity, has made them the primary treatment for non-ruptured TOAs. Before the 1970s, due to the inadequacy of antibiotic therapies, drainage was required for all abscesses [37-39]. At that time, non-surgical interventions were insufficient, necessitating extirpation of all affected organs during surgery (usually total abdominal hysterectomy and bilateral salpingo-oophorectomy [TAH+BSO]]. The morbidity associated with surgery, wound site infections, and premature menopause made this method less satisfactory for patients.

Antibiotic therapy; experts agree on the in-hospital and IV treatment of TOA, as recommended by the CDC [40]. Antibiotic therapy alone, as mentioned above, is effective in approximately 70% of affected women [41-45]. This treatment modality can be insufficient depending on the abscess's characteristics (relatively avascular structure, inadequate antibiotic penetration, low pH]. The high effectiveness of antibiotic therapy alone is also due to the high vascularization of the ovaries. The treatment of TOA is the same as that for PID. Although their pathophysiologies are not clear, TOA and PID originate from similar conditions. The first-line treatment for PID (most TOAs) is second-generation cephalosporins with anaerobic efficacy. The benefit of adding a second anaerobic-effective antibiotic to this treatment is still debated. Therefore, according to the CDC, only metronidazole should be used as an additional treatment for PID [44, 45].

Medical Treatment

The treatment algorithm for the medical management of TOA is not definitive. If antibiotic therapy is to be employed as the sole treatment modality, it should be continued for at least 2 weeks, aligning with the CDC's guidelines for PID treatment [46]. Additionally, many experts recommend continuing antibiotic therapy until the abscess is resolved on subsequent imaging, which could extend the treatment duration to 4-6 weeks. In cases where the abscess does not resolve with antibiotic therapy alone, outpatient antibiotic treatment may be provided. If routine therapy fails to resolve the abscess, a collaborative effort with Infectious Diseases and Clinical Microbiology should be initiated [45].

There is no specific duration recommended for the combination of abscess drainage or surgery with antibiotic therapy. According to studies, using antibiotics for 10-14 days has been found effective for the healing process. Each patient should be evaluated based on their unique clinical scenario [42-44].

For patients selected for outpatient oral antibiotic therapy, the ability to tolerate oral medications and adhere to follow-ups are crucial criteria for regular clinical improvement.

All treatments should be administered in accordance with the bacteria involved (similar to the treatment for PID]. Moreover, the treatment should also

cover sexually transmitted pathogens (*N. Gonorrhea*, *C. Trachomatis*-even though they may be isolated from TOA) and anaerobes [48, 49]. The chosen antibiotic should be able to penetrate the abscess wall and be active within the cavity (e.g., clindamycin, metronidazole, cefoxitin) [50].

If sexually transmitted pathogens are isolated in a patient with TOA, specific treatment for these pathogens should be added. Various antibiotic treatments have been found effective for the medical management of TOA. The first-choice medications are listed in Table 4, based on clinical data (Table 4]. First-choice treatment agents have been observed to have nearly equal effects [51, 52].

Cefotetan and Cefoxitin are primary treatment options for TOA. Resistance of the intestinal flora against Cefotetan and Cefoxitin is on the rise, which often plays a role in the pathogenesis of TOA. New agents are being incorporated into TOA treatment, including Ertapenem (1g IV every 24 hours) or Piperacillin-Tazobactam (3.375g IV every 6 hours]. A randomized controlled trial has shown Ertapenem to be significantly more effective than Cefotetan in preventing surgical site infections in patients undergoing elective intestinal surgery [53]. Additionally, according to the latest guidelines by the American Society of Infectious Diseases, cefoxitin is a viable option for treating moderate intraabdominal abscesses [54].

Some studies indicate that triple antibiotic therapy is more effective than dual therapy. Small series have shown that triple therapy (Ampicillin + Clindamycin + Gentamicin) is more effective compared to dual therapy (Clindamycin + Gentamicin or Cefotetan + Doxycycline) (Triple therapy efficacy: 88%, Clindamycin+Gentamicin: 47%, Cefotetan + Doxycycline: 34%) [55]. Despite the high efficacy observed in patients receiving triple therapy, the small size of the study and the lack of a direct comparison necessitate further research. Drug hypersensitivity or aminoglycoside toxicity (renal failure) should be considered when selecting the treatment.

Clinical trials have also indicated the necessity of adding pathogen-specific drugs in TOA cases where *Actinomyces Israelii* is isolated. In these patients, β-lactam antibiotics (Ampicillin-Sulbactam) should be standardly added to the treatment [21]. After completing the standard treatment, Penicillin should be continued for at least one more month. Doxycycline can be used in patients allergic to penicillin. There's no definitive data on the treatment duration; however, most experts recommend a Penicillin treatment duration of 3-6 months.

Several studies have also identified the rare occurrence of pathogens such as *Candida* species, *Pasteurella multocida*, and *Salmonella* as causes of TOA [40-42]. These pathogens are not typical endogenous agents of the genital flora and are not known etiological factors of TOA. Consultation with the Infectious Diseases department is advised for these cases [42].

Due to the potential for rapid clinical deterioration, the possibility of abscess rupture, and the development of sepsis, patients should be closely monitored for 48-72 hours [29, 50-54].

Minimal Invasive Drainage

Since the 1970s, various methods have been successfully employed to drain intraabdominal abscesses without the need for surgery [30, 34, 45]. There are diverse anatomical approaches for drainage documented in studies, including percutaneous, transvaginal, transrectal, and transgluteal methods. The success of drainage is influenced by the abscess localization, the adequacy of imaging technology, and the practitioner's experience. All aspirated fluids should be sent to the microbiology laboratory for aerobic and anaerobic culture. Various studies have shown success rates ranging from 70% to 100% for TOA drainage performed using CT or USG [43, 44]. For instance, a study involving the USG-guided drainage of 49 women with TOA found a definitive treatment rate of 74% without the need for surgical intervention [48]. Additionally, a separate retrospective study showed that adding antibiotic treatment to percutaneous drainage guided by USG or CT was more effective and shortened the treatment duration compared to antibiotic treatment alone [47]. Controlled studies are needed for definitive conclusions.

Generally, higher success is achieved with small, unilocular fluid collections (achieving clinical improvement without the need for surgery) [48]. For multilocular TOAs, clinical success with drainage treatment is lesser. The success rate in treatment increases with the experience and skill level of radiologists and the advancement of radiological imaging equipment.

As modern drainage procedures have evolved, gy-

necologists can now drain pelvic abscesses via the transvaginal route using the posterior colpotomy method. This method is most suitable for abscesses formed post-hysterectomy at the vaginal cuff. The procedure involves dissecting the rectovaginal septum. Cases of peritonitis and sepsis have been reported following this procedure [34]; hence, it must be conducted with great caution in cases of TOA [46].

Surgery

Intraabdominal TOA rupture is a life-threatening condition that requires emergency surgery [26]. A combination of antibiotic therapy and surgery should be performed in these cases.

Laparotomy is the most commonly used method for treating TOAs. The choice of incision for optimal pelvic visualization is crucial, with the Maylard transverse or vertical midline incision typically employed. Some data suggest laparoscopy as a successful option for patients without abscess rupture [49, 50]. Although laparoscopy has been suggested in small case series as an alternative to laparotomy, data are insufficient. The choice between laparoscopy and laparotomy largely depends on the surgeon's skill. Even without abscess rupture, laparoscopy should only be attempted by experienced surgeons. Surgical treatment of TOA involves extensive and complex procedures involving inflamed tissues and various intraabdominal organs.

The importance of experienced surgeons cannot be overstated. Both laparotomy and laparoscopy for TOA are challenging due to the anatomical distortions caused by inflammation and the fragile nature of the tissues. Involvement of an experienced colorectal surgeon is common practice in these operations. Preoperative considerations should account for these conditions, and if the clinical scenario permits, preoperative bowel preparation should also be undertaken [47-50]. The surgical procedure for TOA includes:

- Confirming the diagnosis of TOA
- Draining as much infected and inflamed fluid and debris from the abscess cavity as possible
- Irrigating the peritoneal cavity extensively to reduce the patient's infection and inflammation load

Cultures for aerobic and anaerobic bacteria should be taken from the abscess material and the peritoneal cavity itself. Tissue pathology should be sent from the abscess cavity to confirm microbiological findings. All removed materials should be sent for pathology, as TOAs, especially in postmenopausal women, can sometimes be associated with malignancy.

Total Abdominal Hysterectomy + Bilateral Salpingo-Oophorectomy (TAH + BSO) as a means to remove all infected tissues is an old method. While this may sometimes be the best method, unilateral salpingo-oophorectomy (USO) is considered sufficient treatment for unilateral TOA [26, 29, 34]. USO is preferred over TAH + BSO due to its preservation of fertility and hormonal functions and lower morbidity. Especially in premenopausal women, USO is chosen to preserve fertility.

TAH+BSO is the preferred method for patients with acute abdomen and sepsis who have completed their fertility. This aggressive approach speeds recovery compared to other less invasive surgical procedures. Moreover, TAH + BSO eliminates the 10-20% chance of abscess recurrence seen with other surgical methods [26, 29, 49]. In complicated TOA cases, treatment should be combined with clinical experience whenever possible.

The risk of wound site infection due to surgical field contamination from abscess rupture is high. During closure, a drain should be placed subcutaneously, monitored until minimal output is observed, and clinical improvement is achieved.

Complications

Abscess rupture is observed in 15% of TOAs [30]. Rupture is a life-threatening situation requiring emergency surgery.

Sepsis is observed in 10-20% of TOAs. Immediate surgical intervention is strongly recommended if a sepsis scenario is observed in TOAs, as delaying surgery can lead to more severe clinical outcomes [46]. Another complication is the development of postoperative wound site infections. Patients with systemic diseases (such as diabetes mellitus) are more prone to developing wound site infections [50].

Gastrointestinal system injuries are among the complications that can occur intraoperatively. Therefore, if surgery is planned for patients with TOA, having a preoperative colorectal surgery team on standby is recommended [51].

Special Clinical Situations in TOA Treatment

In patients with TOA who have an IUD, removal of the IUD is recommended along with the adminis-

tration of antibiotic therapy based on the severity of the disease. *Actinomyces Israelii* is typically the specific agent in TOA cases with IUDs. There is no evidence to suggest that removing the IUD has a positive effect on the treatment of A. Israelii or other pathogens in TOA treatment. However, since *Actinomyces Israelii* proliferates on foreign bodies, removal of the IUD is advised [46, 47].

Although most women with TOA are of reproductive age, TOA can also occur in postmenopausal women. Various case series have shown that a significant portion of TOAs observed in the postmenopausal period are associated with malignancy [46-49]. In one series, genital system malignancy was observed in 8 out of 17 postmenopausal women, while only 1 out of 76 premenopausal women had a genital system malignancy [36]. Therefore, if TOA is detected in the postmenopausal period, patients should be thoroughly evaluated for malignancy, and surgical treatment rather than antibiotic therapy or minimal invasive drainage should be recommended. Intraoperative frozen section consultation is also advised. Additionally, evaluation for pelvic and abdominal metastasis should be conducted. Surgical staging should be performed if the patient consents and sufficient expertise for proper staging is available.

TOA is rare in pregnant women [55, 56]. The approach to a pregnant woman with TOA is similar to that for a non-pregnant woman. Care should be taken to avoid antibiotics with teratogenic effects (such as quinolones) in the treatment.

CONCLUSION

The management of Tubo-Ovarian Abscesses (TOAs) presents a challenging dilemma in gynecological practice, reflecting a critical intersection of infectious disease, reproductive health, and surgical intervention. This review has traversed the multifaceted landscape of TOA management, from epidemiological insights to therapeutic strategies and the complex considerations surrounding minimally invasive techniques and surgical interventions. The evolution of management paradigms, underscored by the increasing reliance on broad-spectrum antibiotics and the strategic integration of minimally invasive procedures, marks a significant stride towards optimizing patient outcomes while minimizing procedural morbidity.

Despite these advancements, TOAs continue to pose significant risks, including the potential for severe complications such as sepsis, infertility, and chronic pelvic pain. The critical balance between the effective resolution of abscesses and the preservation of fertility, particularly in the context of surgical decision-making, remains a pivotal concern. Moreover, the emergence of antibiotic-resistant strains necessitates a vigilant, dynamic approach to empirical therapy selection, emphasizing the importance of ongoing research and the adaptation of treatment protocols to meet evolving microbial challenges.

The significance of early diagnosis cannot be overstated, as it serves as a cornerstone for effective management, potentially mitigating the progression to severe complications. A multidisciplinary approach, incorporating the expertise of gynecologists, radiologists, infectious disease specialists, and, when necessary, colorectal surgeons, is essential for the comprehensive management of TOAs. This collaborative framework facilitates a nuanced understanding of each patient's unique clinical scenario, enabling tailored treatment strategies that optimize therapeutic efficacy while considering individual fertility goals and risk profiles.

Looking forward, there is a pressing need for high-quality, evidence-based research to further refine the management of TOAs. Future studies should aim to elucidate the optimal timing and selection criteria for surgical intervention, assess the long-term outcomes of current treatment modalities, and explore the potential of emerging therapies. Additionally, investigations into the pathophysiology of TOAs, particularly in the context of microbial resistance patterns, could yield valuable insights, paving the way for innovative therapeutic approaches.

In conclusion, the management of TOAs demands a judicious, evidence-based approach, underscored by a commitment to patient-centered care. As we navigate the complexities of this condition, the pursuit of enhanced diagnostic techniques, more effective treatments, and comprehensive management strategies remains paramount. Through continued research and interdisciplinary collaboration, we can aspire to improve the quality of life for affected individuals and mitigate the long-term morbidity associated with TOAs.

Authors' Contribution

Study Conception: GEA; Study Design: GEA, TB; Supervision: TB, HZG; Funding: N/A; Materials: N/A; Data Collection and/or Processing: GEA; Statistical Analysis and/or Data Interpretation: TB, HZG; Literature Review: GEA; Manuscript Preparation: GEA and Critical Review: GEA, TB.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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The European Research Journal 2024;10(5):544-549 Case Report

DOI: https://doi.org/10.18621/eurj.1444689 *Radiology*

Invasive micropapillary type male breast cancer: a case report

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ABSTRACT

Male breast cancer is rarely seen and peaks at the age of 71. Hyperestrogenism, gonadal dysfunction, obesity, and alcohol are among the risk factors for male breast cancer. In hereditary cases, BRCA2 mutation is often observed. The most common symptom is a painless mass that is palpable behind the nipple. Most male breast cancers are ductal carcinomas (81%), papillary carcinomas (13%), and mucinous carcinomas (6%). The invasive micropapillary carcinoma of the breast is important due to its high rate of lymphnode metastasis, recurrence, and distant metastasis. Mortality is 50% higher in men with lymphnode involvement compared to non-involved cases. Furthermore, as the number of affected lymphnodes increases, the prognos is worsens. In this study, we examined a case of invasive micropapillary type male breast cancer with clinical presentation as a palpable mass, lymphnode involvement, and aimed to highlight the radiological considerations that need attention.

Keywords: Breast cancer, male breast cancer, micropapillary type cancer

Male breast cancer is a rare condition, accounting for less than 1% of all male malignancies and only 1% of all breast cancer cases [1]. The average age at diagnosis for men with breast cancer is 67, which is approximately 5-10 years later than the average diagnosis age for women [2]. While most men with breast cancer do not have an identifiable risk factor, factors such as Klinefelter Syndrome, obesity, testicular anomalies, cirrhosis, exogenous estrogen therapy, radiation exposure, occupational exposures, and BRCA mutations are among the risk factors. Gynecomastia, however, is not among the risk factors [1, 3]. The most common symptom is a painless mass palpable behind the nipple. The left breast is more commonly affected than the right, and about 1% of cases are bilateral [1]. In the evaluation of a breast mass in men, ultrasound (USG) is generally the first choice, but mammography can also be used. Mammography can often distinguish between malignancy and gynecomastia. Mammographic features suggestive of malignancy includes piculated margins and eccentricextension. Microcalcifications are less frequently observed in male breast cancer compared to female breast cancer [4]. Most male breast cancers are ductal carcinomas (81%), followed by papillary carcinomas (13%) and mucinous carcinomas (6%) [5]. The term invasive micropapillary breast carcinoma (IMPC) was first introduced to

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How to cite this article: Acar MS, Şimşek S, Urfalı FE, Korkmaz M. Invasive micropapillary type male breast cancer: a case report. Eur Res J. 2024;10(5):544-549. doi: 10.18621/eurj.1444689

Received: March 2, 2024
Accepted: May 10, 2024
Published Online: May 26, 2024

the literature by Siriaunkgul and Tavassoli in 1993 [6]. The World Health Organization's 2012 histological classification of breast tumors requires an invasive micropapillary component of at least 50% to diagnose IMPC. Microscopic papillary growth patterns accompany IMPC types at a rate of 3-6% [7]. The frequency of pure IMPC is reported to be 0.9% in one study [8]. Invasive micropapillary carcinoma of the breast is significant due to its high rate of lymphnode metastasis, recurrence, and distant metastasis [9, 10]. Standard early-stage breast cancer treatment in men involves hormone therapy, chemotherapy, or radiotherapy following surgery. Treatment varies based on the patient's prognostic factors, similar to women [11]. The most important prognostic factors for male breast cancer are tumor size and lymphnode involvement. Lymphnode involvement is 40% higher in patients with tumors measuring between 2-5 cm compared to those with tumor size < 2 cm. Similarly, mortality is 50% higher in men with lymphnode involvement compared to those without. Moreover, prognosis worsens as the number of involved lymphnodes increases [12, 13].

CASE PRESENTATION

A 64-year-old male patient presented to the general surgery outpatient clinic with complaints of swelling and a palpable mass in the right breast. Apart from type 2 diabetes mellitus, the patient had no other significant disease history. Upon physical examination, gynecomastia was noted, along with a palpable mass in the retroareolar area of the right breast and in the right axillary tail. Subsequently, a breast ultrasound was performed. The breast ultrasound revealed a solid lesion in the retroareolar area of the right breast, measuring 15 × 16 mm, with irregular borders and spiculated extensions. The lesion exhibited a hypoechoic appearance, and it raised suspicion for a hypoechoic skin-directed breast cancer, classified as a BIRADS-5 solid lesion (Fig. 1). Additionally, pathological lymphadenopathies (LAP) with a conglomerate appearance were observed in the right axilla, with the largest measuring up to 25 × 43 mm (Fig. 2).

Histopathological diagnosis of the mass lesion under local anesthesia, a tru-cut biopsy was performed on the mass in the retroareolar area of the right breast and on the pathological lymphadenopathies (LAP) in the right axillary tail. The biopsy evaluation of the tissue from the retroareolar region revealed invasive ductal carcinoma. Similarly, the biopsy evaluation of the tissue from the LAP in the right axilla indicated metastasis of invasive ductal carcinoma (micropapillary type) (Fig. 3).

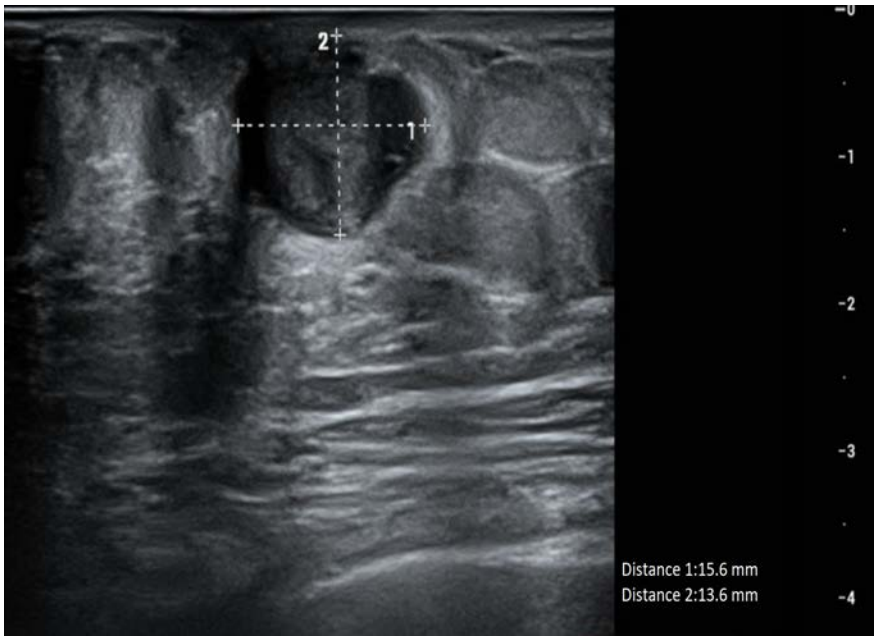

Fig. 1. The ultrasonographic image of the mass lesion in the breast.

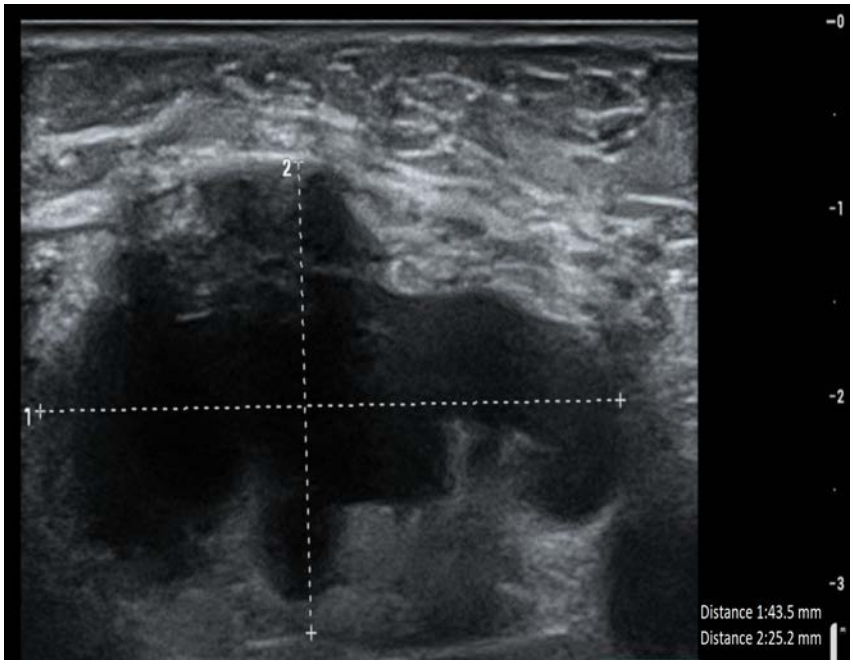

Fig. 2. The ultrasonographic image of the conglomerate lymph nodes in the right axillary fossa.

After the tissue diagnosis of the patient, a whole-body positron emission tomography-computed tomography (PET-CT) scan revealed a mass lesion approximately 15 × 16 mm in size showing pathological fluorodeoxyglucose (FDG) uptake in the retroareolar area of the right breast (Fig. 4). In addition, multiple lymphnodes with the largest approximately 23 × 45 mm size were observed in the right axillary fossa (Fig. 5).

After undergoing whole-body PEC-CT scanning,

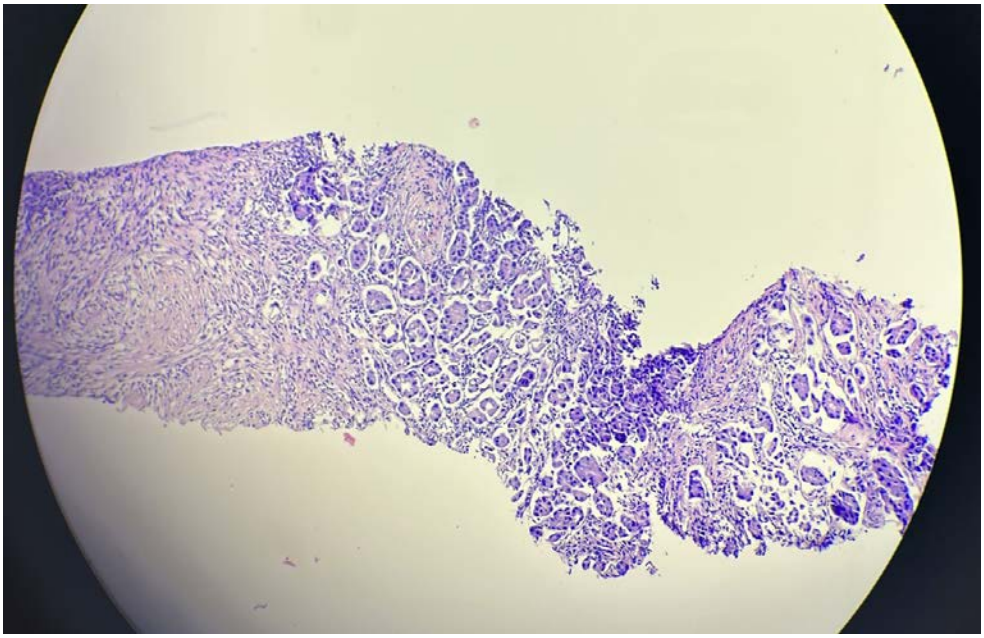

Fig. 3. The pathological preparate image of the biopsy material taken from the right axillary lymph node.

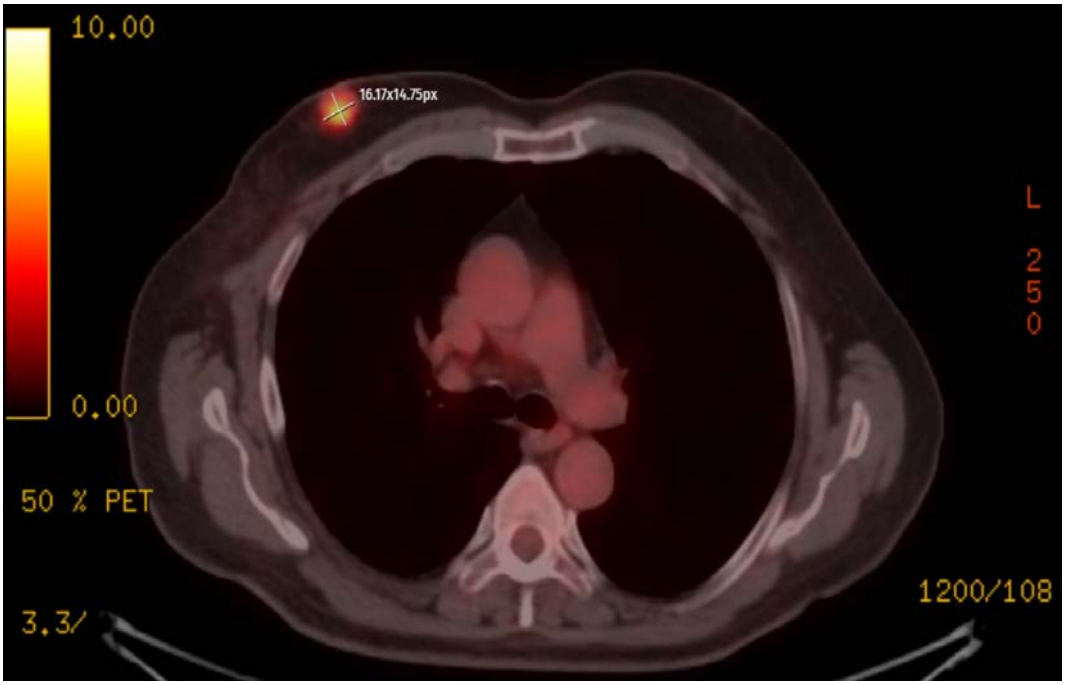

Fig. 4. **The mass lesion showing pathological fluorodeoxyglucose uptake in the retroareolar area of the right breast.**

the patient was started on neo-adjuvant cyclophosphamide + adriamycin therapy to be administered every three weeks. In the follow-up ultrasound (USG) after completing chemotherapy, it was observed that the lesion size in the retroareolar area of the right breast had decreased to 9 × 13 mm, and the largest

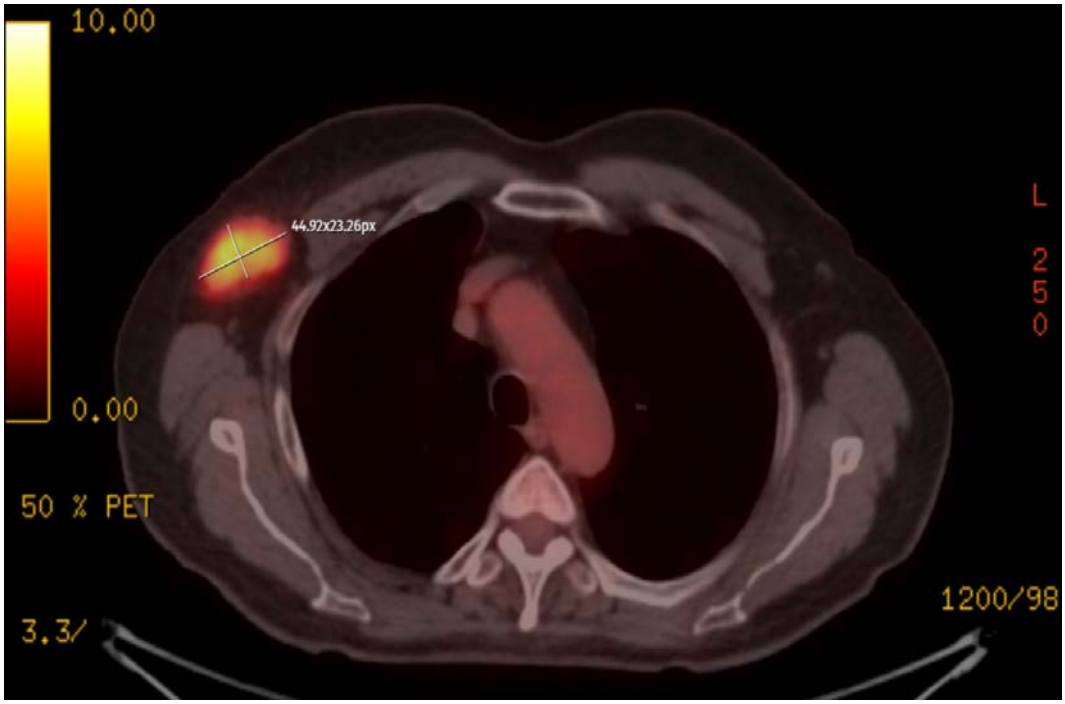

Fig. 5. **Multiple lymph nodes showing pathological fluorodeoxyglucose uptake in the right axillary fossa.**

pathological lymphnode size in the right axillary fossa had decreased to 10 × 20 mm. Following neo-adjuvant chemotherapy, the patient underwent modified radical mastectomy and rightaxillary lymphno dedissection. Adjuvant radiotherapy was applied to the right breas localization and right axillary fossa following surgery in a patient with negative surgical margins. follow-up USG was recommended three months later for further monitoring.

DISCUSSION

As the incidence of male breast cancer cases, which is increasing everyday, most cases are referred to radiology clinics for further evaluation of the mass after being detected through manual examination. Among the initial imaging methods, mammography and ultrasonography are preferred. Following imaging, tru-cutbiopsy under ultrasound guidance is performed for cases suspected of malignancy [14].

Risk factors typically revolve around hormonal imbalances such as androgen deficiency or estrogenexcess. While some patients have organic causes contributing to hormonal imbalances, such as testicular or liverdamage, in most cases, no clear cause can be identified. Mutations in the BRCA tumor suppressor gene are also a risk factor for male breast cancer. BRCA-2 gene mutation is more common than BRCA-1 mutation in this context [15].

Male breast cancer is often detected at later ages and stages, which adversely affects treatment and survival outcomes. The average age of diagnosis for IMPC is 69.8 years, slightly older than in women. In a retrospective study, the incidence of IMPC among included cases of invasive ductal carcinoma was found to be 0.4% [14].

Micro papillary type breast cancer tends to be more aggressive compared to other types of breast cancer, which further emphasizes the importance of early diagnosis and treatment. In a study, it was found that IMPC exhibits lymphotropic behavior, with lymphnode metastasis detected in 73.7% of cases, as in our case. In a study conducted by Luna-More *et al*. [9], the positivity rates for estrogenreceptor (ER), progesteronereceptor (PR), and HER2/neureceptor were reported as 71.3%, 48.3%, and 51%, respectively [9]. However, in a study by Gokce *et al*. [16], it was stated that in IMPC cases showing ER and PR negativity along with c-erb-B2 overexpression, the rate of local recurrence was higher [16]. In our case, ER and PR were found to be negative, and c-erb-B2 overexpression was detected. Furthermore, in the same study, a positive correlation was found between overall survival rate and ER positivity, while nocorrelation was found between PR positivity and c-erb-B2 overexpression with survival.

Diagnosis and treatment of male breast cancer are fundamentally similar to those of female breast cancer, although there are some differences. Numerous studies have demonstrated the benefits of adjuvant, neo-adjuvant hormone therapy, chemotherapy, andradiotherapy. Tamoxifen remains the gold Standard for adjuvant hormone therapy. Selective aromataseinhibitors such as anastrozole, letrozole, andexemestane can also be used in hormone therapy. There are many studies supporting the effectiveness of the combination of adjuvant hormone therapy and chemotherapy. In surgical treatment, radical mastectomy and lymphnode dissection, which are associated with lower rates of local recurrence, are performed [17, 18].

CONCLUSION

In our case, modified radical mastectomy with lymphnode dissection was performed following neo-adjuvant chemotherapy. Subsequently, the treatment process was completed with radiotherapy, and the patient was placed under USG monitoring at regular intervals.

Patient' Consent

Patient was informed about the purpose of the case report, and informed consent was obtained from the patient for this publication.

Authors' Contribution

Study Conception: MSA, SŞ, FEU, MK; Study Design: MSA, SŞ, FEU, MK; Supervision: MK; Funding: ACP, KK; Materials: SŞ; Data Collection and/or Processing: N/A; Statistical Analysis and/or Data Interpretation: N/A; Literature Review: MSA, FEU; Manuscript Preparation: FEU and Critical Review: FEU.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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