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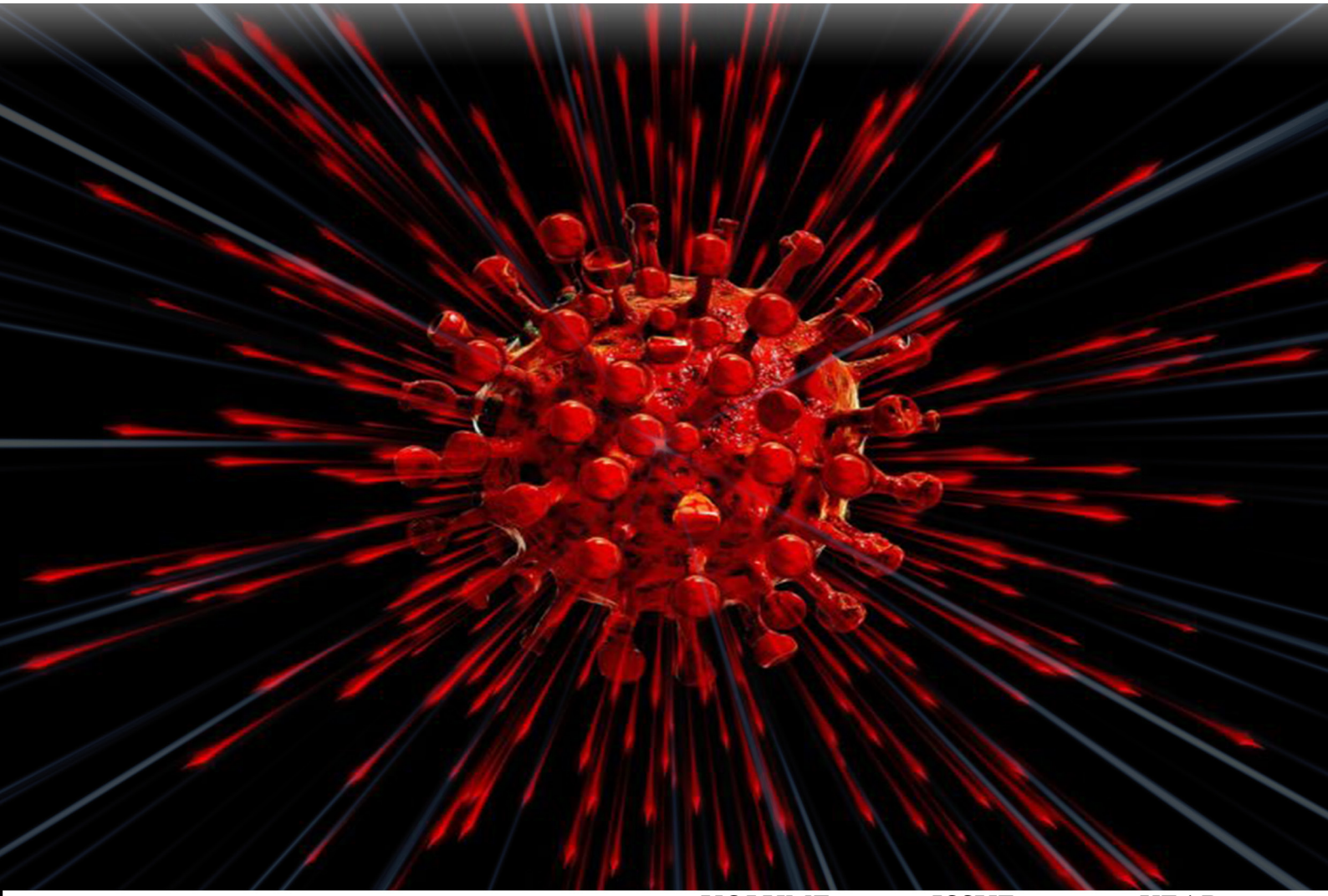

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Original Article

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Termination of pregnancy for fetal anomaly: evaluation of single centre results

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ABSTRACT

Aims: To assess the indications of termination of pregnancies (TOP) for fetal anomaly beyond 22 weeks of gestation.

Methods: It is a retrospective study conducted at Eskişehir City Hospital between January 2021 and December 2023. Our hospital which It serves as a tertiary referral center in the city. During the research period, 4143 births took place, and 495 women were treated in our high-risk unit. During the study period, 34 women underwent elective TOP at at least 22 weeks' gestation. Routine 18–20-week anomaly screening was recommended for all pregnant women.

Results: The sonographic findings revealed the presence of 15 cases of central nervous system malformation, 4 cases of micromelia and thorax hypoplasia, 1 case of diafragma hernia, 3 case of early IUGG, 2 cases of cardiac anomaly, 2 cases of neural tube defect, 3 cases of isolated thorax hypoplasia, 1 case of scoliosis, 1 case of renal agenesis, 1 case of thick nuchal fold, 1 case of partial corpus collosum agenesis. Among 34 cases, there were 1 case of mosaic trisomy 21, case of trisomy 21, 1 case of trisomy 1, 3 cases of trisomy 13, 2 cases of trisomy 18. Array analysis revealed a case of 14 p deletion, 1 case of 16 p 11.2 deletion, 1 case of 1p36 deletion. In all study population 5 cases underwent cesarean delivery. Majority of the terminations were achieved by misoprostol or cervical balloon application.

Conclusion: In conclusion, although late termination of pregnancy by fetocide procedure seems to be safe, earlier detection of fetal structural and chromosomal anomalies is mandatory.

Keywords: Fetosid, termination of pregnancy, fetal anomaly

INTRODUCTION

Advances in medical technology and the introduction of routine prenatal screenings have led to diagnosis of various fetal malformations during prenatal care.[1] In case of untreatable disease or malformation detected during prenatal care, majority of the parents seek for legal termination options of pregnancy due to fetal anomaly. There were many religious, social and ethical questions which were adressed by many articles which was not the scope of this article. Due to the possible abusements, Turkish law allows termination of pregnancy (TOP) in two cases: voluntary and elective since 1983. Abortion is just allowed before the 10th week of pregnancy in unwanted pregnancies. On the other hand, a pregnancy can be terminated at any gestational age if there is a serious maternal condition and/or untreatable fetal condition. There is no upper pregnancy limit in the legislation regarding pregnancy termination. According to the Turkish laws, approval for elective TOP may be given by two experts who declare that there is a maternal or fetal reason justifying TOP (law no. 2827-5, 1983). In majority of the European countries including France; England; Wales; Belgium; Finland; Norway, under limited circumstances; and Sweden, TOP can be performed until term in cases of fatal or serious fetal abnormalities., following approval by the National Board of Health and Welfare.[2] The aim of this study is to evaluate the indications for TOP in fetal anomalies that develop after the 22nd week of gestation in our clinic.

METHODS

It is a retrospective study conducted at Eskişehir City Hospital between January 2021 and December 2023. This

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study was conducted in accordance with the principles of the Helsinki Declaration and approved by the Eskişehir City Hospital Ethics Committee (Date: 22/11/2023, Decision No: 2023-62). Our hospital serves as a tertiary referral center in the city. During the research period, 4143 births took place, and 495 women were treated in our high-risk unit. During the study period, 34 women underwent elective TOP at least 22 weeks' gestation. Routine 18–20-week anomaly screening was recommended for all pregnant women. Ultrasound examinations were performed using Voluson E8 Expert (GE Healthcare, Chalfont St Giles, England). Examinations and invasive procedures were performed by the same specialist. Following completion of the fetal anomaly investigation, the couple was counseled by a multidisciplinary medical panel consisting of experts in obstetrics and gynecology, pediatrics, and pediatric surgery. The counseling included information on the termination procedure in addition to alternative management options. Since our study population includes pregnant women over the 22nd week of pregnancy, fetocide (intravascular potassium chloride injection) is performed in the prenatal period. Medical reports of all TOP cases were reviewed during the study period. Medical and demographic characteristics were recorded.

RESULTS

Mean age, gravidity, parity, number of miscarriages and gestational age at termination took place of the study population were 29.8±6.5 (18-44), 2.41 (1-6), 1.12 (0-5), 0.3 (0-4), 25.2 (23-32) respectively (**Table 1**). There were 13 (38.2 %) cases of primigravid where as 21 cases had at least 1 previous delivery. There were 23 cases with high secıond trimester screening test (32.4 %, 1/270). According to the sonographic findings, there were 15 cases of central nervous system malformation, 4 cases of micromelia and thorax hypoplasia, 1 case of diafragma hernia, 3 case of early IUGG, 2 cases of cardiac anomaly, 2 cases of neural tube defect, 3 cases of isolated thorax hypoplasia, 1 case of scoliosis, 1 case of renal agenesis, 1 case of thick nuchal fold, 1 case of partial corpus collosum agenesis.Among 34 cases, there were 1 case of mosaic trisomy 21, case of trisomy 21, 1 case of trisomy 1, 3 cases of trisomy 13, 2 cases of trisomy 18. Array analysis revealed a case of 14 p deletion, 1 case of 16 p 11.2 deletion, 1 case of 1p36 deletion. In all study population 5 cases underwent cesarean delivery. Majority of the terminations were achieved by misoprostol or cervical balloon application.

Table 1. Demografic characteristics of the study population

	n	Min±Max	Mean±SD
Age (years)	34	18±44	29.79±6.484
Height (cm)	34	150±175	163.65±5.624
Weight (kg)	34	51±113	71.71±15.518
Gravidity	34	1±6	2.41±1.480
Parity	34	0±5	1.12±1.175
Number of miscarriages	34	0±4	.29±.799
Gestational age at termination	34	23±32	25.15±2.862

Min: Minimum, Max: Maximum, SD: Standard deviation

DISCUSSION

Consistent with the previous study, the present study showed that a substantial proportion of TOPs beyond 22 weeks of gestation for fetal anomaly could have been performed earlier with timely diagnosis.[2] Again consistent with the previous studies the majority of anomalies in our study population were central nervous system defects, multiple malformations, and genetic–chromosomal diseases.[2,3-5] Recent reports indicated that structural anomalies had a higher frequency in the TOPs beyond 22 weeks of gestation, while genetic–chromosomal anomalies were more frequent in the early TOP group.[4,6] In our study population, all cases were beyond 22 weeks of gestation, and in all cases fetocide was required before labor induction, among 34 cases chromosomal abnormality was detected in 8 of the cases whereas 23 cases had structural anomaly detected by sonography at 20 weeks fetal anomaly screening. In our study population there were 2 cases of trisomy 21, 2 cases of trisomy 18 and 3 cases of trisomy 13, distribution of chromosomal anomalies was not consistent with the previous reports in which the most frequent chromosomal anomaly was trisomy 21.[2]

In our cohort, there was case with cardiac malformation, the rate in a study from Turkey was 4.6%. In majority of the studies, cardiac malformation rate was reported around 3-10% of the cases.[4-9] It seems that recent advances in ultraound technology and increase in the number of echocardiographers may lead to higher detection rate of cardiac anomalies.[10] The most commonly used method for fetocyst for late termination of pregnancy due to fetal abnormalities (TOPFA) involves injecting potassium chloride (KCl) into the fetal heart and is likely to be painful after the 22nd week of pregnancy. Previods study demonstrated that, fetal umbilical phlebotomy followed by fetocystomy for fetal analgesia therefore appears to be a safe procedure for the mother and allows painless death of the fetus when late termination of pregnancy (TOP) is indicated.[11] Another fetocide procedure was reported to be injection of lidocaine (1%) to create permanent fetal cardiac asystole for the purpose of fetocyst in late termination of pregnancy. Data showed the procedure

to be successful in 92% (46/50) of cases, with complete cessation of cardiac activity, in one case, lidocaine failed and fetocyst was applied with KCl. No maternal side effects were observed. Authors concluded that lidocaine is an effective drug in inducing fetocyst at doses below the toxic dose for the mother.[12]

When a fetal abnormality is diagnosed, the woman may be advised to terminate the pregnancy. It is important to avoid delays in the evaluation process because after the 21st week of pregnancy, fetocyst is required as part of termination of pregnancy, with only a few rare exceptions. According to the stusy, 47 pregnancies were terminated after the 24th week of gestation. In 43 cases, intracardiac potassium chloride fetocide was administered before labor induction. One in 47 women was not referred to a tertiary referral centre; 39 women were referred within 1 week of diagnosis of the abnormality, but in seven cases referral was delayed by more than 2 weeks. Authors concluded that, Inconvenient and avoidable delays occur. In some cases this may mean that the woman must undergo a fetocite, which could have been avoided if she had been referred to a tertiary center more quickly.[13]

As the risk of complications increases with advancing gestational age[14] and according to the recent data indication possibility of identification over half (56%) of major cardiac abnormalities during first trimester screening program, which constituted approximately two-thirds (64%) of all major cardiac anomalies detected antenatally.[15]

Spontaneous vaginal delivery should be a primary option for pregnancy termination with fetal malformations, in most pregnancies complicated by the presence of fetal anomalies, Some fetal malformations may be assiciated with dystocia, bleeding, or disruption of a protective sac. Giant omphaloceles, severe hydrocephalus, and large myelomeningocele and teratomas were reported to be some of the anomalies which may necesitates cesarean delivery. As cesarean delivery is associated with higher cost and complications, For this reason early detection of anomalies is crucial.[16]

In our series majority of the most commonly encountered anomalies were presented with consistent rates with the literature, and although our data and previously published data showed lower complication rates, unwanted psychological impact and health burden may be avoided with timely detection of major anomalies.

The limitations of this study include the relatively small number of patients and the fact that the research was conducted at a single medical centre.

CONCLUSION

In conclusion, although late termination of pregnancy by fetocide procedure seems to be safe, earlier detection of fetal structural and chromosomal anomalies is mandatory.

ETHICAL DECLARATIONS

Ethics Committee Approval

Approved by the Eskişehir City Hospital Ethics Committee (Date: 22/11/2023, Decision No: 2023-62).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Hematological markers for prediction computed tomography findings in mild traumatic brain injury

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ABSTRACT

Aims: This study aims to examine the levels of the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) in mild head injury (mTBI) patients to determine their predictive value for the necessity of head computed tomography (CT).

Methods: mTBI patients admitted to the emergency department demographic details, levels of NLR and PLR, and outcomes from brain CT scans were evaluated. Based on the CT scan outcomes, patients were classified into two groups: one with no detectable abnormalities (group 1) and another with detected abnormalities as acute epidural hematoma, acute subdural hematoma, or subarachnoid hemorrhage (group 2). The levels of NLR and PLR were then compared across these groups.

Results: In the study, out of 221 patients, 131 (59.3%) were male, and the overall mean age was 51.47±13.91 years. The most common cause of admission was traffic accidents, accounting for 70 patients (31.7%). The mean Glasgow Coma Scale score of the patients was 13.99±0.94. Group 2 consisted of 66 patients (29.9%), with 44 (66.7%) having an acute subdural hematoma, 16 (24.2%) with subarachnoid hemorrhage, and 6 (9.1%) with acute epidural hemorrhage. The mean NLR and PLR were 1.85±0.77 and 133.99±51.70, respectively. NLR values in group 2 were significantly higher than those in group 1 ($p<0.000$), whereas no significant difference was found in PLR values between the groups ($p>0.05$). The optimal cutoff value for NLR was determined to be >1.64.

Conclusion: NLR levels, readily derived from standard hematological assessments, function as an objective and inflammatory biomarker. Initial NLR measurements hold the potential for forecasting abnormal findings in head CT scans associated with mTBI patients.

Keywords: Traumatic brain injury, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, computed tomography, mild head injury

INTRODUCTION

The emergency department (ED) is often the first point of care for patients with traumatic brain injury (TBI). TBI is defined as damage to the brain caused by an external mechanical force, common causes include falls, strikes by objects, car crashes, assaults, and self-harm.[1,2] TBI stands as the principal reason for morbidity and mortality in individuals younger than 40 years in both developed and developing countries, imposing a substantial economic burden.[3]

Mild TBI (mTBI) involves a Glasgow Coma Scale (GCS) score ranging from 13 to 15 at 30 minutes after the injury, with short-term or localized neurological disturbances that do not involve loss of consciousness for more than 30 minutes or post-traumatic amnesia for less than a day.[4]

A head computed tomography (CT) scan is an effective diagnostic tool for detecting traumatic intracranial conditions following mTBI.[5] About 10% of mTBI patients develop intracranial complications, 1% of these cases require neurosurgical treatment, and the fatality rate is 0.1%.[6] The decision to perform a CT scan on a patient with mild head trauma is ultimately up to the discretion of a medical specialist.[7] Systematic screening of all mTBI patients in the ED would be costly, and the associated ionizing radiation presents certain health risks.[8]

Identifying a biomarker that facilitates decision-making for conducting head CT scans in patients with mTBI is crucial. Although blood biomarkers such as C-terminal hydrolase-L1, glial fibrillary acidic protein, and S100B

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have been explored for predicting traumatic cerebral injuries on CT scans, they remain expensive and not broadly accessible.[9]

TBI is a complex multi-system condition characterized by interactions between the brain, peripheral, and immune systems.[10,11] Two pathological processes are pivotal in TBI.[12] The initial neurological damage, known as primary injury, occurs directly at the moment of impact and mechanically harms brain tissue. This damage can trigger a secondary pathological process where injured brain cells release various inflammatory factors and neurotransmitters, initiating and perpetuating an inflammatory cascade. This results in neuroinflammation, further exacerbating brain damage, referred to as secondary brain injury.[13]

Recently, the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) have gained attention for their roles in indicating inflammation.[14] These ratios, which are easily calculated from the complete blood count available in routine laboratory tests, are part of our daily medical assessments.

This study aims to examine the levels of the NLR and the PLR in mTBI patients to determine their predictive value for the necessity of head CT.

METHODS

This retrospective analysis was conducted from February 1, 2023, to September 1, 2023, in the ED of our hospital. Ethical approval was granted by the Ankara Etlik City Hospital Clinical Researches Ethics Committee (Date: 27.09.2023, Decision No: 2023-588). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients aged 18 and older who were admitted to the ED with isolated head trauma and mTBI characterized by a GCS score of 13-15 were included in the study. Exclusion criteria for patients included ED admission over three hours post-injury, pregnant patients suffering from drug overdoses, those with a history of neoplastic, cardiac, hepatic, renal diseases, bone marrow dysfunction, ischemic or hemorrhagic stroke, penetrating brain injuries, or those with incomplete medical records. During the patient file review, the study incorporated comprehensive neurological assessments, etiologies of trauma, and symptomatic reports from patients, including headaches, nausea, vomiting, episodes of loss of consciousness, and seizure durations, as documented in their medical histories.

Venous blood samples were systematically drawn from the patients upon arrival and promptly analyzed for a complete blood count in the hospital's laboratory.

Computed Tomography Scan

Noncontrast head CT scans were performed utilizing multislice CT technology within 30 to 60 minutes of ED admission. Data collected included demographic profiles, NLR, PLR, and brain CT scan results. Based on the outcomes of the head CT scans, patients were stratified into two cohorts: group 1, consisting of patients without detectable CT abnormalities, and group 2, comprising patients with identified abnormalities such as acute epidural hematoma, acute subdural hematoma, or subarachnoid hemorrhage. Subsequently, a comparative analysis of NLR and PLR levels was conducted between the groups.

Statistical Analysis

Descriptive statistics for continuous variables encompassed the calculation of mean values, standard deviations, medians, and ranges (minimum and maximum values). For categorical variables, frequencies and percentages were reported. The Shapiro-Wilk test was applied to evaluate the adherence of continuous data to a normal distribution. For comparisons of nominal variables across different groups, the Chi-Square test was utilized. The Mann-Whitney U test facilitated the analysis of differences between two groups in continuous variables. The diagnostic efficacy of NLR and PLR was quantified using Receiver Operating Characteristic (ROC) curve analysis, specifically the area under the curve (AUC). The optimal cutoff point was determined using Youden's Index. Furthermore, the diagnostic accuracy parameters for NLR, including sensitivity, specificity, positive predictive value, and negative predictive value, were calculated. Data analyses were conducted using IBM SPSS for Windows version 20.0 (SPSS Inc., Chicago, IL), and statistical significance was set at a p-value of less than 0.05.

RESULTS

In this study, the cohort comprised 221 patients, of whom 131 (59.3%) were male. The average age across all patients was 51.47±13.91 years. The predominant cause of admission among the patients was traffic accidents, accounting for 70 patients (31.7%) (**Figure 1**). The mean GCS score was 13.99 ± 0.94. Within group 2, there were 66 patients (29.9%), with 44 (66.7%) diagnosed with acute subdural hematoma, 16 (24.2%) with subarachnoid hemorrhage, and 6 (9.1%) with acute epidural hemorrhage. The average NLR among the patients was 1.85±0.77, and the PLR was 133.99±51.70.

In group 2, the GCS scores were significantly lower than those observed in group 1 ($p<0.000$). NLR levels were notably higher in group 2 compared to group 1 ($p<0.000$). However, no significant differences were observed in PLR levels between the two groups ($p>0.05$), as shown in **Table 1**.

Table 1. Comparison of patients in group 1 and group 2					
Parameter	**Group 1 (n=155)**		**Group 2 (n=66)**		**p-value**
Gender	n		%		n
Female	67	43.2	23	34.8	0.246 [c]
Male	88	56.8	43	65.2	
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
Age	49.57±14.34	55 (18-69)	55.94±11.79	58.5 (25-72)	0.001 [b]
GCS	14.04±1.01	14 (6-15)	13.86±0.78	14 (13-15)	0.066 [b]
NLR	1.59±0.52	1.45 (1.00-4.06)	2.48±0.88	2.10 (1.12-4.42)	<0.000* [b]
PLR	150.27±72.91	130.11 (67.34-398.0)	127.06±37.59	112.5 (60.63-206.19)	0.155 [b]

b: Mann-Whitney U test, c: Chi-Square test, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet to-lymphocyte ratio, Min: Minimum, Max: Maximum, SD: Standart deviation

The predictive accuracy of NLR for indicating positive CT findings was statistically significant, with the AUC demonstrating significance (p<0.001). The optimal cutoff value for NLR was determined to be >1.64 (**Table 2**, **Figure 2**).

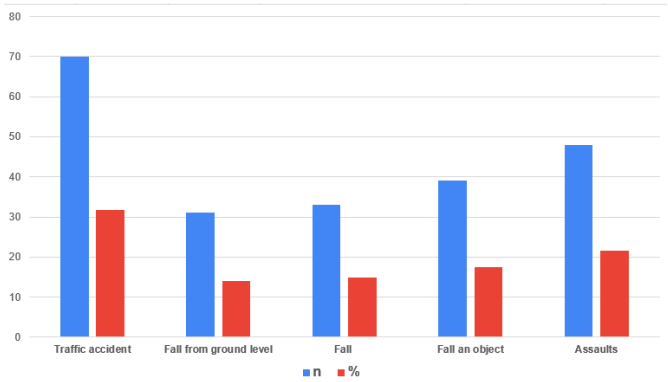

Figure 1. Patient admission cause to among the participants was traffic accidents

Table 2. Diagnostic performance of neutrophil-to-lymphocyte ratio levels in distinguishing positive findings on head computed tomography					
Parameter	**AUC (95% CI)**	**p-value**	**Cut-off**	**Sensitivity**	**Specificity**
NLR	0.805 (0.743-0.867)	<0.000	1.64	84.8% 74.3–91.6	63.2% 55.4–70.4

NLR: Neutrophil-to-lymphocyte ratio

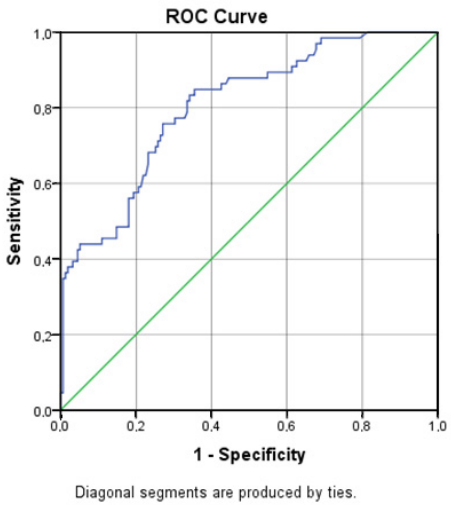

Figure 2. The predictive accuracy of NLR with ROC curve
ROC: Receiver Operating Charasteristics, NLR: Neutrophil- to-lympocyte ratio

DISCUSSION

mTBI constitutes a substantial public health challenge globally, exerting significant impacts on individuals. Noncontrast CT serves as the definitive standard for evaluating mTBI. The decision to perform a CT scan predominantly resides with the emergency physician. Medical professionals tasked with managing mTBI patients encounter complex decisions regarding the advisability of CT examinations. It is imperative to restrict unnecessary head CT scans to mitigate radiation exposure, particularly in patients with mTBI.[15] Moreover, the economic implications of redundant CT scans are not trivial, encompassing costs related to false-positive results and potential patient transfers to alternative medical facilities when CT capabilities are unavailable.[16]

In this study, it was determined that patients with mTBI presenting at the ED exhibited elevated NLR levels when head CT scans showed abnormalities. A threshold value of >1.64 was identified as the optimal cutoff for detecting abnormal head CT findings, achieving a sensitivity of 84.8% and a specificity of 63.2%. Conversely, the PLR levels at admission did not show statistically significant differences between patients with abnormal and normal CT scan results.

In cases of TBI, neurological impairments are initially induced by direct mechanical forces at the moment of impact, constituting the primary injury. These impairments subsequently evolve into a secondary injury phase, during which inflammation plays a critical role in exacerbating brain damage.[16] TBI leads to the disruption of the blood-brain barrier, triggering the mobilization of macrophages, neutrophils, and lymphocytes to the injury site.[17]

Under normal conditions, neutrophils are absent from the brain parenchyma due to the protective function of the blood-brain barrier; however, they are found in limited numbers within the cerebrospinal fluid, pia, and meninges.[18] Neutrophils contribute to increased oxidative stress, further damage to the blood-brain barrier, and the promotion of neuronal cell death.[19] Conversely,

lymphocytes play a crucial role in the repair of damaged brain tissue, not only by secreting growth factors but also by regulating microglial activity.[3]

The NLR is determined by dividing the absolute neutrophil count by the absolute lymphocyte count, both of which are derived from a complete blood count test.[13] NLR serves as a biomarker that integrates two aspects of the immune system: the innate immune response, predominantly mediated by neutrophils, and the adaptive immune response, facilitated by lymphocytes.[20] An increased NLR indicates a predominance of neutrophils relative to lymphocytes, suggesting the presence of an active inflammatory response.

Corbett et al.[21] identified that elevated NLR levels hold prognostic value in severe TBI patients undergoing decompressive craniectomy. In their study involving 144 adult patients, the median GCS score at admission was five. An NLR exceeding 15.63 upon admission was predictive of 28-day mortality. Furthermore, elevated NLR values during the first week of treatment were associated with severe disability in TBI patients.[22] In a longitudinal study, unfavorable outcomes at the 1-year follow-up were observed in 73.8% of head trauma patients. In this cohort, a high admission NLR for severe TBI correlated with poorer clinical outcomes. The sensitivity and specificity of an elevated NLR in predicting adverse outcomes were determined to be 60.2% and 71.1%, respectively.[23]

A comprehensive study on TBI involving 1.291 patients identified several factors as independent predictors of negative outcomes six months post-injury. These factors included age, admission GCS scores, the presence of subdural hematoma, intraparenchymal hemorrhage, traumatic subarachnoid hemorrhage, coagulopathy, and an elevated NLR.[24] Xie et al.[25] conducted a retrospective study with 93 patients suffering from diffuse axonal injury and found that a higher NLR at admission was independently associated with unfavorable outcomes at six months. Furthermore, the combinations of NLR-GCS and NLR-coma duration demonstrated superior predictive performance compared to using NLR, GCS, or coma duration alone. However, it is important to note that the sample size of this study was relatively small, and it primarily included patients with severe TBI. Research has demonstrated that in patients with TBI, those suffering from diffuse axonal injury exhibit significantly elevated NLR compared to patients with other TBI-related conditions such as cerebral edema, intracranial hematoma, subdural and/or epidural hematoma, and subarachnoid hemorrhage.[22]

In a study by Acar et al.[26] involving 200 patients with minor head trauma, significant differences in NLR were observed between those with brain pathologies and those with normal CT scans. A cutoff value of 4.29 was determined to effectively differentiate patients with traumatic brain injuries that involved brain pathology from those without, yielding a specificity of 90%.

In a study by Alexiou et al.[17] involving 130 patients with mTBI, 74 exhibited positive CT findings. The mean NLR at presentation was 5.6±4.8, and significantly higher NLR levels were noted in patients with positive CT findings. ROC analysis established an NLR threshold of 2.5 for detecting positive CT results, with a sensitivity of 78.1% and a specificity of 63%. Furthermore, patients with a GCS score of 14 or lower demonstrated a sensitivity of 26% and a specificity of 98.1% for detecting positive CT findings. Notably, the mean age in this study was 61.6±19.9 years, which is older than the population in our study. It is recognized that age can influence the inflammatory response and outcomes in TBI.[16]

The initial injury mechanism of TBI typically results in the rupture of capillaries and vessels, disrupting the blood-brain barrier. This breach prompts interactions between platelets and either endothelial cells or the subendothelial matrix, culminating in platelet adhesion, activation, and the formation of platelet emboli at the site of injury to promote hemostasis27. A reduced PLR might signal an early imbalance in coagulation and an increase in neuroinflammatory responses.[27]

Moreover, a study encompassing 247 participants found that elevated PLR values were correlated with poor clinical outcomes in individuals with subarachnoid and intracranial hemorrhages.[28] In a study involving 54 children diagnosed with isolated mTBI, it was observed that NLR levels were elevated in children presenting with mild head injuries and abnormal CT scans at the ED. An optimal cutoff value of 2.5 was determined for detecting abnormal head CT scans, with a sensitivity of 54.2% and a specificity of 89.5%. The mean NLR levels at presentation were recorded at 5.6±4.8. Additionally, children with abnormal CT findings exhibited lower PLR levels at presentation compared to those with normal CT scans, although the differences were not statistically significant.[29] Similarly, our study found that patients with abnormal CT findings also had lower PLR levels at presentation compared to those with normal CT scans, with no statistically significant differences. We hypothesize that this may be due to the PLR levels being measured early in the course of mTBI. We believe that conducting further studies with multiple measurements of PLR levels at different time points could provide more definitive guidance on this issue.

Limitations

The limitations of our study include its single-center, retrospective design, and relatively small sample size.

Such a structure might not fully capture the diversity and complexity of mTBI cases seen in a broader, real-world setting where patients often present with varied types of injuries. Understanding the interactions between different injury types is essential for more comprehensive clinical decision-making and for evaluating the efficacy of predictive biomarkers. Additionally, while patients with TBI were admitted to the ED within three hours of their head injury, the precise timing of the head injury itself could not always be accurately determined. There's variability in how much time passed since the injury. The exact timing of trauma could influence NLR and PLR levels, as inflammatory responses evolve over time. More detailed information on injury-to-sample time might refine the interpretation of results. Further research, including prospective studies and multi-center trials, is needed to validate the utility of these biomarkers in broader clinical practice.

CONCLUSION

Limiting unnecessary head CT scans is crucial in the management of mTBI. Inflammation plays a critical role in the pathology of TBI, and the NLR is a rapidly accessible, low-cost, objective, and reproducible inflammatory biomarker, easily calculated from routine hematological tests. The measurement of NLR at admission shows promise for predicting abnormal head CT findings in mTBI cases, potentially averting complications associated with delayed diagnosis and treatment. Furthermore, NLR can be seamlessly integrated into daily clinical practice without incurring additional costs.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Ankara Etlik City Hospital Clinical Researches Ethics Committee (Date: 27.09.2023, Decision No: 2023-588).

Informed Consent

Because the study was designed retrospectively, no written informed consent form were obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Incidence analysis of six diseases in the national newborn screening program: a retrospective study from Adıyaman, Turkiye (2019-2023)

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ABSTRACT

Aims: This study aimed to determine the incidence of diseases included in the national newborn screening program in Adıyaman, Turkey, over the past five years and to evaluate the relative status of Adıyaman compared to national and global data. The study also sought to identify potential risk factors based on demographic variables.

Methods: A retrospective, descriptive study was conducted in Adıyaman, Southeastern Turkey, analyzing heel blood sample data from 52,964 newborns between 2019 and 2023. The study excluded cases with unsuitable or retaken heel blood samples, partial biotinidase deficiency, and partial phenylalaninemia. Annual incidence rates were calculated based on confirmed diagnoses from relevant clinics.

Results: The five-year incidence rates in Adıyaman were found to be higher than the national averages for phenylketonuria (1:2407), congenital hypothyroidism (1:582), biotinidase deficiency (1:481), cystic fibrosis (1:10593), congenital adrenal hyperplasia (1:5864), and spinal muscular atrophy (1:9489). No statistically significant differences were identified based on gender, birth weight, birth week, or maternal nationality, except for biotinidase deficiency, which was significantly higher in infants of Turkish mothers.

Conclusion: The study highlights a higher incidence of screened diseases in Adıyaman than national averages, particularly biotinidase deficiency. Further research is recommended to investigate these findings and to address potential causes, such as consanguineous marriages.

Keywords: Newborn screening, phenylketonuria, biotinidase deficiency, congenital hypothyroidism, incidence

INTRODUCTION

Newborn screening programs are globally implemented for various diseases. Examples of these diseases include hearing impairment, developmental hip dysplasia, endocrine and metabolic disorders, and genetically inherited diseases such as spinal muscular atrophy.[1-3] The criteria for screening a disease were defined by Wilson and Jungler in 1968, and many of these are still in use today. These criteria include the disease being a significant public health issue, having effective treatment available, the screening test being feasible to apply, and being acceptable to society.[4] From a public health perspective, newborn screening programs constitute a critical group within secondary prevention programs. When diagnosed early, the diseases screened in this group generally progress with minimal impact on the individual and society through low-cost treatments and precautions. Individuals who are not diagnosed early may become entirely dependent.[5] This program, which enhances the quality of life for society and individuals, was first implemented in Turkiye for phenylketonuria in 1987 and was nationally expanded in 1993. Later, in 2016, with the addition of hypothyroidism to the screening program, it was named the national newborn screening program. The program was expanded to include six diseases with the addition of biotinidase deficiency in 2008, cystic fibrosis in 2015, and congenital adrenal hyperplasia and spinal muscular atrophy in 2022.[6]

The history of neonatal screening programs is not very old. The first implementation of screening programs can be traced back to 1957 when phenylpyruvic acid was tested in urine. Later, in 1959, phenylalanine levels in the blood were measured, but the test became widely applicable in 1961 when Dr. Guthrie developed the method using a few

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drops of blood from a newborn's heel. Four years later, all newborns in New York State, USA, began to be screened for phenylketonuria.[7] While many countries implement newborn screening programs, approaches to screening programs and the diseases screened can vary. Countries such as Saudi Arabia, the Netherlands, New Zealand, and the UK run national programs, while Australia, Canada, and Sweden implement them at a regional level. Many countries continue the implementation, but the mentioned countries grant regional discretion in their application methods. The content of screening programs also varies globally. Six diseases are currently screened in Turkey, 13 in the UK, 20 in Singapore, 21 in Japan, 24 in Saudi Arabia, 27 in Australia, 30 in Canada, and 36 diseases are screened in the USA.[1,8] The number of diseases screened in developed countries is significantly broader than in our country.

In countries like Turkiye, where consanguineous marriages are common, the need for neonatal screening programs is better understood, considering that genetic, metabolic, and endocrine disorders are more likely to develop. Like other countries with similar marriage traditions, Turkiye continues its efforts to include other diseases in the screening programs.[3,6] To understand the significance of consanguineous marriages, it is worth noting a 2022 study that examined infant mortality in Adıyaman, where 26% of infant deaths were found to be associated with parental consanguinity.[9] The percentage of newborns screened in Turkiye increased from 53% in 1998 to 88% in 2008, and according to the latest data from the Ministry of Health, currently, 97% of newborns in the country are screened for six diseases.[10] Among the diseases screened in Turkiye, congenital hypothyroidism is the most common worldwide, with an incidence of 1:3500-4000 globally, while in Turkey, it is found to be 1:3344. In Turkiye, phenylketonuria is most frequently observed, with an incidence of 1:4500.[3]

This study aims to determine the incidence of diseases included in the neonatal screening program over the past five years, to assess the relative status of Adıyaman compared to Turkiye and world data, and to evaluate any risk factors for the screened diseases according to demographic data. A literature review reveals that incidence studies have been conducted in several provinces in Turkey. However, no study has examined all six diseases in the more traditional culture of the Southeastern Anatolia Region. We believe this study will fill a gap in the literature.

METHODS

This retrospective-descriptive study was planned in Adıyaman province, located in the Southeastern Anatolia Region of Turkey.

Ethical approval for the study was obtained from the Non-interventional Researches Ethics Committee of Fırat University (Date: 14.12.2023, Decision No: 2023-14/32). Due to the study's retrospective nature, patient consent was not obtained, but institutional permission to use the data was obtained from the Adıyaman Provincial Health Directorate. The study adhered to the principles of the Helsinki Declaration.

Heel blood samples from newborns born in hospitals are taken within the first 48 hours in the hospital where the birth occurs before the patient is discharged. The second heel blood sample is taken at Family Health Centers 48-72 hours after the newborn starts oral feeding. Newborns with values above the upper limit in the heel blood results are referred to the relevant clinic by family physicians for diagnosis and treatment. This process is usually completed within approximately 15 days. For newborns with borderline values, heel blood is taken again, and the process continues. Diagnostic values are specified in **Table 1**.

Table 1. Reference values used in evaluating the results of diseases screened under the national newborn screening program

	Normal		Repeat heel blood[a]/Advanced analysis from the same sample[b]/ Venous blood sample[c]	Referral
PKU - Phenylalanine level	≤2 mg/dl		2,1-3,9 mg/dl[a]	≥4 mg/dl or ≥21 mg/dl after repeat blood sample
CH-TSH value	<5,5 mlU/L		5,5-20 mlU/L[a]	>20 mlU/L or ≥55 mlU/L after repeat blood sample; refer to appropriate lab for serum T4, TSH test, depending on the physician's opinion
BD – Biotinidase enzyme activity	>65 U		≤65 U[a]	≤65 U after repeat blood sample
CF-Blood immunoreactive trypsinogen (IRT) value	<90 µg/L		≥90 µg/l[a]	≥70 µg/L after repeat blood sample
CAH-17-hydroxyprogesterone first-step analysis	For babies ≥36 weeks and ≥2500 g	<10 ng/ml	≥10 ng/ml[b]	≥10 ng/ml; in the second-step analysis from the same sample, 21-deoxycortisol + 17-hydroxyprogesterone/cortisol ≥ 1 and/ or 11-deoxycortisol ≥ 10 ng/mL
	For preterm babies 32-35 weeks and 1500-2499 g	<15 ng/ml	≥15 ng/ml[b]	
SMA-SMN1 gene molecular analysis	No mutation		Suspicious[c]	Homozygous mutation; or refer to neurology if a homozygous mutation is confirmed by dry blood spot analysis

PKU: Phenylketonuria, CH-TSH: Congenital hypothyroidism-thyroid stimulating hormone, BD: Biotinidase deficiency, CF: Cystic fibrosis, CAH: Congenital adrenal hyperplasia, SMA: Spinal muscular atrophy, SMN1: Survival motor neuron 1

Table 2. Number of births, diagnosed cases from screened diseases, and incidences of screened diseases by year

	Births	PKU		CH		BD		CF		CAH		SMA	
	n	n	Incidence	n	Incidence	n	Incidence	n	Incidence	n	Incidence	n	Incidence
2019	11 915	6	1:1986	28	1:426	30	1:397	-	-	-	-	-	-
2020	11 056	3	1:3685	18	1:614	34	1:325	1	1:11056	2	1:5528	-	-
2021	11 015	7	1:1574	14	1:787	16	1:688	1	1:11015	-	-	-	-
2022	10 386	3	1:3462	27	1:385	14	1:742	3	1:3462	4	1:2597	1	1:10386
2023	8 592	3	1:2864	4	1:2148	16	1:537	-	-	1	1:8592	1	1:8592
Total	52 964	22	1:2407	91	1:582	110	1:481	5	1:10593	7	1:5864	2	1:9489

PKU: Phenylketonuria, CH: Congenital hypothyroidism, BD: Biotinidase deficiency, CF: Cystic fibrosis, CAH: Congenital adrenal hyperplasia, SMA: Spinal muscular atrophy

All newborns who had heel blood taken in Adıyaman between 2019 and 2023 were included in the study. Thus, the sample size represents the entire population. During data analysis, heel blood samples that were improper and had to be retaken were excluded. Newborns with partial biotinidase deficiency and partial phenylalaninemia were also excluded. The annual and five-year incidences of the diseases were calculated by dividing the number of diagnosed individuals by the number of individuals at risk (number of births) within the specified time period. Birth numbers were obtained from the Turkish Statistical Institute (TÜİK) data. The treatment status of the babies was not questioned, and the annual incidence was calculated based on the babies diagnosed by the relevant clinics. The researchers identified babies diagnosed through the Public Health Management System, and their diagnoses were confirmed through hospital records.

Statistical Analysis

The analyses were conducted using the SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) version 25 software package. Categorical data were presented as n (%), and numerical data were presented as median (min-max) values. The Pearson Chi-square test and Fisher Exact test were used for the comparison of categorical variables between groups. The normality of the distribution of numerical variables was assessed using the Kolmogorov-Smirnov test and visual methods (histogram and probability plots). For numerical data that did not follow a normal distribution, the Mann-Whitney U test was used for comparison between two independent groups. A p-value of less than 0.05 was considered statistically significant in all analyses.

RESULTS

The screening and referral results for six diseases conducted between 2019 and 2023 for newborns residing in Adıyaman province under the NCP were evaluated. As a result of the screenings conducted over these 5 years, 109 (0.2%) of the screened babies were referred for further investigation with suspicion of phenylketonuria, 551 (1.1%) for congenital hypothyroidism, 260 (0.5%) for biotinidase deficiency, 113 (0.2%) for cystic fibrosis, 80 (0.2%) for congenital adrenal hyperplasia, and 5 (0.03%) for spinal muscular atrophy. After referral, 22 babies were diagnosed with phenylketonuria (incidence: 1:2407), 91 with congenital hypothyroidism (incidence: 1:582), 110 with biotinidase deficiency (incidence: 1:481), 5 with cystic fibrosis (incidence: 1:10593), 7 with congenital adrenal hyperplasia (incidence: 1:5864), and 2 with type 1 spinal muscular atrophy (incidence: 1:9489). Among the diseases screened, biotinidase deficiency had the highest five-year incidence in Adıyaman province, while cystic fibrosis had the lowest (**Table 2**).

Table 3. Disease status of newborns screened between 2019-2023 by gender

		Gender (n=53 252)		
		Male, n (%)	Female, n (%)	p
PKU	Normal	27 047 (99.97)	26 147 (99.95)	0.472*
	Diagnosed	9 (0.03)	13 (0.05)	
CH	Normal	25 952 (99.85)	25075 (99.79)	0.083*
	Diagnosed	38 (0.15)	53 (0.21)	
BD	Normal	27 014 (99.8)	26 128 (99.8)	0.987*
	Diagnosed	56 (0.2)	54 (0.2)	
CF	Normal	26 822 (99.99)	58 (99.99)	0.682**
	Diagnosed	2 (0.01)	3 (0.01)	
CAH	Normal	18 904 (99.98)	18 245 (99.98)	1.000**
	Diagnosed	4 (0.02)	3 (0.02)	
SMA	Normal	8 000 (99.98)	7 991 (100.0)	0.500**
	Diagnosed	2 (0.02)	0 (0.0)	

* Chi-square test ** Fisher's exact test applied. PKU: Phenylketonuria, CH: Congenital hypothyroidism, BD: Biotinidase deficiency, CF: Cystic fibrosis, CAH: Congenital adrenal hyperplasia, SMA: Spinal muscular atrophy

The frequency of disease occurrence by gender was evaluated for newborns screened for six diseases between 2019 and 2023. No statistically significant difference was found in the diagnosis rates by gender for all screened diseases (p>0.05 for each comparison) (**Table 3**).

No significant difference was found when comparing the birth weeks and birth weights of those diagnosed and not diagnosed with the six diseases (p>0.05 for each comparison). Although the birth weights were not statistically significant, the birth weights

Table 4. Birth weights of newborns screened between 2019-2023 based on disease status

	Birth week					Birth weight				
	Normal		Case		p*	Normal		Case		p*
	n	median (min-max)	n	median (min-max)		n	median (min-max)	n	median (min-max)	
PKU	53 190	39.0 (23.0-43.0)	22	38.5 (36.0-40.0)	0.824	52 956	3200.0 (500.0-6000.0)	22	3070.0 (2700.0-3600.0)	0.361
CH	51 016	39.0 (32.0-41.0)	90	39.0 (23.0-43.0)	0.869	50 794	3200.0 (560-6000)	90	3145.0 (1310.0-4350.0)	0.193
BD	53 132	39.0 (23.0-43.0)	109	38.0 (32.0-42.0)	0.469	52 899	3200.0 (500.0-6000.0)	108	3155.0 (1084.0-4200.0)	0.179
CF	52 745	38.0 (36.0-40.0)	5	39.0 (23.0-43.0)	0.195	52 514	3200.0 (545.0-6000.0)	5	2930.0 (2360.0-3700.0)	0.474
CAH	37 181	39.0 (35.0-40.0)	7	38.0 (28.0-43.0)	0.639	37 181	3200.0 (1175.0-6000.0)	7	3000.0 (2100.0-3600.0)	0.279
SMA	15 991	38.5 (38.0-39.0)	2	38.0 (23.0-43.0)	0.901	15 991	3365.0 (2900.0-3830.0)	2	3195.0 (570.0-5750.0)	0.649

*Mann-Whitney U test was applied, min: Minimum, max: Maximum, PKU: Phenylketonuria, CH: Congenital hypothyroidism, BD: Biotinidase deficiency, CF: Cystic fibrosis, CAH: Congenital adrenal hyperplasia, SMA: Spinal muscular atrophy

of those diagnosed were lower than those not for all screened diseases (Table 4).

The frequency of disease occurrence among newborns was evaluated according to the district of residence of their mothers. No statistically significant difference was found for the six diseases screened by district of residence, and the frequency of disease occurrence was similar for those residing in the central district and other non-central districts (p>0.05 for each comparison).

The frequency of disease occurrence among newborns was examined according to the nationality of their mothers. No significant difference was found in the frequency of disease occurrence according to maternal nationality for five of the six diseases. However, a significant difference was found in biotinidase deficiency, where the frequency of disease occurrence was significantly higher in babies whose mothers were Turkish citizens (0.22%) compared to those whose mothers were non-Turkish citizens (0.02%) (p=0.009) (Table 5).

Table 5. Disease status of newborns screened between 2019-2023 by mother's nationality

		Nationality		
		Turkish, n (%)	Others, n (%)	p*
PKU	Normal	48925 (99.96)	4316 (99.95)	0.698**
	Diagnosed	20 (0.04)	2 (0.05)	
CH	Normal	47307 (99.81)	3759 (99.92)	0.199
	Diagnosed	88 (0.19)	3 (0.08)	
BD	Normal	48852 (99.78)	4337 (99.98)	0.009
	Diagnosed	109 (0.22)	1 (0.02)	
CF	Normal	48767 (99.99)	4033 (99.98)	0.328**
	Diagnosed	4 (0.01)	1 (0.02)	
CAH	Normal	34288 (99.98)	2893 (99.97)	0.433**
	Diagnosed	6 (0.02)	1 (0.03)	
SMA	Normal	14692 (99.99)	1299 (99.92)	0.156**
	Diagnosed	1 (0.01)	1 (0.08)	

PKU: Phenylketonuria, CH: Congenital hypothyroidism, BD: Biotinidase deficiency, CF: Cystic fibrosis, CAH: Congenital adrenal hyperplasia, SMA: Spinal muscular atrophy

DISCUSSION

Although newborn screening programs hold a significant place as a secondary prevention practice, studies examining the incidence of diseases within these programs are crucial for determining the frequency of these diseases and identifying potential risk factors. A 2022 study in Diyarbakır reported an incidence of phenylketonuria at 1:7878.[5] In Kırşehir, the incidence was found to be 1:7924, while in Sivas, it was 1:1334.[3,7] Globally, incidences vary across different countries and regions (11). For instance, PKU incidences in Europe are 1:4000 in Italy, 1:4545 in Ireland, 1:13434 in Denmark, and 1:112000 in Finland. In Asia, examples include incidences of 1:5000 in Iran and Jordan, 1:14245 in Saudi Arabia, 1:227273 in Thailand, 1:125000 in Japan, and 1:116006 in the Philippines. In the United States, incidence rates vary between 1:15000 and 1:47000 across different studies.[1,12] In our study, the five-year incidence was found to be 1:2407. Turkey's national incidence stands at 1:4500. Generally, phenylketonuria, which is more frequent in Eastern societies, is often attributed to consanguineous marriages.[5] Compared to other studies within Turkiye, the incidence of phenylketonuria in Adıyaman appears to be higher. While the incidence of PKU, a genetic disease, is generally decreasing, there have been fluctuations in Adıyaman over the past five years. Despite the decline, which we attribute to the effectiveness of genetic counseling, the incidence in Adıyaman remains high. We believe this is due to the higher rate of consanguineous marriages compared to the rest of Turkiye.[13] However, prospective studies are recommended to investigate factors such as access to healthcare, particularly in rural areas, and barriers to obtaining genetic counseling.

Congenital hypothyroidism is the most common disease screened worldwide.[3] A study conducted in Kırşehir found a six-year incidence of 1:1132, while a ten-year

incidence of 1:378 was reported in Sivas.[3,7] Incidences of 1:7175 and 1:2000 have been reported in Saudi Arabia and Japan, respectively.[1,14] In our study, the incidence was calculated at 1:1582. Hypothyroidism, a common disease both globally and in Turkiye, is thought to be influenced by preterm births and diet.[14]

A study in Diyarbakır that examined ten years of data found an incidence of biotinidase deficiency at 1:2359.[5] In Kırşehir, the annual incidence, based on six years of data, was reported as 1:2264, while in Sivas, the annual incidence, based on five years of data, was 1:3255.[3,7] Studies conducted in Şanlıurfa and Adana reported incidences of 1:1195 and 1:1177, respectively.[15,16] A study in Saudi Arabia covering six years of data found an incidence of 1:28316.[17] In Turkiye, 1637 newborns were diagnosed with biotinidase deficiency in 2021. According to TÜİK data, the incidence for 2021 was calculated as 1:660 when divided by the number of live births in the same year.[10,18] Two studies in Italy reported biotinidase deficiency incidences of approximately 1:6000.[19,20] In our study, the five-year annual incidence was found to be 1:481. The incidence of biotinidase deficiency can vary in different studies. This variation could be due to the different sample sizes and the timing of the studies, but the high incidence in Adıyaman is noteworthy. As with PKU, consanguineous marriages are considered a factor in biotinidase deficiency, but conducting further studies would be beneficial to better understand the specific increase in its incidence.

A study in Mersin on cystic fibrosis, analyzing three years of data, found an annual incidence of 1:9388.[21] A study in Kırşehir examining data from 2015 to 2020 found no cases of cystic fibrosis diagnosed.[7] When examining Turkey's 2021 data, the annual incidence was calculated at 1:8570.[10,18] While the global incidence of CF is thought to be 1:2500, screening programs have shown a declining trend in this ratio. Incidence studies in Europe have reported 1:1353 in Ireland, 1:25000 in Finland, 1:4500 in Western Europe, and around 1:6000 in Northern Europe.[21] In other parts of the world, incidences have been reported as 1:3000 in Australia, 1:3300 in Canada, and 1:4000 in the United States.[22] In our study, the incidence of CF was calculated as 1:10593. The data appear consistent with those from other cities in Turkiye and the country's incidence.

Congenital Adrenal Hyperplasia (CAH) was included in the national program in 2022 but was already being screened in pilot provinces like Adıyaman and Sivas. In Sivas, examining 2020 and 2021 data, the incidence was calculated at 1:4420.[3] For 2021, the annual incidence in Turkiye was calculated at 1:10383.[10,18] An analysis of eight years of data in Saudi Arabia reported an incidence of 1:7908.[1] In the United States, a 15-year study found an incidence of 1:13493.[23] In India, a 2019 study reported an incidence of 1:2500.[24] In Denmark, a ten-year data review found an incidence of 1:20000.[25] Globally, the incidence of CAH is estimated to range between 1:10000 and 1:20000.[26] In our study, the incidence of CAH was calculated as 1:5864. Studies investigating the incidence of CAH in different regions of Turkiye are scarce, likely due to the recent inclusion of the disease in the screening program. Although a study conducted in the province of Sivas found a low incidence, it would not be entirely accurate to state that CAH is more common in Adıyaman based on a comparison with just one province. However, considering global studies, the influence of consanguineous marriages in Adıyaman, where certain diseases are generally more prevalent, cannot be overlooked. Further research is recommended to explore consanguineous marriages and other potential risk factors.

Spinal muscular atrophy (SMA) screening began nationwide in Turkiye in 2022. The annual incidence for 2022 in Turkiye was calculated at 1:9590.[10,27] A study in the United States examining three years of data found an incidence of 1:19000.[28] A study in Japan reviewing nine years of data found an incidence of 1:100000.[29] However, a study in Germany reported an incidence of 1:6910 among 297163 newborns, with 43 diagnosed with SMA.[30] In our study, examining two years of data, the incidence was 1:9489. The incidence in Adıyaman is consistent with that of Turkiye. However, global studies show a wide range of incidence intervals, indicating the need for more studies on this topic.

Limitations

Due to the study's retrospective nature and the lack of additional information requested from families, it was impossible to establish causal relationships. No statistically significant risk factors were identified when examining the available demographic data. The study's results may not be generalizable since it was conducted only in Adıyaman province. However, the study shows that Turkiye generally screens fewer diseases than developed countries and detects higher incidences of the diseases screened. Additionally, the study has been identified as the first in Turkiye to screen for six diseases, including SMA.

CONCLUSION

In our study, we found that newborns in Adıyaman were generally diagnosed with diseases screened in the National Newborn Screening Program at a higher frequency than the Turkish average. However, biotinidase deficiency, in particular, was slightly more prevalent. Further studies should be planned to investigate the frequency of biotinidase deficiency, specifically in Adıyaman, and

efforts should be made to raise social awareness in Turkey about reducing consanguineous marriages, which is a potential cause.

ETHICAL DECLARATIONS

Ethics Committee Approval

Ethical approval for the study was obtained from the Non-interventional Researches Ethics Committee of Fırat University (Date: 14.12.2023, Decision No: 2023-14/32).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All the authors declare that they have all participated in the design, execution, and analysis of the study and that they have approved the final version.

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Original Article

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Assessing the readability of diabetes information provided by the Turkish Ministry of Health

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ABSTRACT

Aims: The aim of this study is to analyze the readability levels of diabetes-related texts available on the official website of the General Directorate of Public Health of the Turkish Ministry of Health, and to use the findings to guide the preparation of future informational texts.

Methods: This research is a descriptive study based on document analysis, aiming to determine the readability of diabetes-related texts developed by the Turkish Ministry of Health. The data was obtained from publicly accessible educational texts published on the General Directorate of Public Health's website (Cited 2024, June 20. Available from: https://hsgm.saglik.gov.tr/tr/diyabet). The Ateşman readability formula was used to evaluate the readability levels of the texts.

Results: A total of 32 documents under four main headings were examined using the Ateşman formula, and the average readability score of the texts was found to be 61.69±10.15. Based on their readability levels, 28.1% texts were classified as 'Easy', 56.2% as 'Moderately Difficult', and 15.6% as 'Difficult'. Among the 13 texts in the Type 1 Diabetes group, 46.1% were 'Easy' and 53.8% were 'Moderately Difficult'. In the Type 2 Diabetes group, 17.6% were 'Easy', 64.7% were 'Moderately Difficult', and 17.6% were 'Difficult'.

Conclusion: The readability levels of the analyzed texts in our study are generally in the "Moderately Difficult" category; however, some texts were found to be in the "Difficult" readability level. The readability and understandability of educational materials prepared to improve public health are of great importance. Regular review and optimization of the readability levels of educational materials will enhance public health literacy, contributing to the development of healthier individuals and a healthier society.

Keywords: Diabetes mellitus, readability, public health education, ateşman readability index, health information

INTRODUCTION

The transformation and advancements in information technology and the widespread use of the internet over the years have significantly facilitated access to health information. Nowadays, many users research various health-related topics on websites before consulting doctors. A study by Murray and colleagues indicated that 85% of patients research health issues on the internet before a doctor's appointment.[1] However, there is no legal regulation or mechanism governing the sources of health information on the internet, nor is the accuracy of this information monitored. This increases the risk of spreading incorrect or misleading information.[2] In Turkiye, various educational materials are prepared and distributed by the Public Health Directorate of the Ministry of Health to enhance public health awareness and ensure access to accurate information. These informational texts must contain adequate and comprehensible information that citizens can access and understand.[3]

In the effectiveness of chronic disease treatments, it is crucial not only for healthcare professionals to provide treatment but also for patients to be aware of the disease and cooperate with the physician.[4] Readability refers to the ease with which any written text can be understood by the reader.[5] Various measures, formulas, and indices can be used for readability analysis. For this purpose, formulas such as the Smog-Simple measure, Gunning-Fog index, Flesch-Kincaid grade level, and ARI-automatic readability index can be used. The Ateşman readability index, which uses average word and sentence lengths, is suitable for the structure of the Turkish

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language and can be used for Turkish texts.[6,7] According to the Ateşman readability index, texts with a range of 90-100 are classified as very easy; 70-89 as easy; 50-69 as moderately difficult; 30-49 as difficult; and 1-29 as very difficult (Table 1).[8]

Table 1. Readability classification according to the Ateşman readability formula

Readability level	Score range
Very difficult	1-29
Difficult	30-49
Moderately difficult	50-69
Easy	70-89
Very easy	90-100

Diabetes mellitus is a significant health problem with high morbidity, mortality, and treatment costs for both patients and society. With technological advancements, a sedentary lifestyle, and the widespread prevalence of obesity, its incidence is increasing globally.[9] The prevalence of diabetes is rapidly increasing in Turkiye. While the TURDEP I study conducted in 2000 found a prevalence of 7.8% in individuals over 20 years of age, the results of PURE, CREDIT, and TURDEP II studies conducted in 2009 and 2010 found a prevalence of 14-16%.[10] Effective management of diabetes requires not only medical intervention but also active cooperation from patients who must be well-informed about their condition.

The aim of this study is to analyze the readability levels of diabetes-related texts available on the official website of the General Directorate of Public Health of the Turkish Ministry of Health and to use the findings to guide the preparation of future informational texts.

METHODS

This research is a descriptive study based on document analysis, aiming to determine the readability of diabetes-related texts developed by the Turkish Ministry of Health. As publicly accessible information was used, and as it does not entail the utilization of human subjects or patient data ethical approval was waived for this study. All procedures were carried out in accordance with the ethical rules and the principles. The research data was obtained from educational texts published on the General Directorate of Public Health's website (Cited 2024, June 20. Available from: https://hsgm.saglik.gov.tr/tr/diyabet). All documents on the page were examined in four groups: Type 1 Diabetes, Type 2 Diabetes, Gestational Diabetes, and Diabetes due to Other Specific Causes. The texts available in PDF format on the website were downloaded, tables and figures were removed, and the content was transferred to a Microsoft 365 Version 2402 Word document without modification.

To evaluate the readability of the texts, the data was transferred to a free online readability level calculator (http://okunabilirlikindeksi.com/). This calculator uses the Flesch readability formula adapted to Turkish by Ateşman (1997).[7] This formula calculates the readability levels of texts based on the total number of syllables, words, and sentences, as follows: readability score=198.825 - (40.175 x average word length) - (2.610 x average sentence length). The Ateşman Readability Formula accepts groups of words ending with a period (.), question mark (?), exclamation mark (!), and ellipsis (...) as sentences. Sequential dependent clauses separated by commas (,) are considered as a single sentence. The average word length (AWL) represents the average number of syllables per word, while the average sentence length (ASL) represents the average number of words per sentence. Using the Ateşman Readability Formula, a readability score ranging from 1 to 100 is obtained. These scores are categorized into five different levels to determine readability levels. The details of the Ateşman Readability classification are presented in Table 1.

Statistical Analysis

It was conducted using SPSS 24 (SPSS Inc., Chicago, IL, USA) statistical package program. The Kolmogorov-Smirnov test was used to determine the normality distribution. The mean, standard deviation, minimum, and maximum values of the data were calculated. Readability index values were classified according to the Ateşman readability classification (Table 1).

RESULTS

Diabetes-related texts available on the General Directorate of Public Health's website were examined. According to the Ateşman formula, 32 documents under four main headings were analyzed, and the AWL values ranged between 2.48-3.25, while the ASL values ranged between 6-18.2. The average Ateşman readability score was found to be 61.69±10.15. The AWL and ASL values, along with the readability scores of all texts, are presented in Table 2. According to the Ateşman readability levels, 9 (28.1%) of the diabetes-related texts were 'Easy', 18 (56.2%) were 'Moderately Difficult', and 5 (15.6%) were 'Difficult', with an overall readability of 'Moderately Difficult' for all texts. The texts classified as 'Difficult' were related to Gestational Diabetes, Diabetes due to Other Specific Causes, and three specific texts within the Type 2 Diabetes group: 'What Does Type 2 Diabetes Treatment Include?', 'Oral Drug Treatment in Type 2 Diabetes', and 'Diet and Herbal Products in Type 2 Diabetes'. No texts were classified as 'Very Easy' or 'Very Difficult' according to the Ateşman formula. Overall, when evaluated without grouping, 28.1% (n=9) of the texts were 'Easy', 56.2% (n=18) were 'Moderately.

Table 2. Readability scores of texts according to the Ateşman formula

Text title	AWL	ASL	Score	Difficulty
Type 1 diabetes				
1.1. What is diabetes?	2.76	8.2	66.5	M. difficult
1.2. Types of diabetes	2.64	7.1	74.2	Easy
1.3. Symptoms and diagnosis of diabetes	2.48	9.1	75.4	Easy
1.4. General information on T1D and insulin	2.72	6.8	71.8	Easy
1.5. T1D and nutrition	2.72	11.8	58.8	M. difficult
1.6.Diet and herbal products in T1D	2.8	9.8	60.8	M. difficult
1.7. Exercise in T1D	2.68	8.7	68.4	M. difficult
1.8. Sudden low blood sugar	2.56	6.8	78.2	Easy
1.9. Sudden high blood sugar	2.6	7.7	74.3	Easy
1.10. T1D in disease conditions	2.89	12.1	51.1	M. difficult
1.11. Importance of self-monitoring in T1D	2.72	10.6	61.9	M. difficult
1.12. Surveillance in T1D	2.93	10.6	53.4	M. difficult
1.13. Living with diabetes	2.6	8.9	71.1	Easy
Type 2 diabetes				
2.1. What is T2D?	2.76	8.4	66	M. difficult
2.2. Types of T2D	2.72	7.1	71	Easy
2.3. Symptoms and diagnosis of T2D	2.56	12.4	63.6	M. difficult
2.4. Who Is at risk for T2D and what ıs prediabetes	2.68	7.2	72.4	Easy
2.5. Importance of controlling T2D	2.76	8.9	64.7	M. difficult
2.6. Pregnancy and T2D	2.8	13.1	52.1	M. difficult
2.7. What does T2D treatment include?	3.25	8.8	45.3	Difficult
2.8. Healthy nutrition for diabetes	2.72	14	53	M. difficult
2.9. Principles of nutritional therapy in T2D	2.6	10.9	65.9	M. difficult
2.10. Oral medication treatment in T2D	2.85	16.9	40.2	Difficult
2.11. General information on insulin treatment in T2D	2.76	11	59.2	M. difficult
2.12. Essential information on insulin administration in T2D	2.72	12	58.2	M. difficult
2.13. Physical activity in T2D	2.85	11	55.6	M. difficult
2.14. Health ıssues related to T2D	2.68	7.9	70.5	Easy
2.15. Foot care in T2D	2.93	6	65.5	M. difficult
2.16. Living with T2D	2.89	11.1	53.7	M. difficult
2.17. Diet and herbal products in T2D	2.89	13.1	48.5	Difficult
Gestational diabetes	3.01	15.4	37.7	Difficult
Diabetes due to other specific causes	2.68	18.2	43.7	Difficult
Average±SD	2.75±0.14	10.36±2.95	61.69±10.15	

AWL: Average Word Length ASL: Average Sentence Length, SD:Standard deviation M: Moderately, T1D: Type 1 diabetes, T2D: Type 2 diabetes

Difficult', and 15.6% (n=5) were 'Difficult'. In the Type 1 Diabetes group, 46.1% (n=6) of the 13 texts were 'Easy' and 53.8% (n=7) were 'Moderately Difficult', while in the Type 2 Diabetes group, 17.6% (n=3) of the 17 texts were 'Easy', 64.7% (n=11) were 'Moderately Difficult', and 17.6% (n=3) were 'Difficult'.

DISCUSSION

This research is the first study in Turkiye analyzing the texts prepared to inform the public about diabetes. and it found that the average readability level of the informational texts was at a college level and of moderate difficulty.

With technological advancements. access to information has become significantly easier. Studies show that more than 70% of adults search for health information online. and over 30% attempt to diagnose a medical problem for themselves or someone they care for.[11] The increased use of the internet as an information source has led to the important issue of accessing incorrect. misleading. and inconsistent information. Accurate and easily understandable information is essential for managing an individual's health effectively. Individuals who have access to reliable and easily comprehensible health information are better positioned to manage their health. enhance their knowledge and skills. and consequently reduce healthcare costs while improving their quality of life.[15]

It is well known that the educational level of readers plays a critical role in understanding texts. To ensure that health-related texts published online are understood. they must align with the literacy and educational level of the general population.[12] A study conducted in Turkiye in 2010 reported that the average education duration of individuals aged 15 and over was 7.18 years.[13] The 2011 Human Development Report found that the average education duration in Turkish society was 6.5 years.[14] In light of these findings. it is essential to prepare educational materials using clear and comprehensible language. This approach ensures that information is accessible and understandable to a wide audience. thereby enhancing health literacy across the population.

According to Ateşman. the average sentence length in Turkish is 9-10 words. and the average word length is 2.6 syllables.[8] To improve the readability of health-related information. it has been suggested that sentences should be limited to 8-10 words and that simpler words should be used instead of complex medical terms.[16] In our study. the average word length was found to be 2.75 syllables. and the average sentence length was 10.36 words. slightly above the expected values. In the study by Muslu et al.[3] evaluating the readability of the Ministry of Health's brochures on nutrition. the average readability level was found to be 'Moderate' difficulty. Numerous studies in our country have evaluated the readability of web-based patient information materials using the Ateşman readability formula. In the study by Saldırım et al.[17] evaluating the readability of educational materials related to tinnitus. the readability of the texts was found to be 'Difficult'. Another study on

hoarseness found the readability index to be of 'Moderate' difficulty.[18] In the study by Tahir et al.[19] on dizziness. the readability of the evaluated texts was found to be 'Easy'. Similar studies in dentistry found the readability levels to be of 'Moderate' difficulty.[2,20] The difference of our study is that it evaluates the texts prepared by an official state institution aimed at informing the public.

The readability levels of the analyzed texts in our study are generally in the "moderately difficult" category; however. some texts were found to be in the "difficult" readability level. Particularly. the texts related to gestational diabetes and diabetes due to other specific causes were found to be more complex. Considering that the average education level in Turkiye is 6.5 years. the readability levels of educational materials should be reviewed and improved especially in these contexts.[14] We suggest that it would be more appropriate to write the texts at a level suitable for at least 4th and 5th-grade students.

Limitations

This study primarily relied on the Ateşman readability formula. the most widely used index for Turkish texts. to evaluate readability. While this formula is well-suited for analyzing Turkish language texts. its sole use presents a limitation. Another limitation is that the Ateşman formula is based solely on written texts and may be insufficient for evaluating the readability of visual materials. graphics. and tables.[3] With technological advancements. the use of visual elements in educational materials is increasing. which limits the effectiveness of these formulas. Future research should focus on developing more comprehensive evaluation tools that include the readability of tables. graphics. and other visual content and also benefit from incorporating multiple readability formulas to provide a more comprehensive evaluation of text readability.

CONCLUSION

Our study found that the educational materials published by the General Directorate of Public Health regarding diabetes have an average readability level classified as moderate difficulty according to the Ateşman readability formula. The readability and comprehensibility of educational materials prepared to improve public health and enhance individuals' health knowledge levels are of great importance. They should be written in a language that readers can easily follow and comprehend. Writing these materials in a clear. simple. and easily understandable language will facilitate the accessibility and comprehension of this information by a broad segment of society. Regular review and optimization of the readability levels of educational materials will enhance public health literacy. contributing to the development of healthier individuals and. consequently. a healthier society

ETHICAL DECLARATIONS

Ethics Committee Approval

As publicly accessible information was used and as it does not entail the utilization of human subjects or patient data ethical approval was waived for this study.

Informed Consent

As publicly accessible information was used and as it does not entail the utilization of human subjects or patient data informed consent was waived for this study.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design. execution. and analysis of the paper. and that they have approved the final version.

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Original Article

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Evaluating the effects of bleaching on color stability and surface roughness in single-shade and multi-shade resin composites

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ABSTRACT

Aims: This study aimed to evaluate the effects of aging and bleaching procedures on the color stability and surface roughness of single-shade composite resins and compare them with a multi-shade composite resin.

Methods: Fifty composite resin specimens (n=10 per group) from five brands— Omnichroma (Tokuyama, Japan) (OMN), Zenchroma (President Dental, Germany) (ZNC), Charisma Diamond One (Kulzer, Germany) (CHR), Essentia Universal (GC Corporation, Japan) (ESU) and one multi-shade composite resin Filtek Z550 (3M ESPE, USA) (FLT), —were subjected to aging (1-year simulated staining and brushing) and bleaching procedures. Color measurements were taken at baseline, after aging, and after bleaching using a spectrophotometer, while surface roughness was measured using a contact mode profilometer. Data were analyzed using Generalized Linear Models and Tukey's test for multiple comparison, with a significance level set at $p<0.05$.

Results: Statistically significant differences in ΔE values were observed across the composites and time points ($p<0.001$). Single-shade composites generally exhibited higher color change compared to multi-shade composite, with OMN showing the highest ΔE values. In terms of surface roughness, single-shade composites (ZNC and OMN) showed similar roughness to the multi-shade composite (FLT), while ESU and CHR exhibited greater roughness after bleaching.

Conclusion: Single-shade composites showed comparable performance to the multi-shade composite in terms of color stability and surface roughness after aging and bleaching. However, variations in composite responses highlight the importance of material selection in clinical practice, particularly when bleaching procedures are involved.

Keywords: Single-shade composites, multi-shade composites, color stability, surface roughness, bleaching

INTRODUCTION

Composite resins are widely utilized in modern dentistry due to their ability to provide both aesthetic and functional restorations.[1] Achieving tooth-colored and natural-looking restorations is one of the primary uses of composite resins. The broad color spectrum of these materials gives clinicians the flexibility needed to achieve clinically successful outcomes.[1] Composite resins are generally categorized into two main types: multi-shade and single-shade. Multi-shade composite resins are available in various shades to match different tooth colors, which is particularly important for achieving high aesthetic demands in anterior teeth.[2] However, working with multiple shades can be time-consuming and may increase the likelihood of errors.[3] Single-shade composite resins were developed to address these challenges and simplify the procedure.[4] These materials offer the ability to match all tooth colors with a single-shade, allowing clinicians to perform restorations more quickly and effectively.[5] In busy clinical settings, where reducing complexity and chair time is critical, single-shade composites are particularly advantageous. They generally require less chair time and help achieve more consistent aesthetic results.[2,4] Despite their advantages, the performance of single-shade composites in terms of color stability and surface roughness under various clinical conditions, such as aging and bleaching, remains a critical area of investigation. Understanding how these materials respond to common dental treatments is essential for assessing their long-term success in restorative dentistry.[6]

Color stability of composite resins is crucial for the longevity and aesthetic success of restorations. Factors affecting color stability include intrinsic factors, such as

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material composition and filler size, as well as extrinsic factors like exposure to staining agents in food, beverages, and tobacco.[7-9] Single-shade composite resins are known for their ability to match a wide range of tooth shades, but their color stability under various clinical conditions is not fully understood. The impact of common staining agents, such as coffee and tea, on these materials, and the effectiveness of bleaching treatments in restoring their original color, requires further investigation. Comparing the color stability of single-shade composites with multi-shade composites is essential to determine their clinical reliability. Understanding these changes is key to assessing the aesthetic performance of restorations and overall patient satisfaction.

The surface roughness of composite resins plays a significant role in their aesthetic performance and longevity. Mechanical and optical profilometers, scanning electron microscope (SEM), and atomic force microscope (AFM) are widely used devices to measure and evaluate the surface roughness of restorative materials.[10] A smooth surface reduces plaque accumulation, staining, and improves the gloss of the restoration. However, various factors such as aging, brushing, and bleaching procedures can alter the surface roughness of these materials.[11,12] Single-shade composites, like all restorative materials, are subjected to wear and surface changes over time. The impact of aging and bleaching on their surface roughness is particularly important, as increased roughness can lead to more staining and plaque retention, compromising the restoration's appearance and longevity. Assessing the surface roughness of single-shade composites after such procedures, and comparing it with that of multi-shade composites, is crucial to understanding their durability and aesthetic outcomes in clinical practice.[6,13]

Office-type bleaching is commonly used in clinical practice and can significantly affect the properties of composite resins. These treatment simulates long-term wear and exposure to staining agents, allowing researchers to evaluate the durability and aesthetic resilience of restorative materials under realistic conditions.[14] Bleaching treatments, in particular, which are widely used to enhance the appearance of teeth, may alter both the color and surface roughness of composite resins. While composite resins are susceptible to staining during use, it is important to recognize that the bleaching procedures commonly employed for natural teeth are generally ineffective in altering the shade of resin composites.[15] Although bleaching can partially reverse staining, it may also increase surface roughness, leading to a less smooth finish and a higher susceptibility to plaque accumulation and staining.[6,14]

Single-shade composites, despite their aesthetic advantages, may exhibit variability in color stability, especially when exposed to external factors such as bleaching and aging. This variability can be attributed to differences in the internal structure of the composite. For example, some studies have reported that the size and distribution of filler particles in single-shade composites play a significant role in their color stability.[8,14] Additionally, the composition of the resin matrix, particularly the monomer components, may interact with bleaching agents, leading to color changes after aging. Recent literature suggests that bleaching procedures may cause more pronounced color changes in single-shade composites.[6]

The aim of this study is to evaluate the effects of aging and bleaching procedures on the color stability and surface roughness of single-shade composite resins, comparing them with a multi-shade composite resin. Specifically, this study will test the following hypotheses:

1. The color stability of single-shade composite resins after different aging and bleaching procedures will not differ significantly compared to the multi-shade composite resin.
2. The surface roughness of single-shade composite resins after different aging and bleaching procedures will not differ significantly compared to the multi-shade composite resin.

METHODS

No biological materials were used in the laboratory study with composite resin, no personal data are available. Therefore, ethics committee approval is not required. All procedures were carried out in accordance with the ethical rules and the principles.

Study Design

Four single-shade composite resin Omnichroma (Tokuyama, Japan) (OMN), Zenchroma (President Dental, Germany) (ZNC), Charisma Diamond One (Kulzer, Germany) (CHR), Essentia Universal (GC Corporation, Japan) (ESU) and one multi-shade composite resin Filtek Z550 (3M ESPE, USA) (FLT), were analyzed in this study. The category manufacturers, lots, and compositions of the composite resins are presented in **Table 1**. **Figure 1** describes the study design, which shows the flow of the specimens through the different stages of the study. All specimens were subjected to staining, brushing and bleaching simulation. Color measurements were performed with spectrophotometer at baseline (t0), after 1 year brushing and staining (t1), and after bleaching (t2).

Specimen Size Calculation

Utilizing G* Power statistical software, the specimen size was computed. With a confidence level of 95% (1-α), a test power of 95% (1-β), and an effect size (f) of 0.655, the total specimen size required for one-way analysis of variance (ANOVA) has been determined to be 54, with 9 specimens

Table 1. The category, manufacturers, lot numbers, and compositions of the composite resins

Material	Code	Material type	Composition	Filler content wt%	Filler content vol%	Filler size	Shade	Manufacturer	Lot number
Omnichroma	OMN	Nanofilled	Spherical silica-zirconia filler Composite filler 1,6-bis(methacryl-ethyloxycarbonylamino) trimethyl hexane UDMA TEGDMA Mequinol Dibutyl hydroxyl toluene UV absorber.	79%	68%	0.3 µm	Single Shade Universal	Tokuyama, Japan	044EZ0
Filtek Z550	FLT	Nanohybrid	BIS-GMA UDMA BIS-EMA PEGDMA TEGDMA	81.8%	67.8%	0.01–3.5 µm	A2	3M ESPE, USA	NC45123
Zenchroma	ZNC	Microhybrid	Glass powder Silicon dioxide UDMA Bis-GMA, TEGDMA	75%	53%	0.005-3.0 µm	Single Shade Universal	President Dental, Germany	2022003395
Charisma diamond one	CHR	Nanohybrid	Barium Aluminium Boro Fluor Silicate Glass TCD-Urethaneacrylate Silica UDMA TEGDMA Titanium Dioxid, Fluorescent Pigments Metallic Oxide Pigments Organic Pigments Aminobenzoicacidester BHT Camphorquinone	81%	64%	0.05-20 µm	Single Shade Universal	Kulzer, Germany	K010025
Essentia Universal	ESU	Microhybrid	UDMA Bis-MEPP Bis-EMA Bis-GMA TEGDMA PPF Strontium glass Lanthanide fluoride Fumed silica FAISi Glass	91%	61%	0.1 µm	Single Shade Universal	GC Corp, Japan	2007231

Figure 1. Flow chart of the study design

in each group. To account for potential specimen loss, the study was designed with a total of 10 specimens allocated to each group.

Specimen Preparation

A total of 50 specimens (n=10) were prepared using silicon molds of 10x2 mm.[8] After the resin composite was placed in the molds with a slight overflow, a mylar strip and microscope slide were placed on the upper surfaces of the materials and polymerized for 10 s using a curing light (SmartLite Focus, Dentsply Sirona, USA). The slide was then removed, and the materials were polymerized by applying the curing light for 10 s over the mylar strip,

according to the manufacturer's instructions and. the same curing light was used for all polymerization steps and the output of the light was controlled periodically using a radiometer (Woodpecker LED-F, Woodpecker Medical Instrument Co., China) to ensure an intensity of at least 1000 mW/cm^2 throughout the preparation of the specimens. Following the polymerization process, each of the specimens was polished with usin polishing disc (Optidisc, Kerr Corporation, USA) from extra-coarse to extra-fine at speed 10,000 rpm and 10 s each. A new disc was used for each specimen. The specimens were rinsed with water for 10 seconds to clean debris from the restoration surface then were kept in distilled water at 37°C in an incubator for 24 h post-polymerization.[8] All the procedures on the materials were applied by a single operator. In order to control the effect of press-on force on the polishing accuracy, the initial and final measurements of the thickness of each specimen were carried out 3 times by a single operator using an industrial type screw thread digital caliper (0.01 mm) with 0-150 mm measuring range.[10]

Staining Procedure

For the preparation of the coffee solution, 3,6 g of coffee was used per 300 ml of 100°C boiling water. All solutions were allowed to reach 37°C. Eppendorf tubes were preferred to immerse the specimens individually in the study. 1.5 mm eppendorf tubes were filled with the solution and one specimen was placed inside. The tubes were kept in an oven at 37°C for 12 days (t1–1 year) to replicate intraoral conditions. Specimens were turned over and immersed in fresh solutions every day to ensure uniform contact of the specimen with the staining solution and prevent contamination with bacteria and fungus.[8,16]

Brushing Procedure

The specimens removed from the solutions were subjected to brushing simulation with the MF-100 (Mod Dental, Esetron Smart Robotechnologies, Turkiye) brushing simulator. Toothbrush (Colgate Extra Clean 1+1, Colgate Palmolive, USA) and toothpaste (Sensodyne Çok Yönlü Koruma, Haleon, United Kingdom) with a relative dentin abrasivity (RDA) of 142 diluted 1/3 by volume were used in the brushing simulation. The specimens were subjected to 10,000 (t1-1 year) cycles of brushing under a load of 250 g, with a circular motion with a movement diameter of 20 mm, and a movement speed of 30 mm/sec, simulating 1 year of brushing. The toothbrush and paste were changed for each specimen.[17-19]

Bleaching Procedure

The bleaching product (FGM Whiteness HP, FGM Dental, Brazil) was applied to the composite specimens according to the manufacturer's instructions. A 1 mm layer of the bleaching gel was carefully applied to ensure consistent contact with the entire surface of the specimens.[20] The bleaching agent was allowed to remain on the specimens for 15 minutes and was activated every 4 minutes using a micro brush. This procedure was performed in a single session. After the bleaching session, the bleaching agent was removed from the specimens using gauze, followed by thorough rinsing under distilled water for 30 seconds. The specimens were then stored in distilled water at 37°C until color assessment. The gel layer was maintained between 0.5 and 1 mm in thickness to ensure optimal contact with the composite surface.

Color Assessments

Color measurements of the specimens were conducted at three time points: t0, t1, and t2, using a digital spectrophotometer (Vita Easyshade V, Vita Zahnfabrik, Germany). The color evaluation was based on the CIE Lab* color space, which is a three-dimensional system that includes the parameters of lightness (L*), red-green chromaticity (a*), and yellow-blue chromaticity (b*). In this system, L* ranges from 0 (completely dark) to 100 (completely bright), a* represents the red-green axis, and b* represents the yellow-blue axis. For each specimen, three measurements were taken from the center, and the mean values of the L*, a*, and b* coordinates were recorded. The spectrophotometer was calibrated before each measurement session. An 18% grey card (L* =50, a*=0, b*=0) (JJC Photography Equipment Co. Ltd, China) was used as a reference for calibration.

The color differences (ΔE) between different time points were calculated using the CIEDE2000 formula, which provides a more accurate representation of perceptible color differences. These calculations were performed using an online ΔE calculator (http://www.colormine.org/delta-e-calculator/Cie2000).

Profilometric Examination

Quantitative profile analysis and surface roughness of the samples were evaluated using a contact mode profilometer (Surftest SJ-400 Mitutoyo, Japan). At the beginning (t0) and the end of the aging procedures (t1), and end of the bleaching session (t2) surface roughness measurements were made from 3 different points of the samples with a Surftest SJ-400 (Mitutoyo, Japan) profilometer and the average surface roughness values were obtained. During the measurements, the device was set in contact mode, the cut-off length was 0.08 mm, the tracing length was 0,5 mm, and the probe speed was 0.1 mm/sec.

Scanning Electron Microscope Imaging Analysis

One specimen from each group was analyzed using a SEM (Regulus 8230 FE-SEM, Hitachi High Tech Corporation, Japan) at three time points: t0, t1, and t2. Imaging was conducted at a magnification of×5000, and the images were recorded for further analysis. Prior to SEM examination, the specimens were surface coated with a 4

nm layer of gold/palladium particles (Leica EM ACE600C, Leica Microsystems Inc., Canada) to enhance surface conductivity.

Atomic Force Microscopy Examination

Three-dimensional images of the surface topography were obtained from one randomly selected specimen from each group using AFM (Park Systems XE 100, Korea) in non-contact mode. Scans were performed over a 2 µm×2 µm area with a resolution of 4000 data points per line at three time points: t0 (baseline), t1 (after aging), and t2 (after the bleaching session). The non-contact mode was chosen to prevent any potential damage to the specimen surfaces while capturing detailed surface features. The resulting data provided insights into changes in surface roughness and morphology due to the aging and bleaching processes.

Statistical Analysis

The data were analyzed using Minitab 14 and R software. The Shapiro-Wilk test was used to assess normality. For parameters that followed a normal distribution based on composite and time, comparisons were made using Generalized Linear Models, followed by Tukey's test for multiple comparisons. For parameters that did not follow a normal distribution, the Two-Way Robust ANOVA method was applied, with multiple comparisons conducted using the Bonferroni test. The results are presented as mean±standard deviation and median (min–max). Statistical significance was set at $p<0.05$.

RESULTS

The main effect of the composite was found to be statistically significant on the median ΔE values ($p<0.001$). The main effect of time was also found to be statistically significant on the median ΔE values ($p<0.001$). Additionally, the interaction between composite and time was statistically significant ($p<0.001$). At t1-t0, the highest median ΔE value was observed in the OMN composite (14.96), while the lowest value was recorded in the ZNC composite (2.37). At t2-t1, the highest median ΔE value was found in the ESU composite (12.15), whereas the lowest value was observed in the ZNC composite (0.78). At t2-t0, the highest median ΔE value was observed in the CHR composite (3.55), and the lowest value was recorded in the FLT composite (0.90) (**Table 2**).

The main effect of the composite was found to be statistically significant on the median profilometer values ($p<0.001$). The main effect of time was not statistically significant on the median profilometer values ($p=0.267$). However, the interaction between composite and time was statistically significant ($p=0.026$). At t0, the highest median profilometer value was observed in the ESU composite (0.022), while the lowest value was recorded in the OMN composite (0.013). At t1, the ESU composite again exhibited the highest median profilometer value of 0.033, whereas the ZNC composite showed the lowest value of 0.011. At t2, the highest median profilometer value was found in the ESU composite (0.031), and the lowest value was observed in the FLT, ZNC, and OMN composites (0.010) (**Table 3**).

Table 2. Comparison of ΔE values by composite and time

Time	Composite					Total		Q	p
	FLT	ESU	ZNC	CHR	OMN				
t1-t0	12.14 (11.61 - 12.49)^{A}	13.23 (12.05 - 14.96)^{AD}	2.37 (1.53 - 3.25)^{BEF}	3.99 (2.84 - 4.84)^{G}	14.96 (14.28 - 16.16)^{D}	12.03 (1.53 - 16.16)^{a}	Composite	258.507	<0.001
t2-t1	11.8 (11.44 - 13.2)^{A}	12.15 (10.87 - 13.35)^{A}	0.78 (0.19 - 1.43)^{C}	1.33 (0.52 - 3.16)^{BCE}	12.14 (10.25 - 13.1)^{A}	11.53 (0.19 - 13.35)^{a}	Time	362.555	<0.001
t2-t0	0.9 (0.64 - 1.12)^{BC}	1.57 (0.93 - 2.46)^{E}	2.24 (1.21 - 3.65)^{DEF}	3.55 (1.65 - 4.18)^{FG}	3.03 (2.25 - 5.8)^{FG}	2.22 (0.64 - 5.8)^{b}	Composite*Time	1451.512	<0.001
Total	11.8 (0.64 - 13.2)^{a}	12.13 (0.93 - 14.96)^{a}	1.72 (0.19 - 3.65)^{b}	3.22 (0.52 - 4.84)^{c}	12.14 (2.25 - 16.16)^{a}	3.33 (0.19 - 16.16)			

Q: Two-Way Robust ANOVA; Median (min - max); $^{a-c}$: No difference between main effects with the same letter; $^{A-G}$: No difference between interactions with the same letter.

Table 3. Comparison of Profilometer Values According to Composite and Time

Time	Composite					Total		Q	p
	FLT	ESU	ZNC	CHR	OMN				
t0	0.17 (0.08 - 0.22)^{ABCD}	0.22 (0.12 - 0.32)^{ABCDE}	0.21 (0.13 - 0.41)^{ABCDE}	0.19 (0.13 - 0.36)^{BCD}	0.13 (0.09 - 0.17)^{ABC}	0.17 (0.08 - 0.41)	Composite	12.603	<0.001
t1	0.12 (0.09 - 0.16)^{ABC}	0.33 (0.27 - 0.37)^{E}	0.11 (0.06 - 0.2)^{ABC}	0.2 (0.17 - 0.33)^{CD}	0.17 (0.06 - 0.21)^{ABCD}	0.18 (0.06 - 0.37)	Time	1.321	0.267
t2	0.1 (0.08 - 0.15)^{AB}	0.31 (0.21 - 0.44)^{DE}	0.1 (0.07 - 0.25)^{A}	0.19 (0.16 - 0.24)^{CD}	0.1 (0.05 - 0.2)^{A}	0.13 (0.05 - 0.44)	Composite*Time	17.383	0.026
Total	0.11 (0.08 - 0.22)^{a}	0.3 (0.12 - 0.44)^{b}	0.13 (0.06 - 0.41)^{a}	0.19 (0.13 - 0.36)^{c}	0.12 (0.05 - 0.21)^{a}	0.17 (0.05 - 0.44)			

Q: Two-Way Robust ANOVA; Median (min - max); $^{a-c}$: No difference between main effects with the same letter; $^{A-E}$: No difference between interactions with the same letter.

Scanning electron microscopy (SEM) images at 5000x magnification revealed the surface morphology of the composites before and after treatment. The SEM analysis confirmed that there were no significant changes in the surface morphology of the composites, with all groups maintaining a consistent and uniform surface texture. Minor surface irregularities were observed, particularly in the ESU and CHR groups, but these were not substantial enough to impact the overall material performance (**Figure 2**).

The Atomic Force Microscopy (AFM) analysis provided detailed three-dimensional surface topography images of the composite resins at different time points. The AFM results showed that the surface roughness of the composites varied slightly after the aging and bleaching procedures, with minor increases in roughness observed in the OMN and ZNC groups. However, the overall surface topography remained relatively smooth, indicating that the composites retained their structural integrity throughout the study (**Figure 3**).

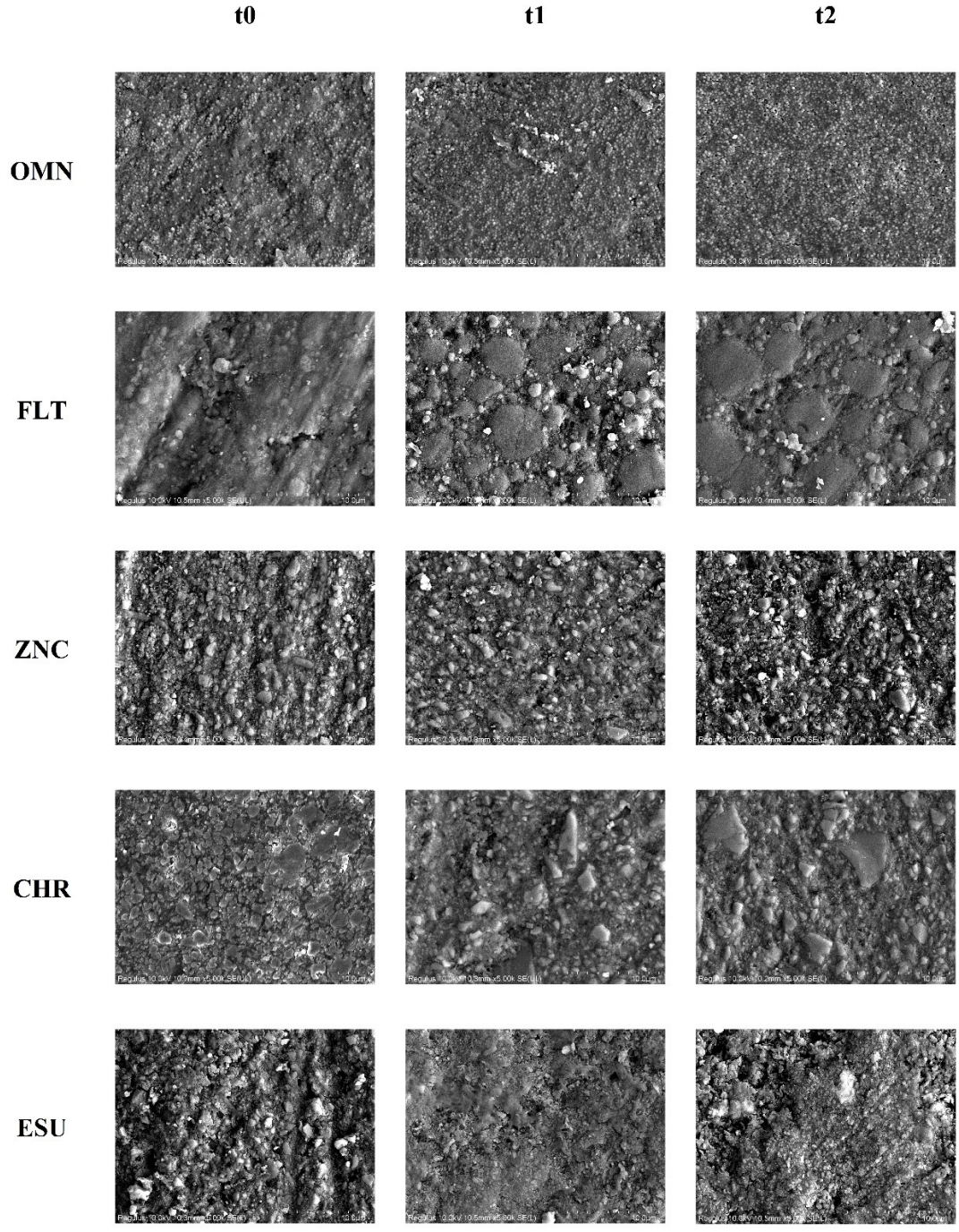

Figure 2. SEM analysis at 5000x magnification reveals the surface morphology of the groups

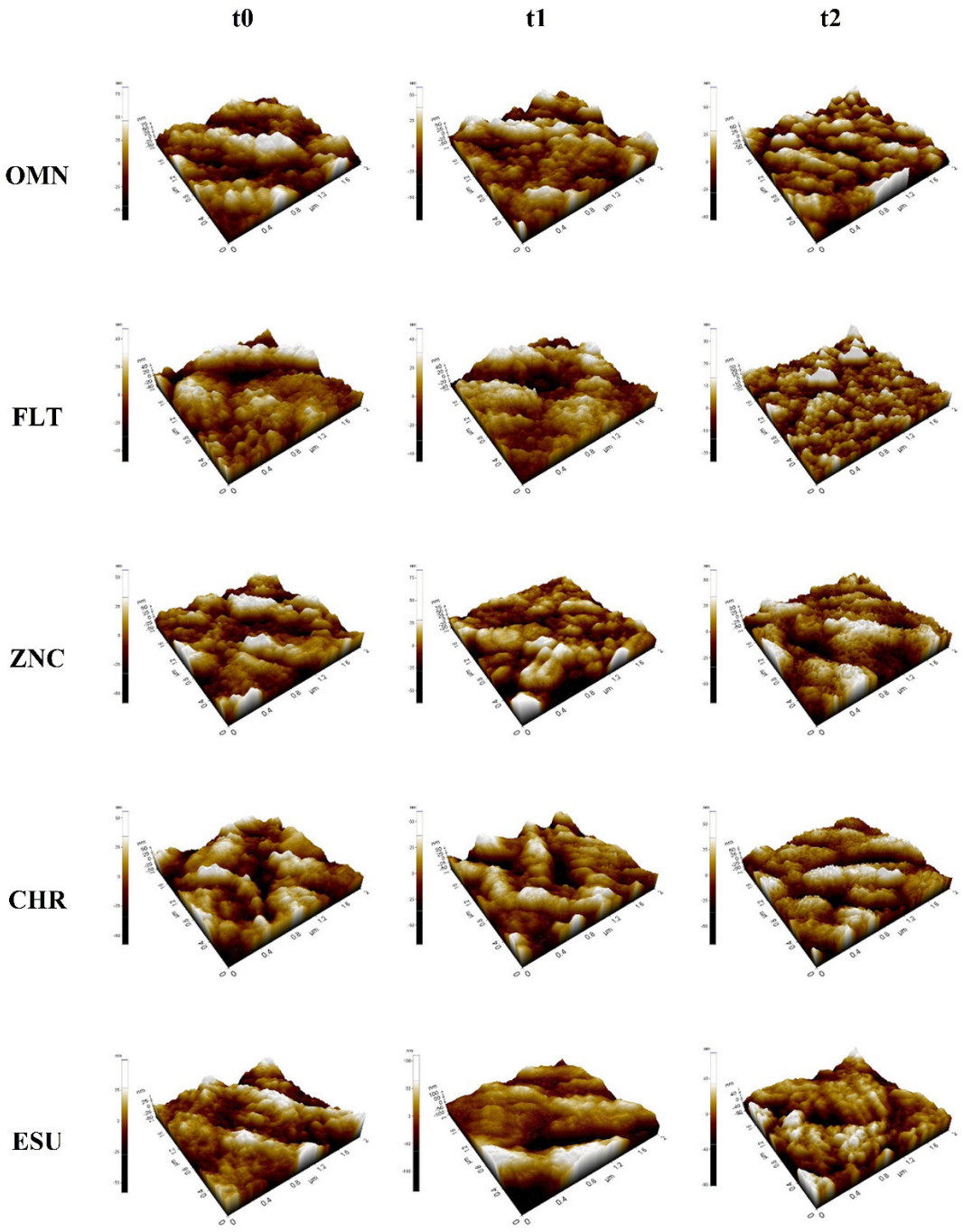

Figure 3. AFM analysis showcasing the three-dimensional surface topography of the groups

DISCUSSION

The widespread use of various shades of adhesive composites for esthetic restorations in both anterior and posterior teeth has highlighted challenges related to the multiple application steps and the significant time required, which can be demanding for clinician.[2,3] To address these issues, manufacturers have recently introduced single-shade resin composites, offering the benefits of reduced chair time and simplified shade selection.[2] However, it has been noted that any discoloration in resin composites can adversely affect their esthetic outcomes.[21,22] The findings of this study indicate that single-shade composite resins, such as OMN and ESU, did not show a significant difference in color stability compared to the multi-shade composite resin, FLT, after aging and bleaching procedures. These results support the acceptance of the color stability hypothesis, demonstrating that single-shade composites perform similarly to multi-shade composites under these conditions. Additionally, the surface roughness of single-shade composite resins, such as ZNC and OMN, did not differ significantly from that of FLT after aging and bleaching treatments. This finding supports the acceptance of the surface roughness hypothesis, confirming that single-shade composites exhibit similar

surface characteristics to multi-shade composites and are a reliable option for clinical use.

Color measurement is commonly performed using the CIELAB color system. The CIE Lab* system is widely adopted due to its standardized methodology, which allows for precise analysis of ΔE* values. This system is known for its ability to detect subtle color changes accurately and offers several advantages, including objectivity, repeatability, and high sensitivity.[23-25] In this study, the CIEDE2000 color difference formula was selected for its ability to provide a more sensitive evaluation of minor to medium color discrepancies, offering a single-number shade pass/fail criterion that improves upon the traditional CIE Lab* system.

Office-type bleaching is widely utilized in dental practices to enhance the aesthetic appearance of teeth. Although bleaching can sometimes effectively remove surface stains from composite restorations and restore their color, it does not lighten composite resins in the same way it affects natural tooth structures.[26] Researches have shown that bleaching treatments can improve the removal of stains from composite resins, but they may also lead to alterations in the color of these materials.[27,28] A study on a single-shade composite (OMN) found no significant differences in L*, a*, and b* values between the composite restoration and the tooth at various time intervals after bleaching, with both visual and instrumental analyses confirming a perfect match between the two.[29] In the current study, the FLT group retained a color closer to its initial shade compared to the single-shade composites, highlighting a significant difference in their response to bleaching. While single-shade composite resins did undergo color change due to the bleaching process, they were less effective at maintaining their original shade compared to the multi-shade composite.[30] The highest ΔE values in OMN can be attributed to the size and distribution of its filler particles and the interaction of its resin matrix with bleaching agents. Increased water absorption in this composite may have also contributed to the color change. The higher surface roughness observed in ESU and CHR may be due to larger filler particles and differing wear rates during bleaching. The abrasive effect of bleaching agents likely contributed to the increased surface roughness as well.

Recent research tested four single-shade composites (OMN, CHR, Vitra Unique, and ESU) on 40 human incisors, using a VITA Easyshade Compact V spectrophotometer to evaluate ΔE. The study reported that all tested composites exhibited acceptable color-matching, with no significant differences between tooth shades and the resin composites.[31] These findings differ partially from our study, likely due to differences in composite selection and specimen preparation methods. While both studies observed similar composite behavior, our analysis revealed statistically significant differences between the composites. In another study, the effect of thickness on the translucency and whiteness of single-shade resin composites (OMN, Vitra Unique, ZNC, and CHR) was compared to a multi-shade composite (Filtek Z250) after thermocycling. The study found that single-shade composites had higher translucency and whiteness values than the multi-shade composite, both before and after aging.[32] However, unlike the present study, bleaching procedures were not performed, which may explain the differences observed in color stability.

It is well-documented that the surface roughness of resin composites can significantly impact their optical properties and ability to adjust color.[6,24] However, previous studies have produced inconsistent results regarding the effect of whitening treatments on the surface roughness of resin composites.[28,33] For instance, some research has found no significant difference in surface roughness after applying 40% hydrogen peroxide to both microhybrid and nanohybrid resin composites.[33] This variation in findings is likely due to several influencing factors, including the type of resin composite, the concentration of the whitening agent, the duration of exposure, the application protocol, and the type of measuring device used. In this study, surface roughness was measured using a contact mode profilometer. The results showed that the surface roughness of the multi-shade composite (FLT) was similar to that of the single-shade composites (ZNC and OMN) after bleaching treatments, while the other single-shade composites (ESU and CHR) exhibited significantly different roughness values. This suggests that different composite materials, whether single-shade or multi-shade, respond variably to bleaching. These findings have important implications for clinical practice, particularly in selecting materials that maintain surface integrity after whitening procedures. Further research is needed to establish standardized protocols for assessing and predicting the impact of bleaching on various composite resins.

SEM and AFM analyses confirmed the morphological changes on the composite surfaces, adding a qualitative dimension to the quantitative findings. SEM images showed that the surface morphology was largely preserved across all groups, while AFM results indicated slight increases in surface roughness. These irregularities, particularly observed in the OMN and ZNC groups, are not considered substantial enough to negatively impact clinical performance. Overall, the findings suggest that the composites maintained their structural integrity and are suitable for long-term use. However, the minor surface irregularities observed in the SEM and AFM analyses should not be overlooked in terms of their

potential impact on clinical performance. Although these irregularities are small, they could negatively affect clinical parameters such as plaque accumulation, biofilm formation, and wear. Increased surface roughness may facilitate plaque buildup, potentially compromising the long-term aesthetic and biological performance of the restorations. Therefore, the surface morphology of composites is crucial not only for aesthetics but also for long-term clinical outcomes.

Limitations

This study has several limitations that should be considered when interpreting the results. First, the study was conducted in vitro, meaning that the conditions simulated in the laboratory may not fully replicate the complex environment of the oral cavity. Factors such as saliva, temperature fluctuations, and mechanical stresses in the mouth could influence the performance of composite resins differently than observed in this study. Second, the aging and bleaching procedures were performed over a relatively short time period. Although these procedures were designed to simulate long-term clinical conditions, they cannot perfectly mimic the cumulative effects of years of use in a real-world setting. Longitudinal clinical studies are needed to confirm the durability and performance of single-shade composites over time.

Clinical Relevance

The results of this study provide valuable insights for dental practitioners when selecting restorative materials, particularly in cases involving aging and bleaching treatments. Single-shade resin composites demonstrated comparable color stability and surface roughness to multi-shade composites, making them a viable option for esthetic restorations. However, variations in the performance of different composites highlight the need for careful material selection, especially in patients undergoing bleaching procedures. Understanding how these materials respond to clinical conditions can improve long-term outcomes and patient satisfaction.

CONCLUSIONS

These findings indicate that while single-shade composites offer significant advantages in simplifying shade selection and reducing clinical time, their performance under various clinical conditions is largely comparable to that of the multi-shade composite. Specifically, the results show that the color stability of single-shade composites, particularly OMN and ESU, did not differ significantly from that of FLT after aging and bleaching treatments. Furthermore, surface roughness measurements revealed that single-shade composites like OMN and ZNC maintained surface qualities similar to FLT, even after extensive aging and bleaching procedures.

ETHICAL DECLARATIONS

Ethics Committee Approval

No biological materials were used in the laboratory study with composite resin, no personal data are available. Therefore, ethics committee approval is not required.

Informed Consent

No biological materials were used in the laboratory study with composite resin, no personal data are available. Therefore, ethics cinformed consent is not required.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Investigation of risk factors for benign or malignant endometrial pathology in patients presenting with abnormal uterine bleeding

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ABSTRACT

Aims: The aim of this study was to determine the risk factors associated with benign or malignant endometrial pathologies by comparing endometrial biopsy results of women presenting with abnormal uterine bleeding (AUB).

Methods: In this cross-sectional study using retrospective record review method, 100 women over 18 years of age who presented to the gynecology and obstetrics clinic with AUB and underwent endometrial biopsy were included. Age, body mass index (BMI), obstetric and gynaecological history, medical history and pathology results were recorded. Endometrial pathology results were classified as normal, benign and malignant. The effects of demographic and clinical characteristics of the patients on the risk of benign and malignant pathology were analysed.

Results: The mean age and BMI of the participants were 48.7±7.7 and 29.3±5.9 kg/m2, respectively. 59 (59.0%) of the patients were in the premenopausal period and 41 (41.0%) were in the postmenopausal period. Ultrasonographic endometrial thickness was below 8 mm in 23 patients (23.0%), between 8-11 mm in 27 patients (27.0%) and 12 mm or more in 50 patients (50.0%). Pathological results were normal in 35 patients (35%), benign pathology in 45 patients (45.0%) and malignant pathology in 20 patients (20.0%). In multivariate analyses, each 1 year increase in the age of the patients increased the risk of developing malignant endometrial pathology 1.17 times and each 1 mm increase in ultrasonographic endometrial thickness increased the risk of developing malignant endometrial pathology 1.16 times. The cut-off point for ultrasonographic endometrial thickness was found to be >12 mm. According to this cut-off point, the sensitivity and specificity of ultrasonographic endometrial thickness in predicting endometrial pathology were found to be 70% and 62.9%, respectively.

Conclusion: It is important to determine the risk factors of malignant disease in women presenting with AUB and to perform invasive methods such as endometrial biopsy in the early period in women with risk factors to affect the success of treatment directly.

Keywords: Abnormal uterine bleeding, endometrial biopsy, benign pathology, malignant pathology, endometrial cancer

INTRODUCTION

Abnormal uterine bleeding (AUB) is one of the most common clinical conditions requiring gynaecological evaluation worldwide.[1] It constitutes one-third of the admissions to gynecology outpatient clinics, and women in premenopausal, perimenopausal and postmenopausal periods may present with AUB.[2] From menarche to menopause. 9 to 14 per cent of all women have a clinical picture of ACS, which has significant effects on the quality of life of patients and may lead to economic losses.[3]

It has been reported that AUB may occur as a result of structural or non-structural uterine diseases. In 2011, the International Federation of Gynecologists and Obstetrics (FIGO) established the PALM-COEIN classification to define AUB pictures and prevent inconsistencies in terminology in the literature.[4] With this classification, PALM refers to structural causes such as polyps, adenomyosis, leiomyoma and malignancy, while COEIN refers to coagulopathy, ovulatory dysfunction, endometrial causes, iatrogenic causes and unclassified pathologies.[4,5] It has been found that most AUBs are not associated with a premalignant or malignant lesion. However, it is reported that AUBs especially in the postmenopausal period and premenopausal AUBs with additional risk factors may be associated with endometrial cancer. Therefore, women presenting with AUB require additional evaluation for endometrial cancer.[6]

AUB is an important clinical presentation seen in premenopausal, perimenopausal and postmenopausal women and constitutes a significant proportion of

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admissions to gynecology outpatient clinics.[7] Studies have shown that most AUBs are not associated with a premalignant or malignant lesion. However, it has been reported that especially postmenopausal women with AUB and premenopausal women with AUB with additional risk factors should be evaluated for endometrial cancer.[6] In the literature, many studies aim to determine the aetiology of bleeding in women presenting with AUB. In these studies, the PALM-COIN classification was created to eliminate the terminology confusion and to create a common language and the etiological factors were revealed with this classification.[8,9] However. studies investigating the risk factors associated with benign or malignant lesions in women presenting with AUB are limited. Our aim was to determine the risk factors associated with benign or malignant endometrial pathologies by comparing endometrial biopsy results of women presenting to a university hospital with AUB.

METHODS

Ethical Aspects of the Study

Ethics committee approval was obtained from Adıyaman University Non-interventional Clinical Researches Ethics Committee (Date: 22.09.2020, Decision No: 2020/08-21) and institutional permission was obtained from the institution where the study was conducted before the study was started. The research was conducted by the Declaration of Helsinki at all stages from design to reporting. The data and information obtained in the study were not used for other than scientific purposes.

Objective

This study aimed to determine the risk factors associated with benign or malignant endometrial pathologies by comparing the results of endometrial biopsy of women presenting to a university hospital with AUB.

Study Methodology

This cross-sectional epidemiological study using a single-centre retrospective record review method was conducted in a university hospital's Gynecology and Obstetrics Clinic. The study data was screened so that the timeline will be between 01.11.2020-10.05.2021.

Population and Sample of the Study

The population of the study consisted of women over the age of 18 who applied to our clinic with the complaint of AUB and underwent endometrial biopsy. In this hospital, approximately 62.000 outpatient obstetrics and gynecology patients were admitted to the Obstetrics and Gynecology Clinic annually, 9.700 inpatients were followed up, approximately 2.000 gynaecological operations. 3.600 deliveries and 2.700 caesarean sections were performed between 01.12.2007-31.10.2020.

The sample size was not calculated before the study. and all women who met the inclusion and exclusion criteria between the study dates were included in the study between 01.11.2020-10.05.2021. During the study period, 350 patients were examined for participation in the study. 150 patients did not meet the inclusion criteria. 48 met the exclusion criteria and 52 patients were excluded due to missing data. As a result, data from 100 patients were included in the analysis.

The inclusion criteria were being 18 years of age or older, presenting with the complaint of AUB, having endometrial biopsy, not being pregnant, and having no cervical pathology, while the exclusion criteria were previous removal of the uterus, having a known history of gynaecological cancer before probe curettage, and having missing data for the study variables.

Data Collection

The data collection form used in the study consisted of 15 questions including age, body-mass index (BMI), obstetric and gynaecological history, medical history and pathology results.

Those who had a history of hereditary breast cancer. ovarian cancer. endometrial cancer and colon cancer in themselves and/or in their family were considered to have a positive cancer history and/or a positive family history of cancer.

Participants were grouped as underweight if their BMI values were below 18.5 kg/m^2, normal weight if they were between 18.5-25 kg/m^2, overweight if they were between 25.0-30.0 kg/m^2, and obese if they were 30.0 kg/m^2 and above.

GE Voluson P8 USG system (GE Healthcare, USA) was used for ultrasonographic endometrial thickness measurement. In the literature. in studies examining the relationship between ultrasonographic endometrial thickness and malignant pathologies in symptomatic or asymptomatic women, the cut-off point was taken at values ranging between 8-15 mm in premenopausal women[10], while it was taken at values ranging between 3-8 mm in postmenopausal women.[11] Since we included premenopausal and postmenopausal women in our study, ultrasonographic endometrial thickness was grouped as less than 8 mm, 8-12 mm and 12 mm or more in descriptive statistics.

Endometrial pathology results were classified as normal, benign and malignant. Pathological examination of the preparations obtained from endometrial biopsy was analysed in the laboratories of the Department of Medical Pathology of the university. Pathological examination was performed with 40X and 100X magnification

using Olympus BX53. Olympus CX41 and Olympus CX31 (Olympus Corporation, Japan) microscopes after hematoxylin-eosin staining.

Procedure

In the study. a retrospective archive search was performed by the researcher himself between 01.12.2007-31.10.2020.

Statistical Analysis

SPSS version 20.0 and MedCalc version 15 statistical package programs were used for data analysis. Mean±standard deviation. median and minimum-maximum values were used for continuous numerical variables and number and percentage were used for categorical variables. The conformity of the numerical variables to normal distribution was checked by Kolmogorov-Smirnov and Shapiro-Wilk tests. Univariate and multivariate logistic regression analysis was used to analyse the risk of benign and malignant endometrial pathology. In regression analysis, univariate analysis was used first. Multivariate analysis was performed with the factors found to be statistically significant in univariate analyses. Odds Ratio (OR) and 95% confidence interval (95% CI) were calculated to evaluate the risk. ROC analysis was used to determine the cut-off point for ultrasonographic endometrial thickness to predict the development of endometrial pathology. The statistical significance limit value $p<0.05$ was accepted.

RESULTS

Among the women in the study. 8 (8.0%) were under 40 years of age, 55 (55.0%) were between 40-49 years of age. 30 (30.0%) were between 50-59 years of age and 7 (7.0%) were 60 years of age or older. and the mean age was 48.7±7.7 years. While 29 (29.0%) of the women were normal weight. 36 (36.0%) were overweight and 35 (35.0%) were obese, the mean BMI was 29.3±5.9 kg/m^2 (**Table 1**).

Hypertension was found in 20 (20.0%). DM in 14 (14.0%). cancer history in 13 (13.0%) and family history of cancer in 18 (18.0%) of the women (**Table 1**).

While 2 (2.0%) of the women had never been pregnant. the median number of pregnancies was found to be 4. In addition. the parity was 0 in 3 patients (3.0%) and the median parity was 4 (**Table 1**).

59 (59.0%) of the patients were in premenopausal period and 41 (41.0%) were in the postmenopausal period. In addition. 1 patient (1.0%) had polycystic ovary syndrome, 22 patients (22.0%) had myoma uteri, 9 patients (9.0%) had intrauterine device and 11 patients (11.0%) had infertility. Ultrasonographic endometrial thickness was below 8 mm in 23 patients (23.0%), between 8-11 mm in 27 patients (27.0%) and 12 mm or more in 50 patients (50.0%). The mean endometrial thickness was 12.9±6.1 mm (**Table 1**).

Pathology results were normal in 35 patients (35%). benign pathology in 45 patients (45.0%) and malignant pathology in 20 patients (20.0%). 15 (42.9%) of 35 patients with normal pathology results had irregular proliferative endometrium. 12 (34.3%) had endometrial destruction findings and 8 (22.9%) had secretory endometrial findings. Of 45 patients with benign pathology. 18 (40.0%) had endometrial polyps. 16 (35.6%) had endometritis. 10 (22.2%) had simple atypical hyperplasia and 1 (2.2%) had myoma uteri. Of the 20 patients with malignant pathology, 11 (55.50%) had endometrioid adenocarcinoma, 5 (25.0%) endometrioid intraepithelial neoplasia. 1 (5.0%) carcinosarcoma. 1 (5.0%) metastasis, 1 (5.0%) serous cystadenoma and 1 (5.0%) clear cell carcinoma (**Table 1**).

Accordingly, age, BMI, presence of hypertension. presence of DM, history of cancer, family history of cancer, number of pregnancies, number of parities, menopausal status, presence of PCOS, presence of myoma uteri, presence of IUD, presence of infertility and USG endometrial thickness had no statistically significant effect on the risk of benign pathological development (**Table 2**).

Each year increase in the age of the patients statistically significantly increased the risk of malignant pathology development by 1.16 times (p=0.002). In addition, the presence of DM statistically significantly increased the risk of malignant pathology by 7.07 times (p=0.026). Past cancer history of the patient statistically significantly increased the risk of developing malignant endometrial pathology by 4.17 times (p=0.044). Postmenopausal patients are statistically significantly 4.33 times more at risk of developing malignant pathology than premenopausal patients (p=0.014). In addition, each 1 mm increase in ultrasonographic endometrial thickness statistically significantly increased the risk of developing malignant endometrial pathology by 1.13 times (p=0.019). BMI, presence of hypertension. history of cancer. family history of cancer, pregnancy, parity, history of PCOS, myoma uteri, IUD and infertility were not found to be associated with the risk of developing malignant pathology (**Table 3**).

In the multivariate logistic regression analysis performed with the risk factors found statistically significant in univariate analyses. age and ultrasonographic endometrial thickness were found statistically significant in terms of the risk of developing malignant endometrial pathology. Accordingly, each 1-year increase in the age of the patients increased the risk of developing malignant endometrial pathology by 1.17 times (p=0.025). which was statistically significant and independent of the

Table 1. The demographic. anthropometric. medical. obstetric. gynaecological and pathological characteristics of women					
Feature		**n**	**%**	**$\bar{X}$±SD Med**	**Min-Max**
Demographic and anthropometric characteristics of women					
Age (year)	Under 40 years old	8	8.0		
	40-49 years old	55	55.0	48.7±7.7	33.0-76.0
	50-59 years old	30	30.0	47.5	
	60 years and over	7	7.0		
BMI	Normal weight	29	29.0		
	Overweight	36	36.0	29.3±5.9	20.0-49.0
	Obese	35	35.0	29.0	
		100	100.0		
Women's medical history					
Hypertension	None	80	80.0		
	There is	20	20.0		
DM	None	86	86.0		
	There is	14	14.0		
History of cancer	None	87	87.0		
	There is	13	13.0		
Family history of cancer	None	82	82.0		
	There is	18	18.0		
		100	100.0		
Obstetric characteristics of women					
Pregnancy	0	2	2.0		
	1	3	3.0		
	2	7	7.0	4.9±2.4	0.0-11.0
	3	18	18.0	4.0	
	4 and over	70	70.0		
Parity	0	3	3.0		
	1	3	3.0		
	2	10	10.0	4.2±2.1	0.0-10.0
	3	30	30.0	4.0	
	4 and over	64	64.0		
		100	100.0		
Gynaecological characteristics of women					
Menopausal status	Premenopausal	59	59.0		
	Postmenopausal	41	41.0		
History of polycystic ovary syndrome	None	99	99.0		
	There is	1	1.0		
Myoma uteri	None	78	78.0		
	There is	22	22.0		
Intrauterine device	None	91	91.0		
	There is	9	9.0		
History of infertility	None	89	89.0		
	There is	11	11.0		
Ultrasonographic endometrium thickness (mm)	Under 8 mm	23	23.0		
	between 8-11 mm	27	27.0		
	12 mm and above	50	50.0		
Ultrasonographic endometrium thickness (mm)				12.9±6.11.5	15.0-34.0
Results of pathology of women		100	100.0		
Pathological result (n=100)	Normal	35		35.0	
	Benign	45		45.0	
	Malignant	20		20.0	
Normal pathology (n=35)	Irregular proliferative endometrium	15		42.9	
	Signs of endometrial destruction	12		34.3	
	Secretory endometrium	8		22.9	
Benign pathology (n=45)	Endometrial polyp	18		40.0	
	Endometritis	16		35.6	
	Simple atypical hyperplasia	10		22.2	
	Myoma uteri	1		2.2	
Malignant pathology (n=20	Endometrioid adenocarcinoma	11		55.0	
	Endometrioid intraepithelial neoplasia	5		25.0	
	Carcinosarcoma	1		5.0	
	Metastasis	1		5.0	
	Serous cystadenoma	1		5.0	
	Transparent cell carcinoma	1		5.0	

SD: Standard deviation, BMI: Body-mass index, Med: Median, Min: Minimum, Max: Maximum

Table 2. Univariate analysis of factors affecting the development of benign endometrial pathology

Feature (n=80)		Risk of benign pathology OR (95% GA)	p*
Age (years)		**1.02 (0.94-1.10)**	**0.648**
BMI (kg/m²)		0.99 (0.92-1.07)	0.779
Hypertension	None	-	0.370
	There is	1.71 (0.53-5.57)	
DM	None	-	0.273
	There is	2.54 (0.48-13.43)	
History of cancer	None	-	0.256
	There is	0.36 (0.06-2.09)	
Family history of cancer	None	-	0.433
	There is	1.67 (0.46-6.10)	
Pregnancy		0.94 (0.77-1.15)	0.551
Parity		0.91 (0.72-1.16)	0.448
Menopausal status	Premenopausal	-	0.087
	Postmenopausal	2.31 (0.89-6.03)	
History of polycystic ovarian dysplasia	None	-	n.a.
	There is	n.a.	
Myoma uteri	None	-	0.225
	There is	0.51 (0.17-1.52)	
Intrauterine device	None	-	0.270
	There is	0.43 (0.09-1.93)	
History of infertility	None	-	0.245
	There is	2.31 (0.56-9.43)	
USG endometrium thickness (mm)		0.98 (0.90-1.07)	0.668

* Multivariate logistic regression analysis was performed, OR: Odds Ratio, BMI: Body-mass index

Table 3. Univariate analysis of factors affecting the development of malignant endometrial pathology

Feature (n=55)		Risk of malignant pathology OR (95% GA)	p*
Age (years)		1.16 (1.06-1.27)	0.002
BMI (kg/m²)		1.03 (0.95-1.11)	0.527
Hypertension	None	-	0.327
	There is	2.00 (0.50-7.99)	
DM	None	-	0.026
	There is	7.07 (1.27-39.41)	
History of cancer	None	-	0.044
	There is	4.17 (1.04-16.73)	
Family history of cancer	None	-	0.096
	There is	3.32 (0.81-13.66)	
Pregnancy		0.94 (0.77-1.15)	0.551
Parity		0.91 (0.72-1.16)	0.448
Menopausal status	Premenopausal	-	0.014
	Postmenopausal	4.33 (1.34-13.99)	
History of polycystic ovarian dysplasia	None	-	n.a.
	There is	n.a.	
Myoma uteri	None	-	0.836
	There is	1.17 (0.26-5.31)	
Intrauterine device	None	-	0.309
	There is	0.32 (0.03-2.92)	
History of infertility	None	-	n.a.
	There is	n.a.	
USG endometrium thickness (mm)		1.13 (1.02-1.25)	0.019

* Multivariate logistic regression analysis was performed, OR: Odds Ratio, BMI: Body-mass index

presence of DM, menopausal status and ultrasonographic endometrial thickness. In addition, each 1 mm increase in ultrasonographic endometrial thickness increased the risk of developing malignant endometrial pathology by 1.16 times (p=0.043), which was statistically significant and independent of age. DM status and menopausal status (**Table 4**).

The cut-off point for ultrasonographic endometrial thickness was found to be >12 mm. According to this cut-off point. the sensitivity and specificity of ultrasonographic endometrial thickness in predicting endometrial pathology were found to be 70% and 62.9%. respectively (**Table 5** and **Figure 1**).

Table 4. Multivariate analysis of factors affecting the development of malignant endometrial pathology

Feature (n=55)		Risk of malignant pathology Adjusted OR (95% GA)	p*
Age (years)		1.17 (1.02-1.33)	0.025
DM	None	-	0.194
History of cancer	There is	5.09 (0.44-59.41)	
Menopausal status	None	-	0.100
USG endometrium thickness (mm)	There is	4.87 (0.74-32.21)	
Age (years)	Premenopausal	-	0.745
DM	Postmenopausal	0.747 (0.13-4.31)	
History of cancer		1.16 (1.01-1.33)	0.043

* Multivariate logistic regression analysis was performed, OR: Odds Ratio

Table 5. ROC analysis and cut-off point of ultrasonographic endometrial thickness values in the detection of malignant endometrial pathology

Criterion	Ultrasonographic endometrial thickness (mm)
Area under the curve (95% CI)	0.680 (0.540-0.799)
Cut-off point	>12
Sensitivity (95% CI)	70.0 (45.7-88.1)
Specificity (95% CI)	62.9 (44.9-78.5)

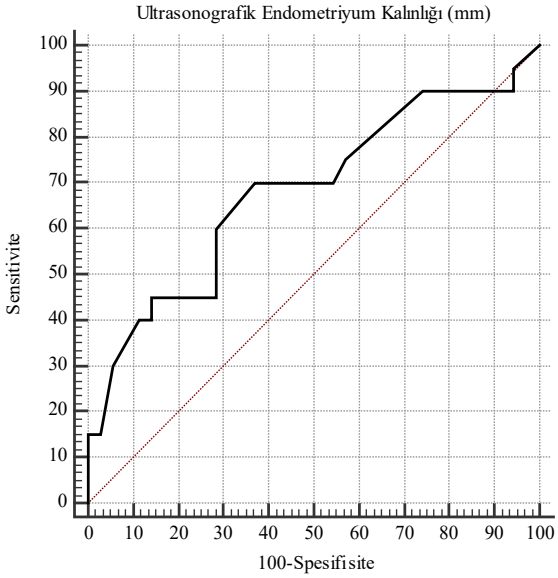

Figure 1. ROC curve of ultrasonographic endometrial thickness in the detection of malignant endometrial pathology

Multivariate logistic regression analysis was performed again according to the risk factors which were found to be statistically significant in univariate analyses and according to being below or above the 12 mm cut-off point found in the ROC analysis for USG endometrial thickness. Accordingly. those with USG endometrial thickness above 12 mm had a statistically significant 5.73-fold higher risk of developing malignant endometrial pathology than those with USG endometrial thickness of 12 mm and below (p=0.034) (**Table 6**).

Table 6. Multivariate analysis of factors affecting the development of malignant endometrial pathology

Feature (n=55)		Risk of malignant pathology Adjusted OR (95% GA)	p*
Age (years)		1.13 (0.99-1.27)	0.063
DM	None	-	0.215
History of cancer	There is	4.78 (0.40-56.46)	
Menopausal status	None	-	0.131
USG endometrium thickness	There is	4.35 (0.65-29.31)	
Age (years)	Premenopausal	-	0.683
DM	Postmenopausal	1.42 (0.26-7.77)	
History of cancer	12 mm and below	-	0.034
	Over 12 mm	5.73 (1.14-28.73)	

* Multivariate logistic regression analysis was performed.

DISCUSSION

According to our study results. each year increase in the age of the patients statistically significantly increased the risk of developing malignant pathology. The presence of DM and past cancer history statistically significantly increased the risk of malignant pathology. Postmenopausal patients were found to be at statistically significantly higher risk of developing malignant pathology than premenopausal patients. Each 1 mm increase in USG endometrial thickness significantly increased the risk of developing malignant endometrial pathology. In multivariate logistic regression analysis. age and ultrasonographic endometrial thickness were found to be statistically significant for the risk of developing malignant endometrial pathology. Each 1 mm increase in USG endometrial thickness increased the risk of developing malignant endometrial pathology. The risk of developing malignant endometrial pathology was found to be statistically significantly higher in women with USG endometrial thickness above 12 mm than in women with USG endometrial thickness of 12 mm or less.

In the multivariate analyses performed according to the findings of the study. each 1 year increase in the age of the patients increased the risk of developing malignant endometrial pathology 1.17 times, and each 1 mm increase in ultrasonographic endometrial thickness increased the risk of developing malignant endometrial pathology 1.16 times. The cut-off point for ultrasonographic endometrial thickness was found to be >12 mm. According to this cut-off point. the sensitivity and specificity of ultrasonographic endometrial thickness in predicting endometrial pathology were found to be 70% and 62.9%. respectively.

Iatrogenic causes and polyps were found to be the most common etiological causes in women presenting with AUB.[12] Apart from this, leiomyomas were found to be the other common etiology of AUB.[13] A history of leiomyoma was present in 22 (22%) of the women included in this study.

In the studies in the literature. PCOS is one of the causes of AUB in women in the reproductive period and is among the etiological causes included in the PALM-COIN classification. In different studies. it has been reported to be detected in 1.3%-19% patients.[12,14] In a meta-analysis study published by Amiri et al.[15] it was found that the risk of endometrial cancer was higher in women with PCOS compared to those without PCOS in all age groups. In this study, only 1 of the women had a history of PCOS. Therefore. it was not identified as a risk factor. In our study, the etiological factors of AUB were not grouped according to the PALM-COIN classification. unlike many studies presented above. In this study. the etiological causes were presented from the

perspective of benign and malignant causes and the risk factors associated with both benign causes and especially malignant etiologies were investigated. When the studies in the literature are analysed. it is seen that benign endometrial pathologies such as polyps. leiomyomas and endometrial hyperplasia are between 24% and 70% of the etiology of AUB.[16,17] In this study, benign pathology was observed in 45% of the patients by the literature.

When malignant pathologies are analysed in the etiology of AUB. It is seen that age is an important factor. Under the age of 50 years. malignant pathology was found in less than 1% of patients. whereas it was found in 10% to 15% of women over the age of 50 years.[18] As expected in this study. age was not found to be a risk factor for benign pathologies by univariate analysis. whereas it was found to be a risk factor for malignant pathologies. Each year increase in the age of the patients was found to increase the risk of malignant pathology development statistically significantly by 1.16 times. In multivariate logistic regression analysis. age was found to be an independent risk factor for the risk of developing malignant endometrial pathology. According to the results of our analysis. each 1-year increase in the age of women statistically significantly increased the risk of developing malignant endometrial pathology by 1.17 times.

Studies have reported that endometrial cancers are more common in postmenopausal women. In a recent study by Clarke et al.[19] the prevalence of endometrial cancer in postmenopausal women was found to be 7.9%. which is approximately 6.5 times (1.2%) higher than premenopausal and perimenopausal women. In this study. in accordance with the literature. the risk of developing malignant pathology in postmenopausal women was found to be statistically significantly 4.33 times higher than in premenopausal women.

In other studies in which endometrial hyperplasia and endometrial cancer risk factors were evaluated. obesity. PCOS. nulliparity and diabetes mellitus were reported as risk factors.[8] Harvey et al.[20] reported that high BMI increased the risk of endometrial cancer in a study. In this study. BMI was not found to be a risk factor for benign and malignant pathologies. When compared with the literature data. we think that the small number of patients in this study was effective in these results. In a metanalysis published by McVicker et al.[21] a significant association between diabetes and endometrial cancer was shown. In this study. it was determined by univariate analysis that the presence of DM increased the risk of malignant pathology statistically significantly by 7.07 times (p=0.026). DM was not found to be a risk factor in multivariate analysis.

In a study conducted by Main et al.[22] it was reported that having at least one or more children significantly decreased the risk of endometrial cancer compared with nulliparity. In the same study. they reported that endometrial cancer RR decreased with the number of pregnancies. In this study. pregnancy and parity were not found to be risk factors for endometrial malignant pathologies. This result is thought to be due to the small number of patients. It has been reported that IUD use may be a factor among iatrogenic causes of AUB and that women give up IUD use because of AUB associated with IUD use.[23] In our study. IUD use was present in 9% of the patients. In our study. benign and malignant diseases risk factors of IUD use in women presenting with AUB were evaluated. IUD use was not found to be a risk factor for both benign and malignant conditions.

Measurement of endometrial thickness by ultrasonography is important in the evaluation of endometrial pathologies in both premenopausal and postmenopausal periods.[13] Studies have reported that endometrial thickness determined by ultrasonography in women of childbearing age is between nearly 4-8 mm in the proliferative phase and 8-14 mm in the secretory phase.[24] The American College of Obstetricians and Gynecologists (ACOG) and the Society of Radiologist in Ultrasound (SRU) consider an endometrial thickness of <4 mm and <5 mm respectively as normal for postmenopausal women. It is stated that the risk of malignancy is quite low under these limit values.[25] In this study. ultrasonographic endometrial thickness was found to be below 8 mm in 23 patients (23.0%). between 8-11 mm in 27 patients (27.0%) and 12 mm or more in 50 patients (50.0%). The mean endometrium thickness was found to be 12.9±6.1 mm.

Ultrasonography is the first examination performed in women presenting with AUB and is performed under emergency conditions. As explained in the previous sections. the phase of the menstrual cycle cannot be evaluated clearly in emergency conditions and this situation negatively affects the standardisation of USG evaluation.[26] Further analyses showed that endometrial thickness had no statistically significant effect on the risk of benign pathology development in our study. However. with univariate risk factor analysis. each 1 mm increase in endometrial thickness statistically significantly increased the risk of developing malignant endometrial pathology by 1.13 times. With multivariate risk factor analysis. each 1 mm increase in ultrasonographic endometrial thickness increases the risk of developing malignant endometrial pathology by 1.16 times in a statistically significant way and independent of age. DM presence and menopausal status.

Different studies in the literature have investigated the sensitivity and specificity of ultrasonography in detecting different clinical pathologies. Kılınç et al.[27] reported the sensitivity and specificity of USG in the diagnosis of endometrial polyp as 78.26% and 51.35%. respectively. In a study conducted by Saccardi et al.[28] patients with endometrial thickness ≥11 mm were compared with patients with endometrial thickness between 5-10 mm by transvainal USG. In the same study it was reported that the risk of endometrial cancer or endometrial hyperplasia with atypia was 2.6 times higher in women with endometrial thickness ≥11 mm than in women with endometrial thickness 5-10 mm. In another meta-analysis endometrial cancer risk was analysed according to the cut-off value of ultrasonographic endometrial thickness 5 mm in asymptomatic postmenopausal women. The sensitivity and specificity of transvaginal USG with a cut-off value of 5 mm were found to be 83% and 72%. respectively.[29] In this study, the cut-off point for ultrasonographic endometrial thickness was determined as >12 mm by ROC curve analysis. According to this cut-off point, the sensitivity and specificity of ultrasonographic endometrial thickness in predicting endometrial pathology were found to be 70% and 62.9%, respectively. It is seen that these values we found are similar to the studies in the literature in which various endometrial pathologies were evaluated ultrasonographically. In addition, in various studies investigating the role of USG in predicting endometrial pathologies, the factors affecting different sensitivity and specificity values were listed as the experience of the practitioner. menstrual periods of the patients. being in menopause and hormonal treatments.[29,30]

It has been reported that ultrasonography or hysteroscopy may not be sufficient to identify endometrial pathologies in women presenting with AUB.[30] In this context. evaluation with endometrial biopsy should be considered in patients aged 40 years and older presenting with AUB in whom the etiology cannot be determined or who do not respond to treatment. In this study. endometrial biopsy was performed in all women presenting with AUB along with ultrasonographic evaluation.

In conclusion. identification of malignant disease risk factors is an important step in women presenting with AUB. In women with risk factors, early application of invasive methods such as endometrial biopsy would be the appropriate approach. It is thought that this study contributed to the literature by investigating and revealing the risk factors of malignant disease.

Limitations

The small number of patients in the study can be considered as a limitation. In addition, the fact that the patients included in the study were not homogenous is one of the limitations of the study.

CONCLUSION

In conclusion, based on the correlation between age and ultrasonographic endometrial thickness and the development of malignant endometrial pathology, it is important to evaluate the patients in terms of possible malignant pathologies, especially in elderly women when the endometrial thickness measurement by USG is above 12 mm. In addition. it is predicted that determination of malignant disease risk factors in women presenting with AUB and early application of invasive methods such as endometrial biopsy in women with risk factors will directly affect the success of treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval

Ethics approval was obtained from Adıyaman University Non-interventional Clinical Researches Ethics Committee (Date: 22.09.2020, Decision No: 2020/08-21).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design. execution. and analysis of the paper. and that they have approved the final version.

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Original Article

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Relationship between ultrasonographic liver steatosis degree and oxidative/nitrosative stress in patients diagnosed with metabolic dysfunction-associated steatotic liver disease

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ABSTRACT

Aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) remains the most common chronic liver disease worldwide. It is considered to be a complication of metabolic syndrome. The main element in intra- and extrahepatic disorders in MASLD is oxidative/nitrosative stress (ONS). The relationship between the increase and decrease in these markers and the degree of liver steatosis defined sonographically has not been specifically studied before.

Methods: Patients in the MASLD spectrum were divided into 3 groups according to the degree of liver steatosis on ultrasonography (US). Patients without liver steatosis on US were taken as the control group. Nitric oxide (NO), malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) were studied in the blood of these patients.

Results: Changes in the degree of liver steatosis on US and changes in the studied parameters were found to be statistically significant. In addition, the cut-off values of NO and MDA were shown to be 8.98 and 2.375, respectively, in distinguishing the healthy control group from the patient group.

Discussion: As the degree of liver steatosis increases on US, NO and MDA levels increase, while antioxidant enzymes CAT and SOD levels decrease. NO and MDA can be used to distinguish healthy and patient groups in the preliminary diagnosis of MASLD.

Conclusion: There is a significant relationship between the degree of liver steatosis on US and ONS parameters.

Keywords: Oxidative/nitrosative stress, metabolic dysfunction-associated steatotic liver disease, ultrasonography

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD) remains the most common chronic liver disease worldwide.[1] MASLD is defined as the accumulation of triglycerides in more than 5% of hepatocytes in individuals who do not consume significant alcohol.[2] In this process, simple steatosis may be present, and it may also progress to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Approximately one-fourth of simple steatosis develops steatohepatitis, while more than one-fourth of patients with steatohepatitis develop significant fibrosis.[3] MASLD can sometimes be a symptom of an underlying disease. However, it is not a disease in itself. And MASLD is not a single disease but encompasses a number of diseases.[4] MASLD is considered a complication of metabolic syndrome because it is associated with hypertension, obesity, insulin resistance and dyslipidemia.[2,5] MASLD does not only affect the liver. The main intra-and extrahepatic complications associated with MASLD include portal hypertension, sarcopenia, cirrhotic cardiomyopathy, hepatorenal syndrome, hepatic encephalopathy, and peripheral neuropathy.[6,7]

The main element in all these disorders is oxidative stress.[8] Under normal conditions, there are antioxidant systems that protect cells from damage by neutralizing oxidative species.[9] Free radicals are atoms or molecules that are

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unstable and reactive. There are two types of free radicals: oxygen-derived (ROS) and nitrogen-derived (RNS).[10] Oxidative/nitrosative stress (ONS) is an imbalance in favor of an increase in ROS/RNS. It is known that ONS is involved in a series of diseases, including liver diseases.[11] The most well-known of the RNS is nitric oxide (NO). Much evidence has been shown that NO plays important physiological and pathological roles in the liver. Among the antioxidant systems, superoxide dismutase (SOD) and catalase (CAT) are important enzymatic antioxidants.[10] ONS damages cellular elements and impairs their functions and contributes to the pathophysiology of many chronic diseases, including MASLD.[12,13] When reactive oxygen species increase, they can consume antioxidant molecules and inhibit antioxidant enzymes such as SOD. As a result, antioxidant systems are reduced in blood, serum, plasma and liver.[14] It has been shown that antioxidant capacity in liver cells is reduced in MASLD patients.[15]

Ultrasonography (US) is a cheap, noninvasive and easily accessible imaging method. US is the most commonly used method in the diagnosis of hepatosteatosis (82-89% sensitivity and 93% specificity). However, if hepatosteatosis is mild or the patient is obese, its sensitivity drops below 30%. Grading (Grade 1-2-3) is also performed with US. Although it was previously thought that this grading had no clinically proven importance and was only frequently used in practice to follow the disease,[16] later studies were conducted showing the correlation of this grading with liver function tests.[17]

Many relationships have been shown in the literature between MASLD and ONS, as mentioned above. This process is still a subject of research. In particular, noninvasive criteria that will contribute to the process will be even more important. In the light of this information, we wanted to shed light on a topic that has not been addressed in the literature. We investigated the relationship between the degree of ultrasonographic liver steatosis and ONS parameters.

METHODS

Ethical Approval And Informed Consent

Ethics committee approval was obtained for this study from the Kahramanmaraş Sütçü İmam University Faculty of Medicine Local Ethics Committee (Date: 13.12 2022, Decision No: 06). The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from patients in the patient group and control group.

Study Design

After obtaining ethics committee approval, the study was conducted prospectively. Informed consent was obtained from patients who met the inclusion criteria. Patients with exclusion criteria were not included in the study from the beginning. After the US procedure, blood samples taken from the patients were examined in the laboratory. The results were analyzed statistically.

Patients

The patient and control groups were selected from patients over the age of 18. The groups were planned to be close in number in terms of gender factor.

Ultrasound Imaging

US examination to assess the degree of liver steatosis was performed after a minimum of 8 hours of fasting. The patient was assessed in the supine position. All US examinations were performed by an experienced radiologist. All examinations were performed using a Canon Aplio a ultrasound device (Canon Medical Systems Corporation, Tokyo, Japan) with a convex probe (Multi-Frequency Slim Face Convex). The criteria we used to determine whether there is liver steatosis or to grade steatosis on US are shown in **Table 1**.

Table 1. Parameters we used to evaluate the degree of liver steatosis in US. PV; portal vein

	Degree of liver steatosis			
Sonographic parameter	**Normal**	**Grade 1**	**Grade 2**	**Grade 3**
Liver echo compared to spleen echo	Darker	Similar	Brighter	Brighter
PV wall echogenicity distinction can be made	Yes	No	No	No
Deep spaces inside the liver can be seen	Yes	Yes	No	No
Diaphragm can be seen	Yes	Yes	Yes	No

Laboratory Parameters

Blood samples were taken from the cases included in the study. These blood samples were centrifuged at 3000 g (relative centrifugal force) for 10 minutes at 4°C to separate plasma and aspirate buffy coat. Erythrocytes were washed 4 times with cold physiological saline and stored at -80°C until the day of analysis. CAT activity in erythrocytes was measured in samples using the method described by Beutler.[18] The dissociation of the H_2O_2 substrate was monitored spectrophotometrically at 240 nm. CAT activity was recorded as Ug/Hb. The method described by Fridovich was used to estimate SOD activities in erythrocytes.[19] Ug/Hb was used to express SOD activity. Lipid peroxidation level was expressed as MDA.[20] MDA levels were expressed as nmol/mL. NO levels in plasma samples were determined with a "sandwich" enzyme-linked immunosorbent assay kit (NO catalogue number MBS2540417 mybiosource elisa kit, USA) according to the manufacturer's protocol. NO levels were expressed as μmol/L.

Exclusion Criteria

Patients with known alcohol use, steatogenic drug use, hepatitis (viral, autoimmune), primary biliary cirrhosis,

alcohol-related liver disease, drug or toxin-related liver disease, liver fibrosis, hypertension, coronary atherosclerotic disease, diabetes mellitus, malignancy, and patients with hypertriglyceridemia in the blood picture were excluded from the study. In addition, patients with any space-occupying lesion in the liver during sonographic examination were excluded from the study. Also, cases with liver steatosis but with significant heterogeneity of steatosis were not included in the study to avoid grading errors.

Statistical Analysis

The conformity of quantitative variables to normal distribution was examined with the Shapiro-Wilk test. Group comparisons for variables not showing normal distribution were performed with the Kruskal-Wallis H test. Dunn-Sidak test was applied for post hoc (pairwise comparisons). Relationships between quantitative variables were examined with the Spearman correlation test. The performance of variables in diagnostic tests was examined with ROC analysis. Statistical significance was accepted as $p<0.05$. Statistical parameters were expressed as Median, (q1-q3), r (correlation coefficient). IBM SPSS version 22 (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States) program was used in the evaluation of the data.

RESULTS

Analyses were conducted with 119 patients who agreed to participate in the study after the patients were excluded by the exclusion criteria. Demographic data of our patients are shown in **Table 2**.

Table 2. Demographic characteristics of our patients according to groups

Degree of liver steatosis	Number and percentage of patients	Age range	Male-female
0	28-(23.5)	19-64	15-13
1	36- (30.3)	20-61	17-19
2	31-(26.1)	19-59	17-14
3	24-(20.2)	20-63	12-12
	119-(100)	19-64	61-58

The significance study of our ONS parameters between groups is shown in **Table 3**. Each of the studied parameters was found to be statistically significantly different between the groups.

When the CAT values of the groups were examined, the median value of the Control group was 17.48 Ug/Hb, the median value of the Grade 1 group was 16.21 Ug/Hb, the median value of Grade 2 was 11.28 Ug/Hb, and the median value of Grade 3 was 9.90 Ug/Hb (**Table 3**).

When the SOD values of the groups were examined, the median value of the control group was 579.41 Ug/Hb, the median value of the Grade 1 group was 486.79 Ug/Hb, the median value of Grade 2 was 511.23 Ug/Hb, and the median value of Grade 3 was 402.03 Ug/Hb (**Table 3**).

When the MDA values of the groups were examined, the median value of the Control group was 2.24 nmol/ml, the median value of the Grade 1 group was 2.31 nmol/ml, the median value of Grade 2 was 2.56 nmol/ml, and the median value of Grade 3 was 3.98 nmol/ml (**Table 3**).

When the NO values of the groups were examined, the median value of the Control group was 8.97 U/ml, the median value of the Grade 1 group was 11.14 U/ml, the median value of Grade 2 was 14.18 U/ml, and the median value of Grade 3 was 14.78 U/ml (**Table 3**).

In addition, the evaluation results for each parameter are shown in figures (**Figure 1**). The NO variable can make a statistically significant distinction between sick and healthy individuals. The value of 8.95 is the cut-off point for sick and healthy individuals. NO can distinguish sick and healthy individuals with high sensitivity and accuracy. (**Figure 2**).

The MDA variable can make a statistically significant distinction between sick and healthy individuals. The value of 2.375 is the cut-off point for sick and healthy individuals. MDA can distinguish sick and healthy individuals with high sensitivity and specificity.

Table 3. Kruskal Wallis H test; a: 0.05; Post-Hoc: Dunn Sidak test; * the difference between the groups is statistically significant; a the difference with the control group is significant; b the difference with the grade 1 group is significant; c the difference with the grade 2 group is significant; d the difference with the grade 3 group is significant

	Group				
	Control	Grade 1	Grade 2	Grade 3	p
CAT Ug/Hb, median (Q1-Q3)	17.48 (14.07-19.35)^{c,d}	16.21 (12.09-20.12)^{c,d}	11.28 (9.04-17.13)^{a,b}	9.90 (9.31-10.40)^{a,b}	p<0.001*
SOD U/gHb, median (Q1-Q3)	579.41 (512.38-771.77)^{c,d}	486.79 (431.24-633.27)^{d}	511.23 (435.34-537.14)^{a,d}	402.03 (351.78-432.71)^{a,b,c}	p<0.001*
MDA nmol/ml, median (Q1-Q3)	2.24 (2.19-2.33)^{c,d}	2.31 (2.26-2.44)^{c,d}	2.56 (2.53-2.61)^{a,b}	3.98 (3.22-4.39)^{a,b}	p<0.001*
NO U/ml, median (Q1-Q3)	8.97 (7.69-10.68)^{b,c,d}	11.14 (10.15-14.11)^{a,d}	14.18 (11.09-15.74)^{a}	14.78 (13.59-16.47)^{a,b}	p<0.001*

CAT: Catalase, SOD: Superoxide dismutase, MDA: MDA: Malondialdehyde, NO: Nitric oxide

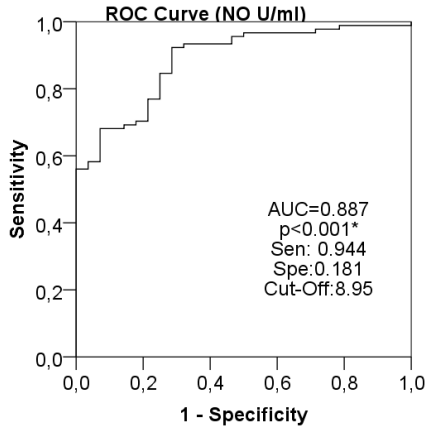

Figure 1. Differences between groups in catalase enzyme

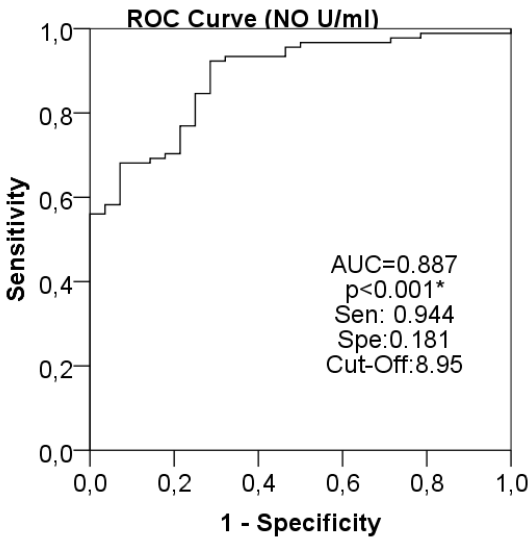

Figure 2. Determination of the cut-off point of nitric oxide values in the patient and control groups.

No statistically significant correlation was found between NO and CAT, SOD and MDA values for each group (**Table 4**).

Table 4. Spearman correlation test; a: 0.05

Group		CAT Ug/Hb		SOD U/gHb		MDA nmol/ml	
		r	p	r	p	r	p
Control	NO U/ml	-0.117	0.554	-0.077	0.696	-0.101	0.611
Grade 1	NO U/ml	-0.325	0.053	-0.147	0.392	0.086	0.616
Grade 2	NO U/ml	0.110	0.555	-0.112	0.549	-0.039	0.835
Grade 3	NO U/ml	0.220	0.302	-0.263	0.214	-0.085	0.694

DISCUSSION

In this study, multiple relationships were found between the degree of sonographic liver steatosis and the ONS parameters focused on in this study. The results obtained will be discussed below in order.

When the differences between the groups for each variable are examined, it is found that this change either decreases or increases, and it shows that the parameters are compatible with the degree of steatosis. Antioxidant enzymes CAT and SOD decrease as the degree of steatosis increases in US. NO and MDA increase as the degree of steatosis increases in US. These findings are compatible with the directions of increase and decrease in the severity of the disease defined in the literature and the directions of increase and decrease defined among ONS parameters, and previously it was defined that NO and MDA increase as the severity of the disease increases, and on the other hand, CAT and SOD decrease as the severity of the disease increases. Similarly, the findings show a change in the opposite direction as the severity of the disease decreases.[14,15,21-25] However, what makes this study special is that it is the first study to show the relationship between the degree of steatosis in US and the defined markers. As seen in **Table 3**, this relationship is very clearly seen in our study. However, a striking point is that although there is a difference between the degree of steatosis in US for each marker in general, some differences are significant between 2 groups (for example, between the control group and the degree of liver steatosis in US as grade 3), some between 3 groups and some between 4 groups. The fact that a statistically significant relationship is shown between four groups shows that the obtained data is very strong. Despite these very strong results, it is debatable why each of these does not show a difference between all groups. One reason for this is that the determination of the degree of steatosis in US is partly subjective. We believe that by resolving this situation, this difference can be shown between all groups. This may be the determination of more objective criteria for the definition of the degree of liver steatosis in US or the almost objective realization of this rating with artificial intelligence techniques through machine learning. Nevertheless, as mentioned, showing these differences between multiple groups is a great achievement and this is probably due to the richness of our exclusion criteria. We showed great care in exclusion and this care is probably reflected in our results. In addition, the results we obtained and shared in this study are suitable for use in clinical practice. This will bring significant convenience to clinicians in patient management.

This study also found values that can be used to distinguish between patient and control groups. In the literature, cut-off points for some markers for many diseases have been examined and used in diagnosis and follow-up. Finding such a cut-off point makes the job of physicians easier.

In this study, we found and show the cut-off value of NO that can be used to distinguish between patient and control groups. The NO variable can make a statistically significant distinction between patients and healthy

individuals. The value of 8.95 is the cut-off point for patients and healthy individuals. NO can distinguish patients and healthy individuals with high sensitivity and accuracy. This value revealed by our study can be used in clinical follow-up. The reasons why this value is different from zero can be questioned. One reason for this is that NO has many roles necessary for the body at low doses. On the one hand, NO is an endothelial relaxant factor. With this feature, it plays an important role in regulating blood pressure. In addition, NO attacks tumor cells, stimulates the brain and acts as a second messenger in various ways. There is a lot of evidence showing that NO plays important physiological and pathological roles in the liver.[10] Our high success in our ROC curve between groups for NO is remarkable. However, it is questionable that the results are still not 100% and it is debatable to increase these values even further. Here again, factors such as patient selection, exclusion criteria, subjectivity of sonographic parameters and number of patients may be effective.

Also, this study has shown that MDA can distinguish between patient and control groups. The MDA variable can make a statistically significant distinction between patients and healthy individuals. The value of 2.375 is the cut-off point for patients and healthy individuals. MDA can distinguish between patients and healthy individuals with high sensitivity and specificity. MDA values have been examined in the literature as a cut-off point for many diseases. However, the data obtained in this study is the first.[26-28] In this study, the value of 2.375, which can be used in clinical diagnosis and follow-up in distinguishing between the normal control group and MASLD, was introduced to the literature. In addition, its high specificity and sensitivity increase its usefulness.

Differences between variables according to groups: No statistically significant correlation was found between NO and CAT, SOD and MDA values for each group.

Limitations

The most definitive way to diagnose nonalcoholic steatohepatitis or assess the stage of fibrosis is to perform a liver biopsy.[29] A limitation of our study is that it is not based on biopsy data.

Our study only dealt with the hepatic steatosis. In this respect, it can be viewed as a narrowed specific group. This is an advantage. On the other hand, it can be considered as a limitation that it does not deal with the continuation of the spectrum.

Heterogeneous steatosis pattern, which is a version of liver steatosis, was not included in the study and was considered as an exclusion criterion: this approach was made to avoid sonographic grading errors of liver steatosis and adds strength to the study in this respect. However, it cannot analyze a group of cases within the MASLD spectrum. This is a limitation.

Not questioning dietary habits; free fatty acids induce ROS production due to high-calorie food intake, and abnormal ROS levels may mediate the progression of MASLD.[30] In our study, obesity was included as an exclusion criterion. However, not focusing on dietary habits is a limitation.

CONCLUSION

There is a significant change in ONS parameters with the change in the degree of liver steatosis on sonography. The obtained data can be further strengthened by reducing sonographic subjectivity. In addition, the cut-off values obtained for NO and MDA can be used with high accuracy in clinical practice to distinguish between groups with and without liver steatosis.

ETHICAL DECLARATIONS

Ethics Committee Approval

Ethics committee approval was obtained for this study from the Kahramanmaraş Sütçü İmam University Faculty of Medicine Local Ethics Committee (Date: 13.12.2022 Decision No: 06).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Letter to the Editor

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Helicobacter pylori, non- *Helicobacter pylori* helicobacters and gastrointestinal diseases

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Keywords: *H. pylori*, non- *H. pylori* helicobacters, gastrointestinal diseasees, children

Dear Editor,

I read with great interest your article titled "Evaluation of hematological parameters in the differentiation of bile reflux gastritis and *Helicobacter pylori (H. pylori)* gastritis in children" by Kıran Taşçı E., published in Anatolian Current Medical, 2023;5(4):445-448. *H. pylori* is a bacterium that remains significant due to its infection of approximately half of the global human population. Recent studies have noted that other *Helicobacter* species of animal origin can also infect humans. The diagnosis of *H. pylori* infection typically involves histopathological methods requiring endoscopy, but these methods may be inadequate for diagnosing other *Helicobacter* species. This text is written to highlight the limitations of histopathological methods in identifying non-*H. pylori* species and to emphasize the importance of considering their zoonotic nature. This letter aims to contribute to the relevant literature.

It is known that *H. pylori* can infect approximately 50% of the global population, leading to conditions such as gastritis, ulcers, and stomach cancer.[1] The article by Kıran Taşçı E.[2] emphasizes that dyspeptic symptoms in children may arise from *H. pylori* infection as well as from various other causes. The same article notes that the use of histopathological methods for identifying dyspeptic disorders necessitates upper gastrointestinal endoscopy. Research indicates that individuals infected with *H. pylori* can be asymptomatic; however, *H. pylori* is most commonly associated with gastrointestinal disorders.[3]

In diagnosing *H. pylori*, there are both invasive and non-invasive test-based methods, each with its own advantages and disadvantages. The sensitivity and specificity of non-endoscopy-based methods are still debated, and research in this area is ongoing. As Kıran Taşçı E. has indicated, endoscopy-based approaches remain crucial, particularly for understanding whether *H. pylori* infection is associated with gastrointestinal diseases. Studies have noted that there are 24 species classified within the Helicobacter genus, with 35 additional species still undergoing classification.[4]

Undoubtedly, the most common and significant *Helicobacter* species colonizing the human stomach and associated with various diseases is *H. pylori*. Recently, *Helicobacter* species such as *H. heilmannii* and *H. felis* have also been isolated from both animal and human stomachs, showing similar symptoms to *H. pylori* and highlighting their zoonotic potential.[5]

Many studies have indicated that *H. pylori* infection is more prevalent among individuals living in rural areas compared to those living in urban settings.[6] This increased prevalence may be attributed to poor hygiene conditions as well as contact with animals .

In conclusion, while *H. pylori* is the most common and significant *Helicobacter* species colonizing the human stomach and causing various symptoms, other species are also worthy of investigation. We recommend that the study of these species should include not only histopathological methods but also molecular techniques. Research into non-*H. pylori Helicobacter* species in humans and their zoonotic characteristics could be important for public health protection.

Sincerely.

Corresponding Author: Serdal Tarhane

ETHICAL DECLARATIONS

Referee Evaluation Process
Externally peer-reviewed.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Financial Disclosure
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Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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