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NAMIK KEMAL MEDICAL JOURNAL

CONTENTS

ORIGINAL ARTICLES/ÖZGÜN ARAŞTIRMALAR

CONTENTS

Investigation of Coronary Artery Disease by Coronary Computed Tomography Angiography and the Diagnostic Value of First-pass Myocardial Perfusion Imaging without Stress

Koroner Arter Hastalığının Koroner Bilgisayarlı Tomografi ile İncelenmesi ve Stressiz İlk Geçiş Miyokardiyal Perfüzyon Görüntülemenin Tanısal Değeri

Semra DELİBALTA[1], Aslı TANRIVERMİŞ SAYIT[2], Çetin ÇELENK[2]

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ABSTRACT

Aim: We aimed to investigate the diagnostic accuracy of first-pass computed tomography (CT) myocardial perfusion imaging (CT-MPI) without stress in combination with coronary CT angiography (CCTA) to detect coronary artery stenosis leading to myocardial ischemia compared to invasive coronary angiography (ICA) as the reference standard.

Materials and Methods: A total of 68 patients and 195 vessels were included in the study. We performed CCTA and first pass CT-MPI without stress on all patients. With ICA as the primary reference, the diagnostic accuracies of CCTA and CCTA plus first pass CT-MPI were expressed in terms of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the detection of vascular territories with significant obstructive coronary artery disease (CAD).

Results: CCTA plus first pass CT-MPI without stress yielded the following results for vascular territory detection with more than 50% coronary stenosis (as determined with ICA): sensitivity, 80%; specificity, 87%; PPV, 61.5%; and NPV, 94.4%. In addition, the ability of the area under the receiver operating characteristic curve for CCTA plus first-pass CT-MPI to distinguish coronary stenosis was markedly improved compared to CCTA alone.

Conclusion: CCTA plus first-pass CT-MPI without stress does not require additional radiation or contrast agent and additionally provides information about myocardial perfusion and coronary stenosis.

Keywords: Coronary artery disease, coronary computed tomography angiography, first-pass myocardial perfusion without stress

ÖZ

Amaç: Miyokardiyal iskemiye neden olan koroner arter darlıklarını tespit etmek için koroner bilgisayarlı tomografi (BT) anjiyografi (KBTA) ile kombinasyon halinde stressiz ilk geçiş BT miyokard perfüzyon görüntülemenin (BT-perfüzyon) tanısal doğruluğunu invaziv koroner anjiyografiye (İKA) kıyasla araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmamızda 68 hasta ve 198 damarı değerlendirildi. Tüm hastalara KBTA ve stressiz ilk geçiş miyokardiyal BT-perfüzyon uygulandı. Referans olarak İKA ile KBTA ve KBTA artı ile birlikte BT-perfüzyonun tanısal doğrulukları, obstrüktif koroner arter hastalığında (KAH) vasküler bölgelerin tespiti için duyarlılık, özgüllük, pozitif prediktif değer (PPD) ve negatif prediktif değer (NPD) cinsinden ifade edildi.

Bulgular: KBTA ve stressiz ilk geçiş miyokardiyal BT-perfüzyon, %50'den fazla koroner darlığı olan (İKA ile belirlenen) vasküler bölgeler için aşağıdaki sonuçları verdi: Duyarlılık; %80, özgüllük; %87, PPD; %61,5, NPD; %94,4. Ek olarak KBTA ve stressiz ilk geçiş miyokardiyal BT-perfüzyon için alıcı işletim karakteristiği eğri altında kalan alanın KAH'yi ayırt etme yeteneği, tek başına KBTA ile karşılaştırıldığında belirgin şekilde iyileşmişti.

Sonuç: KBTA ve stressiz ilk geçiş miyokardiyal BT-perfüzyon, ek radyasyon dozu ve ek kontrast madde gerektirmez ve miyokardiyal perfüzyon ve KAH hakkında ek bilgi sağlar.

Anahtar Kelimeler: Koroner arter hastalığı, koroner bilgisayarlı tomografi anjiyografi, stressiz ilk geçiş miyokardiyal perfüzyon

DELİBALTA et al. First-pass Myocardial Perfusion Imaging

Nam Kem Med J 2023;11(4):301-307

INTRODUCTION

Coronary computed tomography (CT) angiography (CCTA) is a noninvasive, high-quality, cross-sectional imaging method, which is mostly preferred for detecting coronary artery disease (CAD)[1,2]. The use of CCTA to rule out acute coronary syndrome in low- and intermediate-risk populations has been described as a cost-effective and safe method with a high negative predictive value (NPV). CCTA provides both scanning of the coronary lumen and identification of high-risk plaque, such as spotty calcium and low attenuation[3]. Despite the great image quality, CCTA has some limits in cases of severely calcified coronary arteries, motion artifacts, arrhythmias, and high heart rates. CT has another limitation: while coronary artery stenosis can be quantitatively quantified, it is difficult to determine the hemodynamic importance of a particular lesion using CCTA alone[4]. The gold standard for determining the presence of coronary stenosis resulting in lesion-specific myocardial ischemia is fractional flow reserve (FFR), which is evaluated during invasive coronary angiography (ICA). According to the Fractional Flow Reserve vs. Angiography for Multivessel Evaluation research, revascularization decisions based on invasive FFR increased the patient's event-free survival compared to decisions based solely on ICA[5]. However, because of its invasive nature, high cost, and potential inaccuracy in measuring arteries with significant tortuosity and/or coronary calcification, the use of FFR is restricted[6]. Due to these restrictions, precise, noninvasive diagnostic procedures are now required to identify coronary artery stenosis resulting in myocardial ischemia.

Recent research has demonstrated that the multi-detector CT (MDCT) cardiac enhancement pattern of early defects and late enhancement permits noninvasive evaluation of myocardial viability in acute myocardial infarction[7-9]. First-pass CT imaging under pharmacological stress has recently been employed for a quantitative assessment of myocardial perfusion, as further evidenced by more recent investigations[10-12]. However, this examination requires the use of extra contrast materials and radiation. First-pass CT-myocardial perfusion imaging (MPI) without stress has recently been employed as an imaging technique that can provide crucial information about coronary artery stenosis, without the need for additional radiation exposure or contrast materials, and can be carried out concurrently with CCTA[6].

Our objective was to compare the diagnostic efficacy of first-pass CT-MPI without stress in conjunction with CCTA to ICA as the reference standard for identifying coronary artery stenosis causing myocardial ischemia.

MATERIALS AND METHODS

This retrospective study accords with the principles of the Declaration of Helsinki and was authorized by Ondokuz Mayıs University Clinical Research Ethics Committee (protocol no: B.30.2.ODM.0.20.08/819-952, date: 24.10.2019). Consent form was obtained from all patients before the CT imaging.

Patients

The population of this retrospective study consisted of patients who underwent CCTA due to suspected CAD for typical or atypical angina between January 2018 and November 2019. Within 30 days, CCTA and ICA were performed on all patients. Patients with a history of coronary artery intervention or coronary bypass surgery, iodinated contrast medium, beta-blockers, adenosine, or nitroglycerin contraindications, Q waves on resting electrocardiography (ECG), non-sinus rhythm, or previous myocardial infarction were all excluded from the study. The study was comprised of 68 participants with a possible diagnosis of CAD (21 women and 47 males).

Coronary Computed Tomography Angiography

The following CT scans were conducted using a rapid kV-switching dual-energy 64-detector MDCT scanner (GE Healthcare's Discovery CT750 HD scanner, Milwaukee, United States): Retrospective ECG gating, tube voltage of 120 kV, tube current of 450-600 mA, scan field of view of 25, gantry rotation of 0.35 s/rotation, matrix of 512512, slice width of 0.625 mm, and helical pitch range of 0.16-0.22. Based on the patient's heart rate, the pitch was selected. If the patient's heart rate was greater than 65 beats per minute before the CT scan, a single dose of metoprolol (25-100 mg) was given orally 6 hours prior to the scan. The patients were given intravenous beta blockers until the heart rate was below 60 beats per minute, if it did not drop to that level prior to scanning. The patient was in the supine posture during CCTA. A bolus injection of nonionic contrast agent was used to determine the scan delay, allowing for monitoring of the region of interest-the proximal section of the ascending aorta. Intravenous contrast material (iohexol: Omnipaque 350, GE Healthcare) was administered using a standardized weight-based dose injected at 3-5.0 cc/s, followed by a 30-cc saline flush.

We looked for the signs of significant stenosis in the right coronary artery (RCA), left anterior descending (LAD), and left circumflex (LCX) arteries. Significant stenosis was described as a narrowing of the mean luminal diameter of more than 50%.

First-pass Computed Tomography-myocardial Perfusion Imaging

For post-processing, the volume data were moved to a special workstation (AW Volumshare Workstation, GE Healthcare). With the aid of commercially available cardiac evaluation software, first-pass CT-MPI without stress was performed. In

order to reduce motion artifacts, this program was used to produce long-axis and short-axis images via reconstruction with RR intervals of 40-55% and 70-85% of the cardiac cycle. For systole and diastole, the myocardial pictures were captured at 40% and 75% of the RR interval, respectively. The slice thickness was 3 mm. The 16-segment model of the American Heart Association was utilized in the investigation to identify the segments of the left ventricle other than the apical segment[13]. Based on the CT values of the left ventricular myocardium, the first-pass CT-MPI results are displayed in color maps. Cold colors depict hypoenhanced regions with low CT values, whereas warm colors depict hyperenhanced regions with high CT values. The presence of a perfusion defect in at least one segment in any vascular territory was considered a perfusion defect[1].

Invasive Coronary Angiography

ICA was carried out utilizing the transfemoral method in accordance with industry standards. There were at least six projections of the left coronary artery and at least four of the RCA.

Statistical Analysis

Statistical Package for the Social Sciences statistics version 21 for Windows (IBM Inc., Armonk, NY, USA) was used to perform all statistical calculations. The data were expressed as the mean±standard deviation or absolute values and proportions, as appropriate.

Categorical variables, such as age, gender, height, weight, heart rate, and coronary artery risk factors, were expressed as the frequency and percentage. The sensitivity, specificity, positive predictive value (PPV), and NPV of CCTA and CCTA plus first-pass CT-MPI for the detection of vascular regions with severe obstructive coronary artery stenosis were expressed using ICA as the primary reference. The effectiveness of the diagnostic process was assessed for each patient and each vessel (RCA, LAD, and LCX arteries). The diagnosis of CCTA was reclassified depending on first-pass CT-MPI without stress (Figure 1). For every diagnostic testing strategy for which a reference standard was provided, the area under the receiver operating characteristic (ROC) curve (C statistic) was determined. Situations with a type 1 error level below 5% were considered to have a statistically significant diagnostic value while evaluating the area under the curve. A p value <p.05 was considered statistically significant in all statistical analyses.
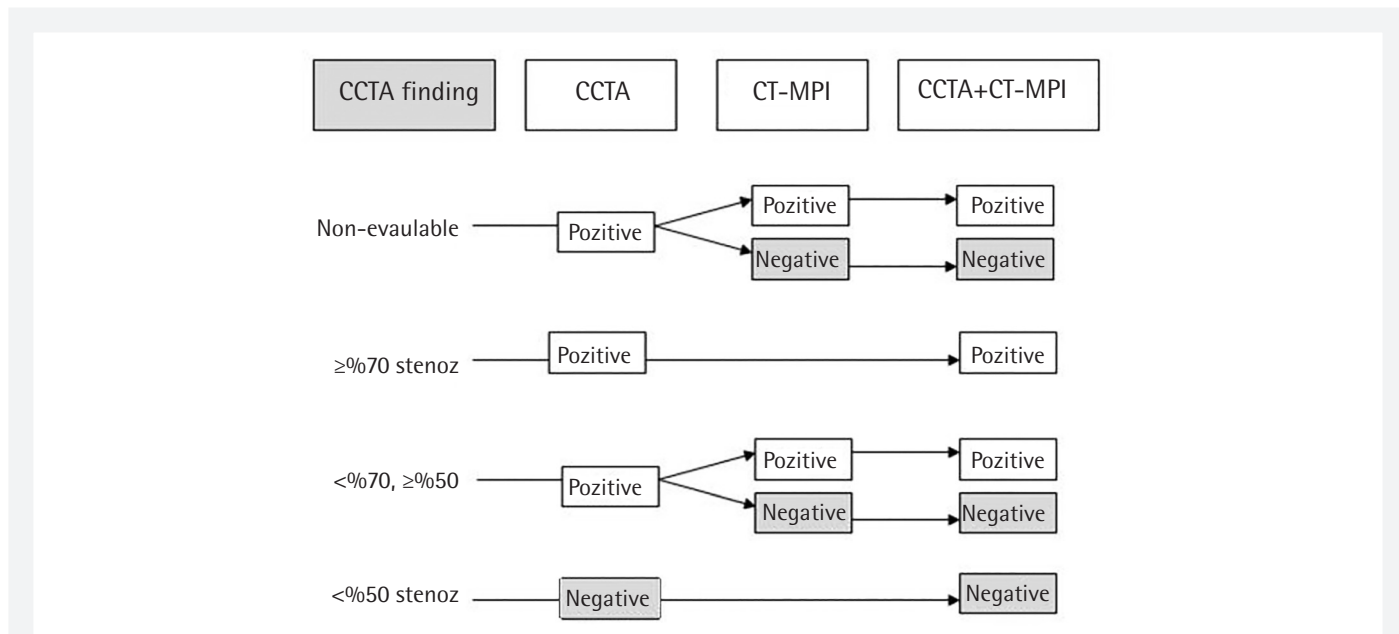
Figure 1. Reclassification criteria. Before first-pass CT-MPI analysis, nonevaluable vessels with CCTA were defined as positive for stenosis using the following criteria: those with no vessel wall definition due to marked motion artefacts, significant structural discontinuity, or heavy calcification and high image noise-related blurring that precluded the acquisition of diagnostic information. After CT-MPI analysis, nonevaluable vessels with CCTA were accepted as positive for stenosis only if they had a CT-MPI defect in the same vascular distribution. Moderate stenosis (50-70%) on CCTA was reclassified as negative if CT-MPI showed no defect in the same distribution. Stenosis with 30-50% luminal narrowing on CCTA was reclassified as positive if CT-MPI showed a defect in the same distribution. CCTA stenosis was not reclassified when no stenosis, <50% stenosis or >70% stenosis was observed on CCTA

CCTA: Coronary computed tomography angiography, CT-MPI: Computed tomography-myocardial perfusion imaging

DELİBALTA et al. First-pass Myocardial Perfusion Imaging

Nam Kem Med J 2023;11(4):301-307

RESULTS

Study Population

A total of 68 patients (58.7±10.8) were included in the study. There were 21 (30.9%) women (mean age: 56.3±12.3 years) and 47 (69.1%) men (mean age: 59.7±10 years). The most common risk factor was hypertension (55.9%), and other risk factors were diabetes mellitus, dyslipidemia, and smoking. The demographic findings of the patients are shown in Table 1.

Analysis of CCTA, CCTA plus First-pass CT-MPI without Stress, and ICA results

A total of 68 patients and 204 vessels (RCA and LAD and LCX arteries) were evaluated by CCTA, first-pass CT-MPI, and ICA. Of the 204 vessels, 9 had stents and were not included in the study. Six of the vessels with stents were RCAs; two were LCX arteries; and one was an LAD artery. Ultimately, 195 vessels were included in the study.

According to ICA, 24 of the 195 vessels (12%) had severe stenosis (75-100%). On a per-vessel basis, 9 (37.5%) of these were in the RCA; 10 (41.6%) were in the LAD artery; and 5 (20.8%) were in the LCX artery. A total of 16 of the 195 vessels had moderate stenosis (50-69%): 4 (25%) of them were in the RCA; 8 (50%) were in the LAD artery; and 4 (25%) were in the LCX artery. In total, significant stenosis (50-100%) was detected in 40 vessels: 18 (45%) of these 40 vessels were in the LAD artery; 13 were in the RCA (32.5%); and 9 (22.5%) were in the LCX artery. According to ICA, the most common risk factor in patients with significant stenosis was hypertension (57.6%), followed by dyslipidemia (50%).

With CCTA, greater than 70% luminal diameter narrowing was detected in 26 (13%) of the 195 vessels. In addition, 40 of the 195 vessels had moderate stenosis (between 50% and 70%). Among them, 21 vessels (11%) could not be clearly evaluated due to calcification or movement artefacts. In total, 66 (34%) vessels had obstructive stenosis (luminal narrowing greater than 50%). A proportion of 23% of these vessels were RCAs; 56% were LAD arteries; and 21% were LCX arteries. According to CCTA plus first-pass CT-MPI, 52 (27%) of the 195 vessels had obstructive stenosis (greater than 50% narrowing). A proportion of 35% of these vessels were RCAs;
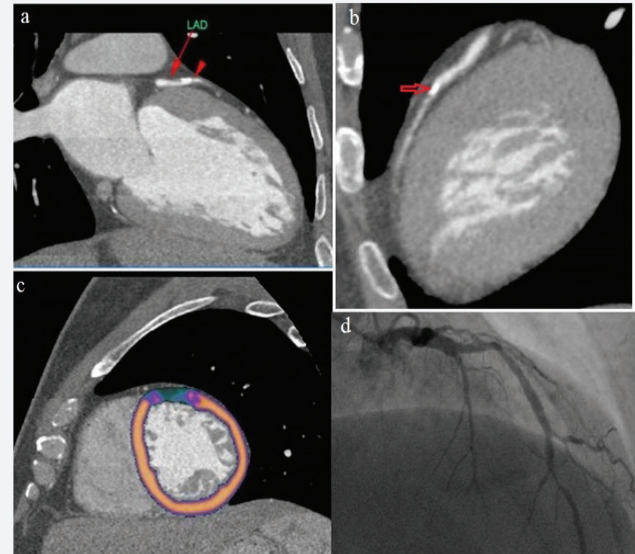
Figure 2. a, b) CCTA showed a calcified plaque in the proximal segment of the LAD artery and greater than 50% luminal narrowing in the distal segment of the LAD artery due to mixed plaque. c) First-pass CT-MPI without stress revealed a perfusion defect in the mid-anterior segment of the left ventricle. d) Invasive angiography shows greater than 50% luminal narrowing in the distal segment of the LAD artery

LAD: Left anterior descending, CCTA: Coronary computed tomography angiography, CT-MPI: Computed tomography-myocardial perfusion imaging

Table 1. Demographic findings of the patients (n=68)		
Variable	**Mean±SD**	
Age (years)	58.7±10.8	
Hypertension (n=38)	Systolic blood pressure (mmHg)	137.17±20.09
	Diastolic blood pressure (mmHg)	84.92±9.89
Serum lipid biomarkers (n=28)	Total cholesterol (mg/dL)	181.11±46.85
	LDL cholesterol (mg/dL)	102.20±39.89
	HDL cholesterol (mg/dL)	44.73±15.86
	Serum triglycerides (mg/dL)	174.82±89.23
	Serum creatine (mg/dL)	0.88±0.19
Variable	**Number (%)**	
Smoker (n)	29 (42.6%)	
Patients with stent placement (n)	9	
Diabetes mellitus (n)	29 (42.6%)	
LDL: Low-density lipoprotein, HDL: High-density lipoprotein, SD: Standard deviation		

Nam Kem Med J 2023;11(4):301-307

DELİBALTA et al. First-pass Myocardial Perfusion Imaging

48% were LAD arteries (Figure 2); and 17% were LCX arteries (Figure 3).

CCTA yielded the following results for vascular territory detection with more than 50% coronary stenosis (as determined with ICA): sensitivity, 92.5%; specificity, 70.9%; PPV, 45.1%; and NPV, 97.3%.
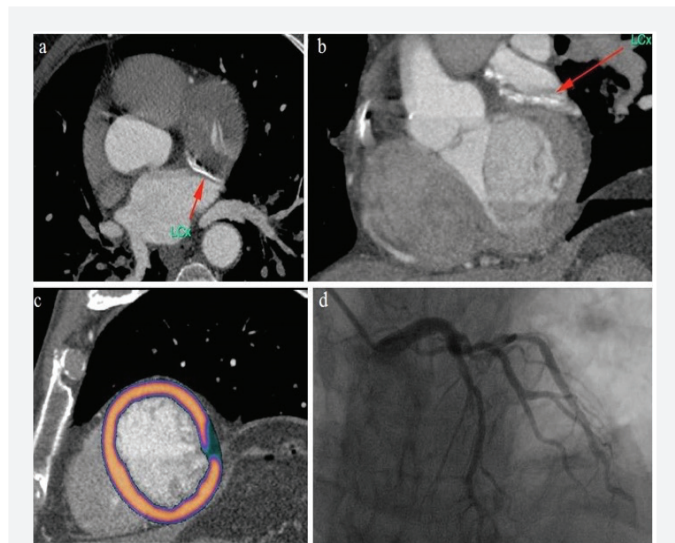
Figure 3. a, b) CCTA shows a calcified plaque and an irregularity in the LCX artery wall. The LCX artery also has motion artefacts. c) First-pass computed tomography myocardial perfusion imaging (CT-MPI) without stress revealed a perfusion defect in the left ventricular mid-anterolateral segment. d) Invasive angiography showed greater than 50% luminal narrowing in the LCX artery

LCX: Left circumflex coronary, CCTA: Coronary computed tomography angiography, CT-MPI: Computed tomography-myocardial perfusion imaging

For vascular territory detection with higher than 50% coronary stenosis (as measured by ICA), CCTA plus first-pass CT-MPI without stress produced the following results: sensitivity, 87%; specificity, 87%; PPV, 61.5%; and NPV, 94.4% (Table 2).

When compared to CCTA alone, the area under the ROC curve with CCTA plus first-pass CT-MPI was significantly better in differentiating stenotic coronary arteries, increasing from 0.817 to 0.835 in the per-vessel analysis.

DISCUSSION

In this study, we found that CCTA plus first-pass CT-MPI was more useful in defining stenotic coronary arteries that might cause myocardial ischemia than CCTA alone in patients without a history of CAD. Myocardial vascular volume may have been reduced as a result of substantial epicardial coronary artery stenosis, according to a decrease in myocardial signal intensity during diastole. We proved the diagnostic utility of first-pass CT-MPI conducted with CCTA to assess substantial coronary artery stenosis in patients without myocardial infarction based on this assumption.

Coronary capillaries are primarily responsible for intramyocardial perfusion. The ideal coronary blood flow is disturbed by stenotic epicardial coronary flow. Due to the need to preserve capillary hydrostatic pressure, capillary resistance rises, which reduces myocardial blood volume and attenuates perfusion[14]. The main cause for decreasing the blood flow in coronary artery stenoses, which is more than 75%, is deterioration in the capillary vessels rather than stenosis itself[1]. It has also been shown that myocardial perfusion is affected by coronary artery stenosis without stress[14]. Hydrostatic blood pressure is controlled by coronary capillary microvessels to preserve equilibrium. Epicardial coronary pressure is maintained constant in moderate coronary artery

Table 2. Diagnostic accuracy of CCTA and CCTA plus CT-MPI without stress for the detection of significantly stenotic coronary arteries in 195 vessels

	CCTA Per vessel (n=195)	CCTA+ first pass CT-MPI Per vessel (n=195)
No of results		
True positive	37	32
True negative	110	135
False positive	45	20
False negative	3	8
Statistical analysis		
Sensitivity	92.5	80
Specificity	70.9	87
Positive predictive value	45.1	61.5
Negative predictive value	97.3	94.4
C statics	0.817	0.835
CCTA: Coronary computed tomography angiography, CT-MPI: Computed tomography-myocardial perfusion imaging		

DELİBALTA et al. First-pass Myocardial Perfusion Imaging

Nam Kem Med J 2023;11(4):301-307

stenosis by rising capillary resistance. However, in patients with severe coronary artery stenosis without myocardial infarction, reducing myocardial blood volume becomes more evident. These actions can be seen as a reduction in myocardial perfusion in first-pass CT-MPI[1,6].

Myocardial ischemia is not necessarily brought on by the morphological luminal constriction of the coronary artery. It is not easy to decide whether moderate coronary artery narrowing causes myocardial ischemia. According to recent research, CCTA combined with adenosine stress CT-MPI is a useful imaging technique for identifying coronary artery stenosis. However, this imaging technique needs additional contrast agent, and radiation is one of the important disadvantages[15]. First-pass CT-MPI without stress is a simultaneous imaging method with CCTA that does not need additional radiation or contrast agent to evaluate coronary arteries, especially those with severe calcification or intermediate stenosis. Evaluating first-pass CT-MPI together with CCTA can help show coronary artery stenosis leading to ischemia[6].

Matsuoka et al.[16] used 64-slice MDCT at rest to analyze 75 patients with probable CAD in order to find myocardial ischemia, which is often shown by myocardial perfusion scintigraphy (MPS). They claimed that using pharmacological MPS as the reference standard, first-pass CT-MPI at systole alone exhibited good performance in diagnosing CAD patients with a sensitivity of 90%, a specificity of 83%, a PPV of 86%, and an NPV of 88%[16]. They reported that rest perfusion abnormalities, which occur in systole, provided very similar information to that of adenosine stress studies. In this study, the degree of stenosis assessed with CCTA could not be clearly evaluated due to severe calcifications in 15% of patients. However, first-pass CT-MPI was used to provide information on the ischemia and perfusion status in the region of a severely calcified coronary artery. Therefore, they reported that first-pass CT-MPI at rest, a noninvasive method, showed the typical ischemia enhancement pattern. In addition, it overcomes the limitations of CCTA and provides the significant advantage of increasing the diagnostic accuracy for CAD.

Yoshida et al.[17] evaluated 70 patients with single-vessel disease, who underwent CCTA followed by ICA. The signal densities at diastole were measured to assess cardiac enhancement. According to their findings, segment-based analysis using 64-MDCT of major stenoses revealed sensitivity, specificity, and accuracy for segments without calcified lesions of 92%, 100%, and 99.7%, respectively, and those for calcified lesions of 95.2%, 50%, and 77.1%, respectively. They calculated the percentage drop in the signal densities to account for cardiac enhancement, and they reported the values as 95.2%, 85.7%, and 91.4%, respectively, for segments with calcified lesions. As a result, they observed that first-pass CT-MPI provided new

diagnostic information about severe coronary artery stenosis, particularly in the presence of calcified plaque. It does this by identifying the diminished myocardial enhancement of the lateral ventricle wall in diastole[17].

Osawa et al.[1] evaluated 45 patients with suspected CAD with CCTA and CCTA plus first-pass CT-MPI without stress. As color maps, they assessed the signal concentrations during diastole. Comparing diagnostic accuracy to ICA allowed for evaluation. In comparison to CCTA alone, they found that CCTA + first-pass CT-MPI improved diagnostic performance. Per-vessel analysis showed a rise in the sensitivity, specificity, PPV, and NPV from 81% to 85%, 87% to 94%, 63% to 79%, and 95% to 96%, respectively. Additionally, the area under the ROC curve for detecting CAD increased from 0.84 to 0.89 (p=0.02)[1]. They reported that first-pass CT-MPI could be particularly useful in the evaluation of coronary artery segments with severe calcification and motion artefacts. Particularly in patients with extensive calcification or motion artifacts, it can contribute to the diagnostic value of identifying coronary artery stenosis. They reported that CCTA with CT-MPI performed more effectively as a diagnostic tool than CCTA alone[1]. In our study, CCTA plus first-pass CT-MPI had a sensitivity of 80%, specificity of 87%, PPV of 61.5%, and NPV of 94.4%. In addition, the area under the ROC curve for detecting CAD was 0.835 likely as the other studies in literature. In our study, the number of patients with a false positive decreased, and accordingly, the PPV increased.

There are benefits to first-pass CT-MPI. It can provide information about myocardial perfusion without the use of drugs. It is not necessary to increase the radiation dose or contrast agent for first-pass CT-MPI compared to stress CT-MPI performed with pharmacological agents[1]. However, first-pass CT-MPI has some disadvantages. Due to poor filtration of the contrast medium in the myocardium, it has a significant false-positive rate. Furthermore, first-pass CT-MPI is unable to distinguish between reversible and irreversible myocardial ischemia. Although there are many studies in the literature that compare CCTA to ICA, few studies have evaluated CAD with CCTA plus first-pass CT-MPI.

Study Limitations

The limitations of our study include the small number of patients and the lack of interobserver variability among radiologists in this study. Studies with large sample sizes can contribute to the literature on the contribution of first-pass CT-MPI to CAD diagnosis.

CONCLUSION

In conclusion, our study has shown that CCTA plus first-pass CT-MPI is superior to CCTA alone in the diagnosis of obstructive

CAD. As a result, in cases of suspected coronary artery stenosis, first-pass CCTA plus CT-MPI can provide significant additional information in terms of severe coronary artery stenosis and as a complement to CCTA in clinical practice without the need for additional radiation doses or additional contrast agent. It can also provide information about myocardial perfusion and ischemia.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University, and it complies with the principles of the Declaration of Helsinki (protocol no: B.30.2.ODM.0.20.08/819-952, date: 24.10.2019).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.D., A.T.S., Ç.Ç., Design: S.D., A.T.S., Ç.Ç., Data Collection or Processing: S.D., Analysis or Interpretation: S.D., Ç.Ç., Literature Search: S.D., A.T.S., Ç.Ç., Writing: S.D., A.T.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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Epidemiology of Leishmaniasis Disease in Şanlıurfa Between the Years of 2010 and 2019

Şark Çıbanı Hastalığının Şanlıurfa'da 2010-2019 Yılları Arasındaki Epidemiyolojisi

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ABSTRACT

Aim: In the present study, by making use of Geographical Information Systems, it was aimed to examine the changes in the number of cutaneous leishmaniasis cases in Şanlıurfa between the years 2010 and 2019 by the regions and to define the risky areas.

Materials and Methods: The present study has a descriptive design. The universe of study consisted of patients, who applied to Leishmaniasis Diagnosis and Treatment Center (LDTC) and received the diagnosis of leishmaniasis and treatment in this facility between the years of 2010 and 2019. Patients' age, gender, place of residence, total number of lesions, lesion location, lesion width, lesion clinic, and type of treatment were used in the present study. The maps were prepared involving only 68 neighborhoods having 10 or more cases.

Results: Of 10,706 patients who applied to LDTC between the years of 2010 and 2019, 45.7% were male and 54.3% were female. It was determined that 80% of the cases detected between the years of 2010 and 2014 were residing in Şanlıurfa center. Considering the years between 2015 and 2019, 69% of the cases were living in Eyyübiye, one of the central districts. Given the heat maps, it was determined that the places, where the patients were living, vectorially shifted towards the southeast between the years of 2010 and 2015.

Conclusion: While the northwestern neighborhoods in Şanlıurfa were risky in the early 2010s, the risk shifted to southeastern neighborhoods in the following years. It is thought that, in the coming years, majority of the cases would be in these neighborhoods because of the number of immigrants and environmental factors.

Keywords: Cutaneous leishmania, epidemiology, mapping

ÖZ

Amaç: Bu çalışmada Coğrafi Bilgi Sistemleri'nden yararlanılarak Şanlıurfa'da 2010-2019 yılları arasında şark çıbanı olgu sayılarındaki değişimin bölgelere göre incelenmesi ve riskli alanların belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Bu çalışma tanımlayıcı bir tasarıma sahiptir. Çalışmanın evrenini 2010-2019 yılları arasında Şark Çıbanı Tanı ve Tedavi Merkezi'ne (ŞÇTTM) başvuran ve bu merkezde şark çıbanı tanı ve tedavisi alan hastalar oluşturmaktadır. Bu çalışmada hastaların yaş, cinsiyet, ikamet yeri, toplam lezyon sayısı, lezyon yeri, lezyon genişliği, lezyon kliniği ve tedavi türü bilgileri kullanılmıştır. Haritalar yalnızca 10 veya daha fazla olguya sahip 68 mahalleyi içerecek şekilde hazırlanmıştır.

Bulgular: 2010-2019 yılları arasında ŞÇTTM'ye başvuran 10.706 hastanın %45,7'si erkek, %54,3'ü kadındı. 2010-2014 yılları arasında tespit edilen olguların %80'inin Şanlıurfa merkezde ikamet ettiği belirlendi. 2015-2019 yılları arası dikkate alındığında olguların %69'unun merkez ilçelerden Eyyübiye'de yaşadığı tespit edildi. Isı haritalarına bakıldığında 2010-2015 yılları arasında hastaların yaşadıkları yerlerin vektörel olarak güneydoğuya doğru kaydığı tespit edildi.

Sonuç: Şanlıurfa'da 2010'lu yılların başında kuzeybatı mahalleleri riskli iken, ilerleyen yıllarda risk güneydoğu mahallelerine kaymıştır. Göçmen sayısı ve çevresel faktörler nedeniyle önümüzdeki yıllarda olguların çoğunluğunun bu mahallelerde olacağı düşünülmektedir.

Anahtar Kelimeler: Kutanöz leishmania, epidemiyoloji, haritalama

Nam Kem Med J 2023;11(4):308-313

BEYAZGÜL et al. Epidemiology of Leishmaniasis in Şanlıurfa

INTRODUCTION

Leishmaniasis refers to a group of diseases caused by Leishmania-type protozoa. Three main forms of this disease are cutaneous leishmaniasis (CL), visceral leishmaniasis, and mucocutaneous leishmaniasis[1].

Localized CL is the most widely seen clinical manifestation. It begins in the form of a small nodule or papule 2-8 weeks after a sandfly bite. Although it generally tends to limit itself, a scar might develop[2]. Response of the host might cause different atypical forms (erythematous volcanic ulcer, lupoid, eczematous, erysipeloid, verrucose, dry, zosteriform, paronychial, sporotrichoid, cancriform, and circular) and there may sometimes be secondary infections. CL is also known as the "great masquerader" since it can mimic many dermatoses[3,4].

More than 1 billion individuals worldwide live in endemic regions in terms of leishmaniasis. More than 1 million new CL cases are seen annually[1]. In Turkey, approximately 2500 new CL cases are detected annually and the annual morbidity rate is 3/100,000. Most of the cases are found in Şanlıurfa, Adana, Osmaniye, and Hatay provinces[3,5].

Epidemiological maps play an important role in monitoring the disease and preparing protection and control plans addressing it. Nowadays, epidemiological maps can provide more useful information with the support of Geographical Information Systems (GIS). GISs are the systems qualitatively and quantitatively representing the distribution of a disease. They allow for the preparation of disease-related risk maps by making use of spatial and temporal models[6-8].

In the present study, by making use of GIS, it was aimed to examine the changes in the number of CL cases in Şanlıurfa between the years of 2010 and 2019 by the regions and to define the risky areas.

MATERIALS AND METHODS

The present study has a descriptive design. This is a retrospective study and a consent form was not required as the results were obtained from the patients' records. The universe of study consisted of patients, who applied to Leishmaniasis Diagnosis and Treatment Center (LDTC) and received leishmaniasis diagnosis and treatment in this facility between the years of 2010 and 2019. LDTC is the main center, where this disease is treated, in Şanlıurfa province. More than 90% of leishmaniasis patients are treated in this facility.

No sample selection was performed for the present study and all the registered patients were involved. In total, 10,706 patients were followed up for treatment by LDTC between the years of 2010 and 2019.

The data used in this study were obtained from the patients' electronic files kept in LDTC. Patients' age, gender, place of residence, total number of lesions, lesion location, lesion width, lesion clinic, and type of treatment were used for evaluation in the present study.

Lesion locations were categorized as the face, neck, trunk, upper extremity, lower extremity, and genital area. For those having multiple lesions, each lesion was considered separately.

Clinical situations of lesions were classified as nodule, papule, ulcerated, and recidive.

The treatment methods were grouped as intralesional (IL) and intramuscular (IM).

Şanlıurfa province has 13 districts. Since this province gained the status of metropolitan and city administration changed in 2012, the central districts were specified as Şanlıurfa Center for the LDTC records addressing the year 2014 and before. For the records of the year 2015 and after, the city center of Şanlıurfa started to be administered as three districts named Haliliye, Karaköprü, and Eyyübiye[9].

The data were used by grouping as 2010-2011, 2012-2013, 2014-2015, 2016-2017, and 2018-2019.

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval no. E-49866, date: 19.04.2023 was obtained from the Ethics Committee of the Faculty of Medicine of Harran University. Moreover, the approval for using the data of leishmaniasis patients was obtained from the Provincial Directorate of Health of Şanlıurfa.

Statistical Analysis

Data analysis was performed using descriptive statistics in Statistical Package for the Social Sciences version 20.0 package software. Mapping was performed using Microsoft Excel 2016 3D Maps. Coordinates of the neighborhoods were tagged on the map after obtaining from the Parcel Search Application of the General Directorate of Land Registry and Cadaster[10]. The change of color from blue to red in neighborhoods with given coordinates indicates an increase in the number of cases.

The descriptive statistics were calculated using all the patient data. However, the maps were prepared involving only 68 neighborhoods having 10 or more cases. The patients from locations other than Şanlıurfa were not involved in mapping. Using the criteria specified, 81% of the cases were used in the mapping process.

RESULTS

Of 10,706 patients who applied to LDTC between the years of 2010 and 2019, 45.7% were male and 54.3% were female. The mean age was found to be 20.55±18.17 years (Figure 1).

A single lesion was found in 58.1% of the patients, while 2 lesions were found in 21.2%. The patient having the highest number of lesions had 33 lesions.

BEYAZGÜL et al. Epidemiology of Leishmaniasis in Şanlıurfa

Nam Kem Med J 2023;11(4):308-313

Of the lesions, 48.5% were found in the face, 1.9% in the neck, and 2.5% in the trunk. Moreover, 45.3% of lesions were found in the upper extremities and 17.6% in the lower extremities. Only 2 cases were found to have a lesion in the genital area.

Of the lesions, 45.2% were ulcerated, 44.8% were nodules, and 8.8% were papules. Of the patients, 98.0% received IL treatment, whereas 0.4% received IM treatment and 1.6% received none of these treatments.

The distribution of cases by the years is presented in Table 1. The highest number of cases was found in the year 2013 (15.3%), whereas the lowest number was observed in the year 2014 (7.6%) (Table 1).
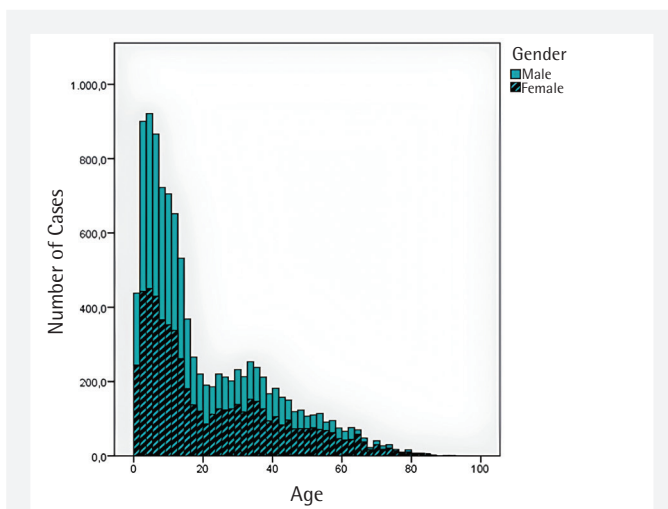
Figure 1. Distribution of the number of cases by age and gender

Ninety-nine percent of the patients were living in Şanlıurfa. It was determined that 80% of the cases detected between the years of 2010 and 2014 were residing in Şanlıurfa center. Besides the city center, the second highest (13.7%) percentage of patients were those living in Birecik district (Table 2).

It was determined that 80% of the cases found in the period 2010-2014 were in the city center, whereas the same percentage was 85.8% for the period 2015-2019. Increases were observed in both percentages and numbers of cases in Harran, Viranşehir, and Ceylanpınar districts in the period 2015-2019.

Considering the years between 2015 and 2019, 69% of the cases were living in Eyyübiye, one of the central districts, followed by the district Ceylanpınar with a percentage of 3.6% (Table 2).

For the period between 2010 and 2019, the three neighborhoods with the highest numbers of cases were Devteyşti (11.8%), Selçuklu (11.3%), and Osmanlı (10.8%) (Table 3).

Table 1. Distribution of cases by years

Year	Number	Percentage
2010	1198	11.2
2011	826	7.7
2012	1019	9.5
2013	1634	15.3
2014	815	7.6
2015	1016	9.5
2016	947	8.8
2017	1.216	11.4
2018	1192	11.1
2019	843	7.9
Total	10706	100.0

Table 2. Distribution of cases by the districts between 2010 and 2019

District	2010–2014		District	2015–2019	
	Number	Percentage		Number	Percentage
Şanlıurfa center	4350	80.0	Eyyübiye	3568	69.0
			Haliliye	570	11.0
			Karaköprü	302	5.8
Birecik	749	13.7	Birecik	104	2.0
Bozova	93	1.7	Bozova	58	1.1
Harran	91	1.7	Harran	147	2.8
Akçakale	59	1.1	Akçakale	58	1.1
Suruç	37	0.7	Suruç	16	0.3
Viranşehir	18	0.3	Viranşehir	133	2.6
Ceylanpınar	15	0.3	Ceylanpınar	185	3.6
Halfeti	12	0.2	Halfeti	13	0.2
Hilvan	6	0.1	Hilvan	6	0.1
Siverek	4	0.1	Siverek	7	0.1
Toplam	5434	100.0		5167	100.0

In the years of 2010 and 2011, the highest numbers of cases were detected in Saha (11.0%), Akşemsettin (8.4%), Direkli (8.1%), Buhara (7.6%), and Devteyşti (7.6%) neighborhoods. For the years of 2012 and 2013, the highest numbers of cases were seen in Devteyşti (27.5%), Osmanlı (8.6%), Akşemsettin (7.4%), and Direkli (7.2%) neighborhoods. In the period 2014-2015, the highest numbers were found in Osmanlı (16.4%), Devteyşti (8.1%), and Yenice (7.2%), whereas the highest numbers of

Table 3. Distribution of 10 neighborhoods with the highest numbers of cases between the years 2010 and 2019			
Neighborhood	District	Number	Percentage
Devteyşti	Haliliye	1259	11.8
Selçuklu	Eyyübiye	1211	11.3
Osmanlı	Eyyübiye	1151	10.8
Direkli	Eyyübiye	618	5.8
Yenice	Eyyübiye	519	4.8
Akşemsettin	Eyyübiye	477	4.4
Süleymaniye	Haliliye	328	3.1
Hayati Harrani	Eyyübiye	313	3.0
Buhara	Eyyübiye	290	2.7
Saha	Birecik	279	2.6
Other neighborhoods		4261	39.8
Total		10706	100.0

cases in the period 2016-2017 were seen in Selçuklu (24.2%), Osmanlı (13.8%), Yenice (9.9%), Direkli (6.4%), and Devteyşti (5.1%) neighborhoods. Finally, in the period 2018-2019, the highest numbers of cases were found in Selçuklu (28.6%), Osmanlı (11.4%), and Devteyşti (5.7%) neighborhoods. The change in the numbers found in neighborhoods is illustrated in the heat map shown in Figure 2.

Given the case distribution, it can be seen that there were two main foci consisting of Akşemsettin-Süleymaniye-Devteyşti neighborhoods and Selçuklu-Osmanlı neighborhoods and that there were many small foci around them. Examining the change by years, it was determined that these foci became sometimes significant and sometimes indistinct.

Given the heat maps, it was determined that the places, where the patients were living, vectorially shifted towards the southeast between 2010 and 2015, the number of cases became more prominent in Karaköprü district (arrow), Yenice neighborhoods became indistinct, and the vector "place of residence" shifted towards the northwest (in the same line) again since the year 2016.

The distribution of patients by the place of residence for the period 2010-2019 is presented in Figure 3 as stacked columns. Most of the cases were found in Devteyşti (11.8%), Selçuklu (11.3%), Osmanlı (10.8%), Direkli (5.8%), Yenice (4.8%), Akşemsettin (4.4%), Süleymaniye (3.1%), Hayati Harrani (3.0%), Buhara (2.7%), and Saha (2.6%) neighborhoods. It was determined that the number of cases decreased in Devteyşti, Direkli, and Süleymaniye neighborhoods after the year 2013 and increased in Selçuklu and Osmanlı neighborhoods after the years 2010-2011.

Figure 2. Heat map of the cases between the years of 2010 and 2019

Figure 3. Stacked column presentation of cases for the period 2010-2019

BEYAZGÜL et al. Epidemiology of Leishmaniasis in Şanlıurfa

Nam Kem Med J 2023;11(4):308-313
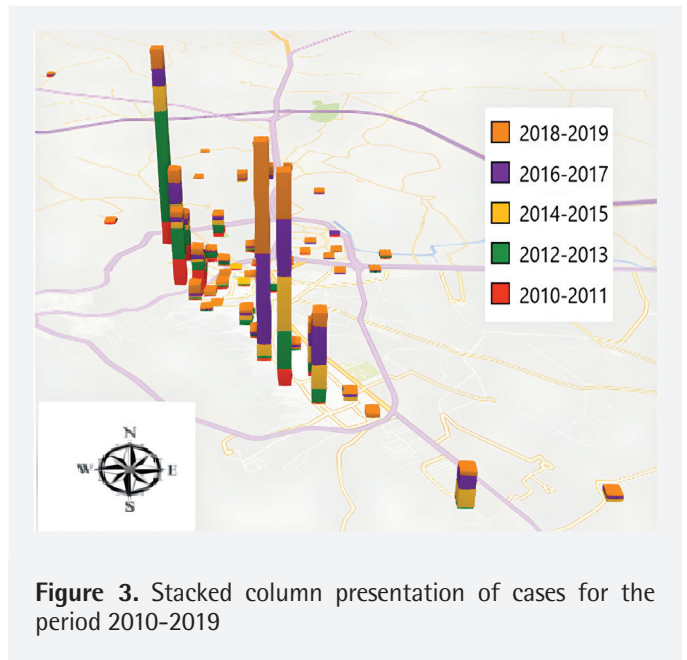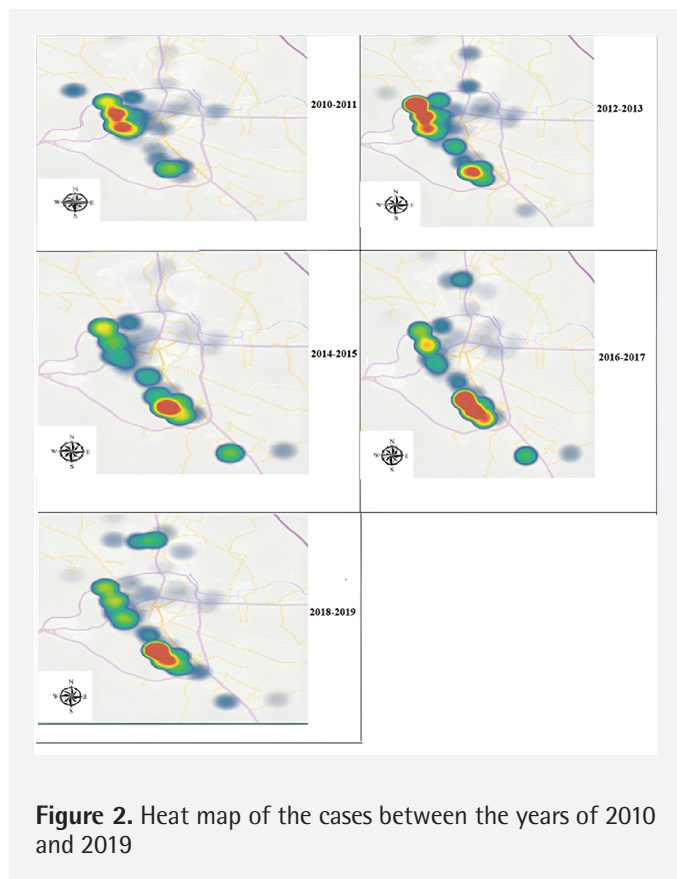
DISCUSSION

The majority of patients consisted of children, adolescents, and young adults. Since leishmaniasis is a disease that causes immunity, it can be stated that, in places where the disease is endemic, individuals have the disease at young ages and then do not become ill again. The finding that almost half of the patients had lesions on the face and upper extremity regions suggests that parasite-transmitting flies bite open surfaces on the body more frequently. Since the region is very hot in the summer season, as a cultural habit, locals sleep in open areas such as balconies and gardens and it increases the possibility of being bitten.

Şanlıurfa is the province, where leishmaniasis disease is seen the most in Turkey, and almost half of the cases seen annually in Turkey are seen in this province[11]. It was revealed that the number of cases was specifically high in neighborhoods where the infrastructure was insufficient, houses were constructed with insufficient materials, there were barns, individuals frequently contacted with animals, and the level of squatting was high.

In Şanlıurfa province, 48.1% of the population lives in central districts[12]. The cases were found more frequently in those central districts. It is known that Eyyübiye district, which was found to have the highest number of cases, has poor socioeconomic and educational status[13]. In a previous study, it was reported that almost 80% of the parents of children having leishmaniasis had an educational level of elementary school or lower[14]. Even though they are central districts, the individuals living in these districts exhibit rural-specific behaviors such as keeping small cattle and cattle in houses. On the other hand, the animal market in Eyyübiye district and the animal barns in houses and nearby regions facilitate the reproduction of sand flies causing the transmission of this disease[3,15]. In a study carried out in Brazil, it was observed that the majority of leishmaniasis patients were found to live in rural areas and have low educational levels[16]. Low educational level, low health literacy, realizing the disease, and effort to have treatment are important determinants in maintaining the treatment. A delayed diagnosis and treatment of this disease play an important role as a source in the infection chain in the transmission of this disease[17]. Moreover, there are also studies reporting that Syrian immigrants live in Eyyübiye and Haliliye districts' Devteyşti, Ahmet Yesevi, Süleymaniye, Bağlarbaşı, Şehitlik, Cengiz Topel, Şair Nabi, Yeşildirek, İpekyolu, Sancaktar, İmam Bakır, Yavuz Selim, Eyyüpnebi, Hayati Harrani, Eyüpkent, Akşemsettin, Yenice, Muradiye, Direkli, and Kurtuluş neighborhoods[18,19]. It is known that the migration of refugees might cause changes in the health patterns of receiving country. Hence, in a previous study carried out in Birecik in the period between 2014 and 2015, it was reported that 76.40%

of leishmaniasis cases were Syrian, while another study carried out in Gaziantep reported that leishmaniasis cases significantly increased after the migration of immigrants[20,21]. Similarly, it was determined that 96.6% of leishmaniasis cases in Lebanon were Syrian[22].

In Şanlıurfa, the neighborhoods where Syrian immigrants live are the places where leishmaniasis has already been observed. However, it is thought that difficulties in living conditions of immigrants negatively contributed to this process. On the other hand, there are also studies suggesting that this vector might have shifted[23].

Many negative factors such as poor living conditions, crowded life, having no access to medical services, and communication difficulties affect immigrants more. From the aspect of controlling leishmania, offering an effective-accessible healthcare service is very important for immigrants. Active surveillance and selective active surveillance activities should be considered in places where there are intense border activities between the countries.

It was stated that the level of knowledge on the contagiousness and preventability of leishmaniasis was low among leishmaniasis patients. Most of them also have a low educational level[17]. Since leishmaniasis patients also carry the factor, they are a source of disease too. In case of any delay in the treatment, these individuals might possibly transmit the disease to those who live with them in the same house, or the individuals they have close contact. It is especially important to inform those who live in endemic regions about the disease and to specify how important early application is for the treatment. In particular, it is important to carry out active surveillance in neighborhoods where the number of cases is high, to detect the cases, and to initiate the treatment in order to eliminate the source of the disease[17].

The cases were aligned in the northwest-southeast line. In parallel with this alignment, it is emphasized that the main wind direction in Şanlıurfa is the northwest[24]. Thus, it is stated that sand flies might be whiffled by the wind[25] and their activities throughout the day are affected by the wind[26]. In a study carried out in Libya, it was reported that the number of leishmaniasis cases caused by flies decreased at locations where the windspeed was higher[27]. Although there is no such finding in the present study, it should be studied if there is a causal relationship. While the northwestern neighborhoods in Şanlıurfa such as Devteyşti, Akşemsettin, Direkli, and Süleymaniye were risky in the early 2010s, the risk shifted to southeastern neighborhoods such as Selçuklu, Osmanlı, Yenice, and Hayati Harrani in the following years. It is thought that, in the coming years, majority of the cases would be in these neighborhoods because of the number of immigrants and environmental factors. Healthcare managers should conduct surveillance works and provide training about the disease

Nam Kem Med J 2023;11(4):308-313

BEYAZGÜL et al. Epidemiology of Leishmaniasis in Şanlıurfa

in neighborhoods having high number of cases, as well as determining and eliminating the infrastructural problems in those neighborhoods and making it easier for immigrants to access healthcare services in neighborhoods where high number of immigrants live.

Study Limitations

The cases in this study were only those who applied to LDTC. Patients who did not apply to this center were not included in the study.

CONCLUSION

While the northwestern neighborhoods in Şanlıurfa were risky in the early 2010s, the risk shifted to southeastern neighborhoods in the following years. It is thought that, in the coming years, majority of the cases would be in these neighborhoods because of the number of immigrants and environmental factors.

Ethics

Ethics Committee Approval: Ethical approval no. E-49866, date: 19.04.2023 was obtained from the Ethics Committee of the Faculty of Medicine of Harran University.

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: B.B., İ.K., Design: B.B., İ.K., Data Collection or Processing: B.B., İ.K., F.B., Analysis or Interpretation: B.B., İ.K., F.B., Literature Search: B.B., İ.K., F.B., Writing: B.B., İ.K., F.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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Protective Effects of ACE/NEP Dual Inhibitor Omapatrilat for Indomethacin-induced Gastric Ulcer

ACE/NEP Dual İnhibitörü Omapatrilatın İndometazinle İndüklenen Mide Ülserinde Koruyucu Etkileri

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ABSTRACT

Aim: The renin-angiotensin-aldosterone system (RAAS) plays important roles in oxidative stress and various gastroenterological mechanisms. Omapatrilat, an RAAS-acting agent, inhibits both neprilysin neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE) and may therefore affect protective mechanisms against gastric ulcer. Therefore, this study examined the gastroprotective role of omapatrilat in a mouse model of gastric ulcer induced by indomethacin to reveal pharmacological and biochemical changes resulting from omapatrilat treatment.

Materials and Methods: Forty-two BALB/c mice were divided into seven groups: control, 40 mg/kg omapatrilat only, 25 mg/kg indomethacin only, indomethacin and 40 mg/kg famotidine, and three groups with indomethacin and 10-40 mg/kg omapatrilat. All chemicals were administered by oral gavage in 0.5 mL of 0.9% NaCl solution at the determined doses. Stomach ulcers were induced by indomethacin in mice treated with famotidine (40 mg/kg) and omapatrilat (10-40 mg/kg). Stomach tissue samples were examined macroscopically. Oxidative stress biomarkers of malondialdehyde (MDA), glutathione (GSH), NEP and ACE levels as well as superoxide dismutase (SOD) activity were measured.

Results: The best antiulcer activity was measured with 40 mg/kg omapatrilat, where the gastric damage observed in the ulcer groups was significantly reversed, and gave the most similar results to the specific famotidine treatment. In relation with the increasing omapatrilat dose, SOD activity was corrected as well as GSH and MDA levels. Also the levels of ACE and NEP decreased back towards those measured in the control group. Therefore, these macroscopic and biochemical findings indicating reversal of gastrotoxicity and gastric ulcer indications demonstrate the role of omapatrilat's NEP and ACE inhibition in indomethacin toxicity, and its strong gastroprotective potential.

Conclusion: Dual inhibition of NEP and ACE by omapatrilat may suppress oxidative stress associated with indomethacin-induced gastric ulcer. Therefore, the protective effect of omapatrilat in the treatment of ulcers may lead to the search for new treatment strategies.

Keywords: Gastric ulcer, indomethacin, neprilysin, oxidative stress, omapatrilat

ÖZ

Amaç: Renin-anjiyotensin-aldosteron sistemi (RAAS), oksidatif stres ve çeşitli gastroenterolojik mekanizmalarda önemli roller oynar. RAAS etkili bir ajan olan omapatrilat, hem neprilisin nötr endopeptidazı (NEP) hem de anjiyotensin dönüştürücü enzimi (ACE) inhibe eder ve bu nedenle mide ülserine karşı koruyucu mekanizmaları etkileyebilir. Dolayısıyla bu çalışmada, omapatrilat tedavisinden kaynaklanan farmakolojik ve biyokimyasal değişiklikleri ortaya çıkarmak için farelerde indometazin tarafından indüklenen bir mide ülseri modelinde omapatrilatın gastroprotektif rolü incelendi.

Gereç ve Yöntem: Kırk iki BALB/c faresi yedi gruba ayrıldı: Kontrol, sadece 40 mg/kg omapatrilat, sadece 25 mg/kg indometazin, indometazin ve 40 mg/kg famotidin, diğer üç grup ise indometazin ve 10-40 mg/kg omapatrilat. Tüm kimyasallar belirlenen dozlarda 0,5 mL %0,9 NaCl solüsyonu içinde oral gavaj ile verildi. Mide ülserleri, famotidin (40 mg/kg) ve omapatrilat (10-40 mg/kg) ile tedavi edilen farelerde indometazin tarafından indüklendi. Mide dokusu örnekleri makroskobik olarak incelendi; ve oksidatif stres biyobelirteçleri malondialdehit (MDA), glutatyon (GSH), NEP, ACE seviyeleri ve ayrıca süperoksit dismutaz (SOD) aktivitesi ölçüldü.

Bulgular: En iyi antiülser aktivite 40 mg/kg omapatrilat ile ölçüldü, burada ülser gruplarında gözlenen gastrik hasar önemli ölçüde tersine çevrildi ve spesifik famotidin tedavisine en benzer sonuçları verdi. Artan omapatrilat dozuna bağlı olarak, SOD aktivitesinin yanı sıra GSH ve MDA seviyeleri düzeldi. ACE ve NEP seviyeleri kontrol grubundaki seviyelerle benzerdi. Dolayısıyla; gastrotoksisite ve gastrik ülser endikasyonlarının tersine çevrildiğini gösteren bu makroskobik ve biyokimyasal bulgular, omapatrilatın NEP ve ACE inhibisyonunun indometazin toksisitesindeki rolünü ve güçlü gastroprotektif potansiyelini göstermektedir.

Sonuç: NEP ve ACE'nin omapatrilat tarafından ikili inhibisyonu, indometazinle indüklenen mide ülseri ile ilişkili oksidatif stresi baskılayabilir. Dolayısıyla; omapatrilatın ülser tedavisinde koruyucu etkisi yeni tedavi stratejileri arayışlarına yol açabilir.

Anahtar Kelimeler: Gastrik ülser, indometazin, neprilisin, oksidatif stres, omapatrilat

INTRODUCTION

Indomethacin, which is a non-steroidal anti-inflammatory drug (NSAID), is frequently used in the treatment of severe inflammatory diseases such as osteoarthritis, rheumatoid arthritis, tendinitis, ankylosing spondylitis and traumatic synovitis[1]. Indomethacin is widely prescribed for the treatment of inflammatory pain, but its anti-inflammatory effects are overshadowed by the fact that it causes significant gastrointestinal ulceration[2]. Indomethacin has also been found to increase oxidative stress and impair blood flow in the stomach[3]. Patients with gastric ulcer prefer to discontinue indomethacin treatment because of its side effects[4].

Indomethacin is a known inhibitor of cyclooxygenase enzymes (COX-1 and COX-2) and its effects on stomach ulceration have been shown to be specifically tied to its COX-1 inhibition[3]. The renin-angiotensin-aldosterone system (RAAS), a multi-hormonal system that coordinates blood pressure regulation and electrolyte balance, has also recently been shown to play a role in the etiology of gastric ulcers[5,6]. RAAS also takes part in pathophysiological phenomena such as inflammation and oxidative stress[7-9]. Angiotensin II (Ang II), the end product of RAAS, is also produced during vascular inflammation and endothelial dysfunction due to oxidative stress. Ang II production increases vascular nicotinamide adenine dinucleotide phosphate oxidase activity and further supports superoxide formation. The resulting superoxide raises oxidative stress and initiates endothelial damage[7].

Omapatrilat, a RAAS-active agent of which Ang II is a key mediator, inhibits both neprilysin/neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE)[10]. NEP inhibition results in some benefits such as stronger vasodilation[11], reduced oxidative stress, and reduced expression of inflammatory cytokines[12,13]. Previous research has observed that omapatrilat can prevent endothelial dysfunction[14], and also provide kidney and cardiovascular protection[15,16]. As such, omapatrilat could potentially be an effective treatment against gastric ulcer.

However, relationship between gastric ulcer and ACE/NEP inhibition has not been elucidated. In this study, we examine omapatrilat's gastroprotective roles against indomethacin-induced gastric ulcers, the effects of NEP and ACE inhibition by omapatrilat on various oxidant/antioxidant parameters, and the relationship between this dual NEP and ACE inhibition and the antiulcer role of omapatrilat.

MATERIALS AND METHODS

Chemicals

Famotidine, indomethacin, and omapatrilat were purchased from Nobel A.S. (Turkey), Merck Sharp & Dohme Corporation (Turkey), and Sigma-Aldrich (Germany), as powder materials, respectively.

Animals

42 BALB/c male mice weighing 40-50 grams were held in steel cages under standard conditions (7 am-8 pm light period, 55% relative humidity, and 21 ± 2 °C) throughout the experiments and they were given standard pellet feed and tap water ad libitum. All animal care and protocols were confirmed by Experimental Animal Ethics Committee of Atatürk University (decision no: 157, date: 02.08.2018).

Preparation and Treatment

Mice were separated into seven groups (n=6), fasted for 24 hours, and administered the following chemicals:

Group 1: Control,

Group 2 (OMA): Omapatrilat (40 mg/kg),

Group 3 (ULCER): Indomethacin (25 mg/kg),

Group 4 (ULCER+FAMO): Indomethacin (25 mg/kg) + Famotidine (40 mg/kg),

Group 5 (ULCER+OMA10): Indomethacin (25 mg/kg) + Omapatrilat (10 mg/kg),

ERKAYMAN et al. Inhibiting ACE/NEP Pathway Against Gastric Ulcer

Nam Kem Med J 2023;11(4):314-321

Group 6 (ULCER+OMA20): Indomethacin (25 mg/kg) + Omapatrilat (20 mg/kg),

Group 7 (ULCER+OMA40): Indomethacin (25 mg/kg) + Omapatrilat (40 mg/kg).

All chemicals were given through oral gavage of 0.5 mL of 0.9% NaCl solution. Omapatrilat was administered at doses of 10, 20 and 40 mg/kg, as it was determined in previous experimental studies that omapatrilat could affect oxidative stress parameters at similar doses[17,18]. The applied dosage of indomethacin was selected according to the standard literature information[3]. This indomethacin (25 mg/kg)[19] dosage was applied 10 minutes after famotidine (40 mg/kg)[19] or omapatrilat (10, 20 and 40 mg/kg)[17,18] were administered[20]. The mice were then sacrificed after a 6-hour period with a 50 mg/kg lethal dose of thiopental anesthesia. The stomachs of the mice were removed, opened along the larger curvature surface, and then washed with saline solution (0.9% NaCl). Macroscopical images of the ulcerous areas on the collected stomach tissue samples were measured on millimeter paper[21]. Antiulcer activities were calculated as the percentage decrease of ulcerated area in each group compared to the control.

Biochemical Measurements

Following the surgical steps, about 75 mg of ground gastric tissue was homogenized in 1 mL of phosphate buffered saline in eppendorf tubes using a homogenizer (TissueLyser II by QIAGEN) and then centrifuged. Total protein concentrations were evaluated using the Lowry method. (Sigma Aldrich Total protein kit TP0300-1KT).

MDA levels[22] and SOD activity[23] were evaluated in standards and each sample's supernatant at room temperature in duplicate according to modified methods of the methods of the enzyme-linked immunosorbent assay (ELISA) reader as previously described[24,25].

ACE and neprilysin activities and GSH level were determined using YLA0163MO, YLA1760MO, and YLA0167MO ELISA kits, respectively, all at room temperature and in duplicate. All ELISA kits were used according to manufacturer's instructions and measured using a BioTek Epoch Microplate Spectrophotometer.

Statistical Analysis

Biochemical quantities were compared using one-way analysis of variance (ANOVA) and Duncan's multiple comparison test with the help of IBM Statistical Package for the Social Sciences software version 20.0. Differences among the groups were considered to be significant at $p<0.05$. All results were expressed as mean±standard deviations.

RESULTS

Gastric Ulcer in Stomach Samples

Macroscopic images of stomach tissues from each experimental group are shown in Figure 1. Tissues obtained from Groups 1 (healthy control), 2 (healthy+omapatrilat), and 4 (ulcer+famotidine) showed no signs of ulcer formation and no visually significant differences. As expected from indomethacin administration, numerous ulcerated areas of different shapes and sizes comprised of mucosal defects were observed throughout the whole stomach tissue samples obtained from Group 3 mice (Indomethacin control group). These foci presented with distinct boundaries and were mostly surrounded by swollen spots. The number and sizes of these spots were reduced with increasing omapatrilat dosage in Groups 5 (OMA10), 6 (OMA20), and 7 (OMA40), pretreated with 10, 20, and 40 mg/kg of omapatrilat, respectively. These findings are given in Table 1 with measured areas of ulcerated spots and corresponding anti-ulcer activity in each experimental group.
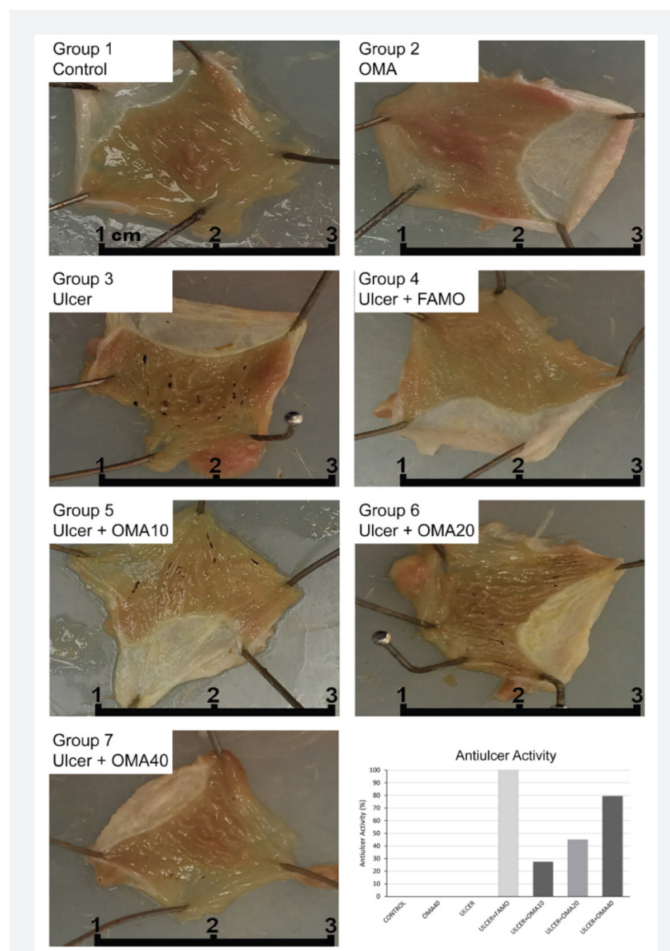
Figure 1. Macroscopic images of stomach tissue samples from mice groups where ulcerations are observed (scale bars indicate a length of 2 cm)

Table 1. Measurements of ulcerated areas and antiulcer activities

Groups	Ulcerated area (mm2)	Antiulcer activity (%)
Control	0.0[a]	-
OMA 40	0.0[a]	-
ULCER	29.0±2.0[e]	-
ULCER+FAMO	0.0[a]	100.0
ULCER+OMA 10	21.0±2.0[d]	27.5
ULCER+OMA 20	16.0±1.2[c]	44.8
ULCER+OMA 40	6.0±0.3[b]	79.3

Results quoted as mean ± standard deviation. Antiulcer activities were calculated as the percentage decrease of ulcerated area in the corresponding group in comparison with the control. Comparisons performed using one-way ANOVA and Duncan's multiple comparison test. Means with the same letter are not significantly different p<0.05

Biochemical Results

SOD, MDA, and GSH measurements are graphically shown in Figures 2, 3, 4. SOD activity measured in the treatment groups with 20 and 40 mg/kg omapatrilat were the closest to those measured in healthy groups (1 and 2). The least SOD activity was measured with indomethacin application in Group 3. In addition, Group 5, which was pre-treated with 10 mg/kg of omapatrilat, exhibited similar SOD activity as with in Group 3. Similar results were obtained for the GSH measurements. The highest GSH level was measured in the Groups 1 and 2. GSH

Figure 3. Effects of omapatrilat treatment on GSH level in indomethacin-induced gastric ulcer (results quoted as mean±standard deviation. Comparisons performed using one-way ANOVA and Duncan's multiple comparison test. Means with the same letter are not significantly different p<0.05)

GSH: Glutathione
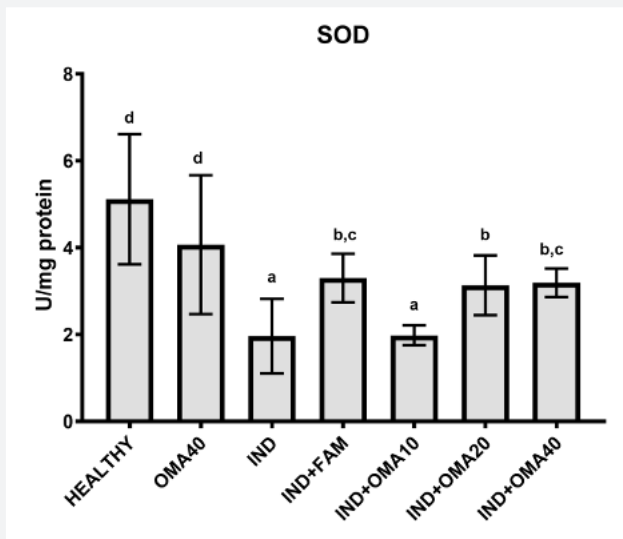
Figure 2. Effects of omapatrilat treatment on SOD activity in indomethacin-induced gastric ulcer (results quoted as mean±standard deviation. Comparisons performed using one-way ANOVA and Duncan's multiple comparison test. Means with the same letter are not significantly different p<0.05)

SOD: Superoxide dismutase
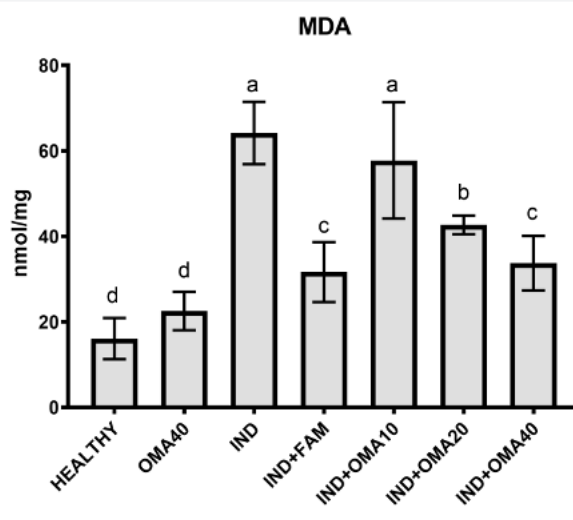
Figure 4. Effects of omapatrilat treatment on MDA level in indomethacin-induced gastric ulcer (results quoted as mean±standard deviation. Comparisons performed using one-way ANOVA and Duncan's multiple comparison test. Means with the same letter are not significantly different p<0.05)

MDA: Malondialdehyde

ERKAYMAN et al. Inhibiting ACE/NEP Pathway Against Gastric Ulcer

Nam Kem Med J 2023;11(4):314-321

level in Group 3 was significantly reduced due to indomethacin administration, and was mostly recovered in Group 4 with the standard famotidine treatment. In omapatrilat-treated groups, while Group 5 only showed minor improvement in the GSH level, in Groups 6 and 7 GSH levels were statistically similar to that measured with the standard treatment in Group 4. Omapatrilat's effectiveness against oxidative stress was also observed in the MDA measurements. While Groups 1 and 2 exhibited the lowest MDA levels, the highest MDA levels were measured in Groups 3 and 5. Standard treatment in Group 4 and the 40 mg/kg omapatrilat treatment in Group 7 resulted in the closest MDA levels to those recorded in Groups 1 and 2. These results in SOD activity as well as in GSH and MDA levels indicate omapatrilat's gastroprotective potential against ulcer.

NEP and ACE levels are shown in Figures 5, 6. The lowest NEP and ACE levels were measured in Groups 1 and 2; and the highest levels were measured in Group 3 due to indomethacin, as expected. By increasing the dosage of omapatrilat from 10 to 40 mg/kg, these enzyme levels were significantly reduced, indicating that omapatrilat dually inhibits both NEP and ACE. Both ACE and NEP enzyme levels were increased in line with ulcer formation.

DISCUSSION

In this study, we have observed that omapatrilat, an inhibitor of both NEP and ACE that acts through the RAAS pathway,

reduces indomethacin-induced gastric ulcer damage. Omapatrilat's connection with oxidant/antioxidant parameters was demonstrated. The relationship between gastrotoxic effect mechanism of indomethacin and the antiulcer effect of omapatrilat was also studied. Omapatrilat was shown to reduce both NEP and ACE levels in the stomach tissue. It was observed that omapatrilat treatment increased levels GSH and SOD activity while decreasing MDA levels. Therefore, omapatrilat was shown to prevent the damage induced by oxidative stress in gastric ulcer.

Indomethacin, which is used in the treatment of many inflammatory diseases, is a NSAID. A well-known side effect of indomethacin, gastric ulcer formation, is commonly used in experiments to induce inflammation. As such, in our experiments, indomethacin is used not only as an anti-inflammatory drug, but also to create an ulcer model in animals[26]. Indomethacin has been shown to cause significant damage to gastric tissue in animals at doses of 10, 20, and 25 mg/kg[27]. Similarly, our results also showed clearly visible ulcer areas in the group receiving only the 25 mg/kg indomethacin. No ulcer formation was observed in the famotidine group. It was seen that omapatrilat showed an antiulcer effect in a dose-dependent manner. The best antiulcer effect was recorded at an omapatrilat dose of 40 mg/kg.

Ang II, which induces inflammation and oxidative stress in RAAS, is an important end product[28]. In addition, Ang II narrows
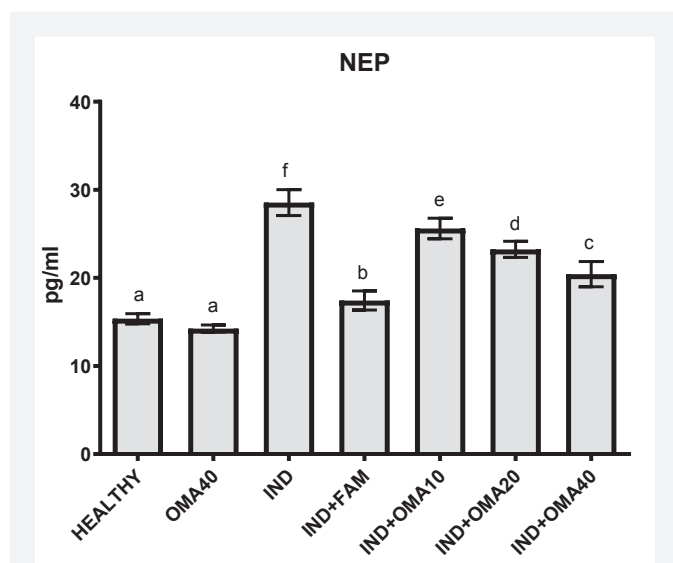
Figure 5. Effects of omapatrilat treatment on NEP levels in indomethacin-induced gastric ulcer (results quoted as mean±standard deviation. Comparisons performed using one-way ANOVA and Duncan's multiple comparison test. Means with the same letter are not significantly different p<0.05)

NEP: Neutral endopeptidase
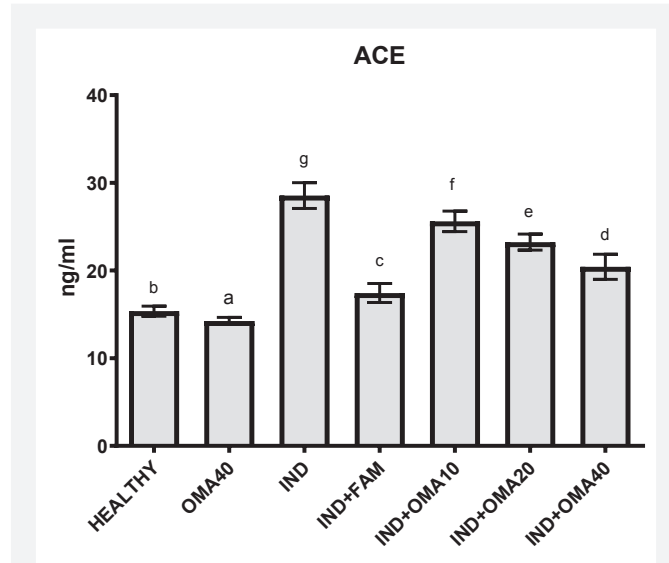
Figure 6. Effects of omapatrilat treatment on ACE levels in indomethacin-induced gastric ulcer (results quoted as mean±standard deviation. Comparisons performed using one-way ANOVA and Duncan's multiple comparison test. Means with the same letter are not significantly different p<0.05)

ACE: Angiotensin converting enzyme

the gastric mucosal vasculature by activating the receptor in the gastrointestinal tract[29]. In the gastrointestinal system, RAAS has been reported to have functional effects on the stomach[30], colon[31], and intestine[32]. The effects of RAAS-acting agents including ACE inhibitors and Angiotensin receptor blocker (ARB) drugs on different ulcer models have previously been studied. For instance, captopril, the first ACE inhibitor used in hypertension treatment, showed antioxidant effects on aspirin-induced gastric lesions[33]. It has been determined that ARBs prevent ulcerations by protecting gastric blood flow and reduce sympathoadrenal activation[34]. ARBs have also been shown to suppress gastric damage due to ischemia/reperfusion injury in rats[35]. Angiotensin-(1e7), a physiological antagonist of Ang II, was also effective against stress-induced gastric lesions in rats[9]. According to these results, it is suggested that the inhibition of RAAS at different stages may play an important role during ulcer treatment. Potential anti-inflammatory, gastro-protective or anti-oxidative effects can be cited as underlying causes of the gastroprotective nature of RAAS inhibition in various ulcer models. Similar to these findings, here we show that omapatrilat blocks RAAS by inhibiting ACE and protects the stomach from possible oxidative stress due to Ang II.

It is known that the toxic effect of indomethacin on the stomach is much more severe than that of other NSAIDs[36]. It has been reported that this effect of indomethacin on the gastrointestinal system is due to the inhibition of prostaglandin (PG) synthesis[37]. However, the COX theory, which plays a role in the gastric mucosal damage by NSAIDs, cannot adequately explain this damage[38]. For instance, in some studies, although PGE2 formation was prevented largely with repeated administration of indomethacin, it was observed that the mucosal lesion decreased[39]. This indicates that there may be factors other than PG in the antiulcer activity mechanism. Based on this idea, GSH and MDA levels as well as SOD activity were measured to determine whether oxidant/antioxidant parameters played a role in the gastric mucosal adaptation mechanism.

In some studies, it has been demonstrated that reactive oxygen species (ROS) play a role in the etiopathogenesis of gastric damage caused by indomethacin[40]. Tissue damage due to ROS begins with the formation of lipid radicals in the cell membrane. Then, this radical first transforms into lipid hydroperoxides, and finally into toxic products such as malondialdehyde, alkane, and aldehyde[40,41]. The detrimental effects of ROS have required cells to develop distinct and potent detoxification mechanisms through a regulatory network of antioxidant enzymes and non-enzymatic compounds[42]. Among these antioxidants, GSH prevents tissue damage by maintaining ROS concentrations below certain levels[43]. Therefore, as expected, we observed that the highest GSH level was measured in the control group. In

the indomethacin group, where the damage was the highest, the GSH level was measured to be the least. Additionally, it was seen that omapatrilat increased the GSH level in the ulcer groups in a dose-dependent manner. With this increase in the antioxidant levels, it is shown that omapatrilat helps to maintain the oxidant/antioxidant balance.

SOD, another key antioxidant, is shown to act as a protective factor in controlling indomethacin-induced damage[44]. Similar to the GSH results above, we also measured the highest SOD activity in the control group, and the lowest SOD activity in the indomethacin group. As expected, we observed that omapatrilat resulted in an increase in SOD activity in a dose-dependent manner. In our experiments, the increase in SOD activity in ulcerated mice treated with omapatrilat demonstrates omapatrilat's antioxidant and antiulcer effects. Our measurements have shown that 40 mg/kg omapatrilat results in statistically similar findings to those from standard famotidine treatment.

It is reported that excessive toxic oxygen radicals in tissues activate lipid peroxidation that leads to the formation of MDA, an oxidative stress biomarker[45]. MDA directly damages the cell membrane structure and other cell components by producing reactive aldehydes, resulting in tissue damage and various diseases[46]. Previous studies have shown high levels of MDA in ulcerated stomach tissue[47]. Similarly, in our experiments, MDA levels were found to be as much as 4 times higher than the control group in the indomethacin group, and were gradually reduced in groups treated with omapatrilat, again in a dose-dependent manner. As with SOD activities, MDA levels were found to be statistically similar with 40 mg/kg of omapatrilat and the specific famotidine treatment. Similar results were reported in another study where reduction in SOD activity and GSH levels in indomethacin-induced ulcer groups and an accompanying increase in the MDA level were observed. These changes were reversed due to treatment with aliskiren, a renin inhibitor that affects the RAAS pathway[5]. These findings show that omapatrilat, which is also a RAAS-acting agent, contributes to the reduction of oxidative stress by promoting antioxidant activity. Our findings of ACE and neprilysin enzyme levels were also similar to the oxidative stress biomarkers discussed above. In comparison with the control group, it was seen that indomethacin significantly increased ACE and neprilysin levels, which were then subsequently reversed to healthy levels with famotidine. With omapatrilat administration in ulcerated mice, we recorded similar decreases in ACE and neprilysin levels that were proportional to the omapatrilat dosage from 10 to 40 mg/kg. Resulting ACE and neprilysin levels with specific famotidine treatment and with 40 mg/kg of omapatrilat were both significantly less than those measured in untreated ulcerated mice, but still above those measured in the control group. Omapatrilat administration did

ERKAYMAN et al. Inhibiting ACE/NEP Pathway Against Gastric Ulcer

Nam Kem Med J 2023;11(4):314-321

not alter ACE or neprilysin levels in healthy mice. As a result, omapatrilat reduced the formation of ulcers by preventing Ang II production, which plays a key role in ulcer etiology. In recent studies, drugs that affect the RAAS, such as aliskiren and enalapril, have been shown to be successful in increasing antioxidant levels. Among these drugs, omapatrilat, which is the first drug known to inhibit both NEP and ACE enzymes simultaneously, is a recently developed RAAS effective agent[10]. As such, we have investigated the possible effects of ACE/NEP pathway on gastric ulcer due to indomethacin, and have seen significant increases in the levels of both enzymes in the ulcer group. This increase in ACE and NEP levels can be interpreted as a defense mechanism accompanying gastric damage.

Study Limitations

The limitations of our study are that the experimental study was conducted only on one ulcer model and the tissues could not be examined histopathologically.

CONCLUSION

In conclusion, the best antiulcer activity of omapatrilat was demonstrated at a dose of 40 mg/kg in our experiments. Our macroscopic results, oxidant/antioxidant levels, and biochemical measurements show that omapatrilat demonstrates significant anti-ulcer effect at 40 mg/kg dosage by inhibiting both neprilysin and ACE levels, which have approached to those measured in the control group. These findings indicate that the dual inhibition of NEP and ACE by omapatrilat suppressed the oxidative stress related to indomethacin-induced gastric ulcer. These results reveal for the first time the combined role of ACE and NEP enzymes in gastric ulcer and the protective effects of omapatrilat against oxidative stress. Therefore, we think that the protective effects of omapatrilat in the treatment of ulcers may lead to the search for new treatment strategies.

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Ethics

Ethics Committee Approval: All animal care and protocols were confirmed by Experimental Animal Ethics Committee of Atatürk University (decision no: 157, date: 02.08.2018).

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.G.E., E.Ç., Concept: R.A.U., Z.B.A.M., A.B., Design: R.A.U., Z.B.A.M., A.B., Data Collection or Processing: K.G.E., E.Y., Analysis or Interpretation: E.Y., A.B., E.Ç., Literature Search: K.G.E., Writing: K.G.E.

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Immunohistochemical Examination of Placental NRG-1 Expression in Pregnant Women Diagnosed with Preeclampsia

Preeklampsi Tanısı Alan Gebelerde Plasental NRG-1 Ekspresyonunun İmmünohistokimyasal Olarak İncelenmesi

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ABSTRACT

Aim: Preeclampsia is a multisystemic disease characterized by hypertension, proteinuria, increased vascular damage and permeability, and superficial placental invasion that occurs during pregnancy. Neuregulin-1 (NRG-1) is an important ligand for embryogenesis, angiogenesis, nervous system development, myogenesis, and gonadogenesis. In our study it was aimed to examine the importance of NRG-1 in preeclampsia using light microscopy.

Materials and Methods: Study groups: Group 1 (control group) (n=10): healthy normal pregnants, Group 2 (preeclampsia group) (n=10): pregnant women diagnosed with preeclampsia. Hematoxylin Eosin and Periodic Acid-Schiff stainings were done. NRG-1 immunostaining was performed and scored.

Results: In the control group, the syncytiotrophoblast layer, villus stroma, fetal vascular structures and intervillous space were histologically normal. It was observed that the lumen of the villi in the placentas of preeclamptic pregnant women was narrowed and the number of villi was less than that of normal pregnant women. A significant increase in the number of nuclear chains was detected around the syncytial node and villi. It was observed that the number of fetal capillaries and fetomaternal barriers decreased. Intervillous and perivillous fibrin deposition was clearly observed. In the preeclampsia group, it was determined that the staining intensity with anti-NRG-1 around the villus was significantly less than the normal pregnant placentas in the control group (p<0.05).

Conclusion: Preeclampsia can cause pathological changes in the placenta at the light microscopic level, as well as a decrease in NRG-1 expression. We think that NRG-1 may be an important marker for the preeclampsia process.

Keywords: Preeclampsia, NRG-1, placenta, immunohistochemistry

ÖZ

Amaç: Preeklampsi; gebelikte ortaya çıkan hipertansiyon, proteinüri, artmış damar hasarı ve geçirgenliği ve yüzeyel plasenta istilası ile karakterize multisistemik bir hastalıktır. Neuregulin-1 (NRG-1), embriyogenez, anjiyogenez, sinir sistemi gelişimi, miyogenez, gonadogenez için önemli olan bir liganddır. Çalışmamızda NRG-1'in preeklampsideki öneminin ışık mikroskobik olarak incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışma grupları: Grup 1 (kontrol grubu) (n=10): sağlıklı normal gebelerden, Grup 2 (preeklampsi grubu) (n=10): preeklampsi tanısı alan gebelerden oluşmaktadır. Hematoksilen-Eozin ve periyodik asit-Schiff boyamaları yapıldı. NRG-1 immün boyaması yapılarak skorlandı.

Bulgular: Kontrol grubunda, sinsityotrofoblast tabakası, villus stroması, fetal vasküler yapılar ve intervillöz aralık normal histolojik yapıda izlendi. Preeklamptik gebelere ait plasentalardaki villus lümeninin daraldığı ve villusların normal gebelere göre sayıca daha az olduğu görüldü. Sinsityal nod ve villusların etrafında nükleer zincir sayısında belirgin artış tespit edildi. Fetal kapiller sayısının ve fetomaternal bariyer sayısının azaldığı gözlendi. İntervillöz ve perivillöz fibrin birikimi belirgin olarak izlendi. Preeklampsi grubunda, villus etrafındaki anti-NRG-1 ile boyanma yoğunluğunun kontrol grubundaki normal gebe plasentalarına göre anlamlı olarak daha az boyandığı tespit edildi (p<0.05).

Sonuç: Preeklampsi, plasentada ışık mikroskobik düzeyde patolojik değişikliklere neden olabildiği gibi, NRG-1 ekspresyonunda da azalmaya neden olmaktadır. NRG-1'in preeklampsi süreci için önemli bir belirteç olabileceğini düşünmekteyiz.

Anahtar Kelimeler: Preeklampsi, NRG-1, plasenta, immünohistokimya

INTRODUCTION

Preeclampsia is a multisystem disease that occurs in 5-10% of pregnant women and is characterized by maternal hypertension, proteinuria, increased vascular damage and vascular permeability, and is one of the main causes of perinatal deaths and disabilities[1,2]. Preeclampsia is defined as *de novo* hypertension accompanied by new-onset proteinuria after the 20th week of gestation. Hypertension is blood pressure measured above 140/90 mmHg at least two times. Proteinuria is defined as the leakage of 300 mg of protein in the urine in 24 hours[3]. Preeclampsia is a disease specific to the human species, and preeclampsia or similar pregnancy pathologies are not observed in other species[4]. Although the cause of preeclampsia is still not fully determined, it is accepted that more than one factor plays a role in it.

The placenta is an organ that continues to exist during pregnancy, with functions such as nutrient delivery to the fetus through the mother, thermoregulation, ensuring fetal immunity, hormone secretion, waste removal and gas exchange[5]. Considering that preeclampsia is a disease specific to pregnancy and that the findings completely regress with the evacuation of the placenta, it has been accepted that the most important role in the development of preeclampsia belongs to the placenta[6]. It is thought that endothelial damage, decreased perfusion and vasospasm in placental vessels play an important role in the pathogenesis of this disease[3,7]. It has been determined that in preeclampsia, there is an increase in placenta-derived oxidative stress and an increase in the levels of oxidative stress products in the maternal circulation, as well as a decrease in antioxidant activity[8]. Recently, many studies have been conducted on the effects of the placenta on the fetus. For this purpose, various placental cells, proteins, hormones and factors have been examined. These factors affecting the development of the fetus and placenta reveal a lot of important information about the fetus and placenta[9].

The roles of angiogenic proteins, cell adhesion molecules and the inflammatory system in microvascular dysfunction are of great importance in the pathophysiology of preeclampsia[3]. Neuregulin-1 (NRG-1), a current signaling pathway molecule investigated in the pathogenesis of various diseases, is a ligand that binds to ErbB receptors, provides activation and mediates intercellular interactions[10,11]. NRG-1 produces fifteen different proteins and is important for angiogenesis, embryogenesis, myogenesis, gonadogenesis and nervous system development[11,12]. NRG-1 sends signals to target cells by interacting with transmembrane tyrosine kinase receptors of the ErbB family. In this way, it stimulates proliferation, migration and differentiation in cells[13]. When scientific publications made to date are examined, it is understood that preeclampsia is an important disease among pregnant women. Although there are studies in the literature examining the connections of NRG-1 with various diseases, there is no study investigating its role in preeclampsia. The immunohistochemical distribution and expression level of NRG-1 in normal pregnant women and pregnant women diagnosed with preeclampsia are unknown. Therefore; in our study, we aimed to examine the importance of NRG-1 in preeclampsia by comparing NRG-1 expression in the placenta of normal pregnant women with the placenta of pregnant women diagnosed with preeclampsia.

MATERIALS AND METHODS

Groups

The study was carried out with placenta tissue samples taken from volunteer pregnant women who applied to Muğla Sıtkı Koçman University Faculty of Medicine, Obstetrics and Gynecology outpatient clinic and wanted to participate by reading and signing the informed consent form. Study groups: Group 1 (control group): Healthy normal pregnant women without any disease (n=10). Group 2 (preeclampsia group): Pregnant women diagnosed with preeclampsia (n=10). Control group consists of volunteer healthy pregnant women who applied to the outpatient clinic and did not have coronary artery, liver, kidney disease or diabetes. Pregnant women were given a routine pregnancy examination by a Obstetrics and Gynecology specialist. The diagnosis of preeclampsia was made after the 20th week of gestation with blood pressure of 140/90 mmHg and above and proteinuria of 0.3 g/24 hours and above.

Criteria for inclusion in the study:

- Healthy control group - 38-40. gestational week - Volunteer to participate in the research - Between 18-40 years old,

- Preeclampsia group - 38-40. gestational week - Volunteer to participate in the research - Between 18-40 years of age - Diagnosed with preeclampsia.

Criteria for exclusion from the study:

Those who have a severe physical illness, use of alcohol, cigarettes and drugs, body mass index >30, gestational

KİLİS et al. Placental NRG-1 Expression in Preeclampsia

Nam Kem Med J 2023;11(4):322-327

diabetes, pregestational diabetes, liver and kidney failure, any endocrine disorder, hematological disease, a history of gastric or intestinal surgery and received medical treatment for any reason in the last three months, cases with chronic inflammation or infection, and with fetal biometry outside the 10-90% percentile on obstetric ultrasound were excluded from the study.

Histological Analysis

Tissue samples taken from the placentas of the pregnant women who participated in the study after giving birth by cesarean section or vaginal delivery in the 38th to 40th week for analysis were washed with physiological saline in the operating room, then transferred to 10% formaldehyde solution and brought to the Histochemistry Laboratory of the Department of Histology and Embryology. After the placenta tissues were fixed in formaldehyde, they were subjected to routine histological follow-up procedures. The samples were first cut into 3-4 mm pieces and fixed in plastic tissue tracking cassettes at room temperature for 48 hours. After the fixation process was completed, the pieces were washed in running tap water for 24 hours, then dehydrated in increasing alcohol levels (70%, 80%, 96%, 100%), made transparent in xylene and embedded in paraffin. 5 μm sections taken from paraffin blocks with the help of a microtome device were placed on slides for histochemical and immunohistochemical examinations. After these prepared slides were kept in an oven at 37 °C for 2 hours to increase the adhesion of the tissue to the slide, Hematoxylin-Eosin (H-E) and Periodic-acid Schiff (PAS) staining methods were applied to observe the general histological structure. The sections were examined and photographed with a Nikon Eclipse 80i light microscope and Nikon image analysis system.

In this study, immunohistochemical staining method was applied using anti-NRG-1 antibody. After the sections were deparaffinized in an oven at 58 °C for 1 hour, they were kept in xylene for 15 minutes, then passed through a decreasing alcohol series (100-70%) and taken into distilled water. The sections that were kept in distilled water for 5-10 minutes and the sections that were placed in boiling citrate buffer to reveal the antigens were treated with citrate for 7 minutes and left to cool without being removed from the buffer. After the cooled sections were washed first with distilled water and then with phosphate buffer solution (PBS) for 1-2 minutes, the borders of the tissue sections were drawn with a hydrophobic pen (pappen) and placed in a humid medium container. Then, hydrogen peroxide was applied to the sections and it was waited for 10 minutes. Boiled water was placed on the platform to keep the medium warm and the lid of the platform was closed. Then, hydrogen peroxide was removed from the tissue and the sections were washed with PBS 3 times for 5 minutes, then the protein block was added and left for 5

minutes. Then, the protein block was removed by shaking the sections very well.

Before washing the sections, anti-NRG-1 (sc-393006) primary antibody, diluted at the rate recommended by the company, was added and kept in a humid environment for 2 hours. Then, after washing with PBS 3 times for 5 minutes, it was treated with secondary antibody for 10 minutes and washed with PBS 3 times for 5 minutes. In the next step, after streptavidin was treated with horse-radish peroxidase complex for 10 minutes, it was washed with PBS 3 times for 5 minutes and incubated with substrate-chromogen (AEC; 3, aminoethyl 9, carbazole) solution for 3 minutes (until a color reaction was observed). After washing with PBS 3 times for 5 minutes and shaking in distilled water, Mayer's hematoxylin stain was applied to the sections (1 minute). The sections were washed first in tap water and then in distilled water, and then sealed with entellan.

Evaluation of IHC staining: Immunostaining coverslips were evaluated under a light microscope (Nicon Eclipse 80i, Japan). Antibody-labeled areas on the coverslips were examined at x20 magnification. The staining intensity of the cells was scored as no staining (-), weak (+), moderate (++) and strong (+++) and photographs were taken with the image analysis system.

Statistical Analysis

Statistical analysis of the data was performed with Statistical Package for the Social Sciences 14 statistical program. Whether the data showed normal distribution was evaluated with the Kolmogorov-Smirnov test. Data that did not show normal distribution were analyzed using the Kruskal-Wallis variance test, and pairwise comparisons were performed using the Mann-Whitney U test. $P<0.05$ was considered statistically significant.

RESULTS

Histopathological Findings

Placental chorionic villi were examined histologically in sections stained with H-E and PAS. In the sections of the control group, the syncytiotrophoblast layer, villus stroma, fetal vascular structures and intervillous space were observed with a normal histological structure. It was determined that the number of free villi increased from the maternal side to the fetal side. The syncytiotrophoblast layer surrounding the villi was observed as a regular, thin layer. Syncytial knot and syncytial nuclear chain structures were detected very rarely. Fetal capillaries in the villi were seen to be numerous and scattered throughout the villus stroma. Fetomaternal barriers were observed to be thin and smooth (Figure 1).

When the H-E and PAS stained sections of preeclamptic pregnant women are examined; it was observed that the

Nam Kem Med J 2023;11(4):322-327

KİLİS et al. Placental NRG-1 Expression in Preeclampsia
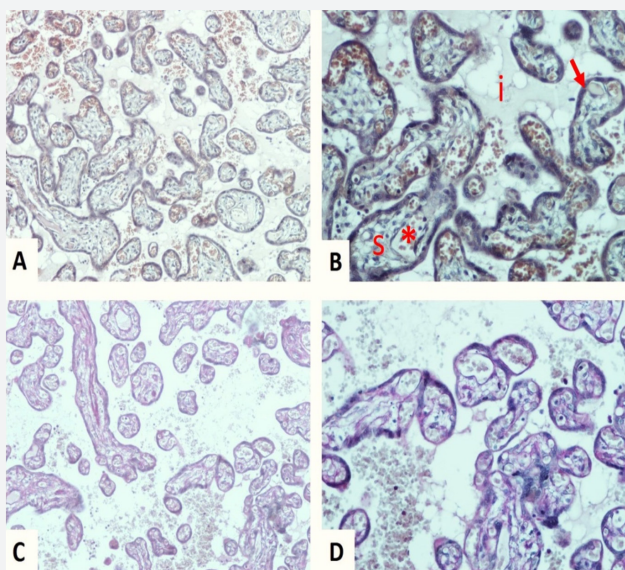
Figure 1. Placenta sections of the control group (taken from normal pregnant women). A) H-E; x10. B) H-E; x20. C) PAS; x10. D) PAS; x20. Star; fetal vascular structure, arrow; syncytiotrophoblast, s; stroma, i; intervillous space

H-E: Hematoxylin-Eosin, PAS: Periodic-acid Schiff
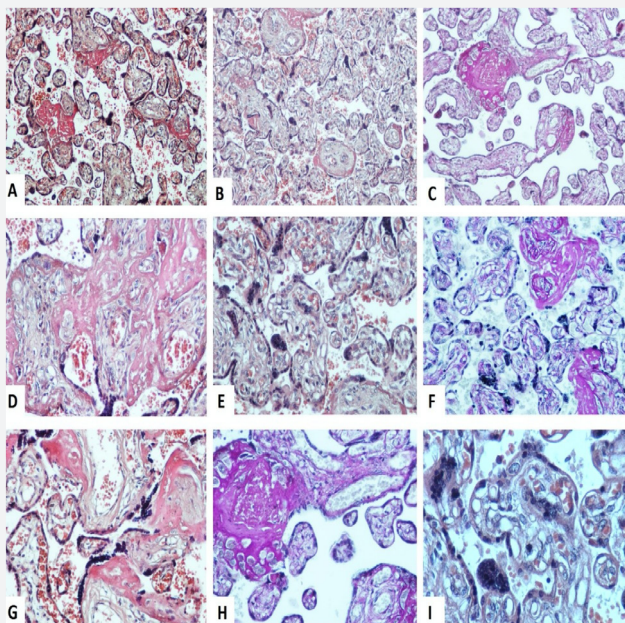
Figure 2. Placenta sections of pregnant women with preeclampsia (Group 2). A) H-E; x10. B) H-E; x10. C) PAS; x10. D) H-E; x20. E) H-E; x20. F) PAS; x20. G) H-E; x20. H) PAS; x20. I) H-E; x40

H-E: Hematoxylin-Eosin, PAS: Periodic-acid Schiff

villus lumen in the placentas narrowed and the villi were less in number than in normal pregnant women. A significant increase in the number of nuclear chains was detected around the syncytial nodes and villi. It was observed that the number of fetomaternal barriers and the number of fetal capillaries decreased. There was dilatation in some of the veins. It was observed that the terminal villous stroma was rich in collagen. Intervillous and perivillous fibrin accumulation was clearly observed (Figure 2).

Immunohistochemical findings were evaluated according to anti-NRG-1 staining intensity. In the placenta, anti-NRG-1 expression was observed in the nucleus and cytoplasm of the cells. Placental NRG-1 expression in the control group was observed to have strong (+++) staining in the trophoblast cells around the villi, and moderately weak (++) staining in the villus mesenchymal connective tissue cells and capillary endothelium. In the preeclampsia group, the intensity of anti-NRG-1 staining around the villi was found to be significantly less compared to the normal pregnant placentas in the control group (p<0.05) (Table 1, Figure 3).

DISCUSSION

Preeclampsia is a disease seen during pregnancy being, one of the leading causes of maternal and perinatal mortality and

Table 1. Anti-neuregulin-1 immunoreactivity

Groups	Staining intensity in trophoblasts	Villus mesenchymal staining intensity
Group 1: Normal	+++	++
Group 2: Preeclampsia	++	+
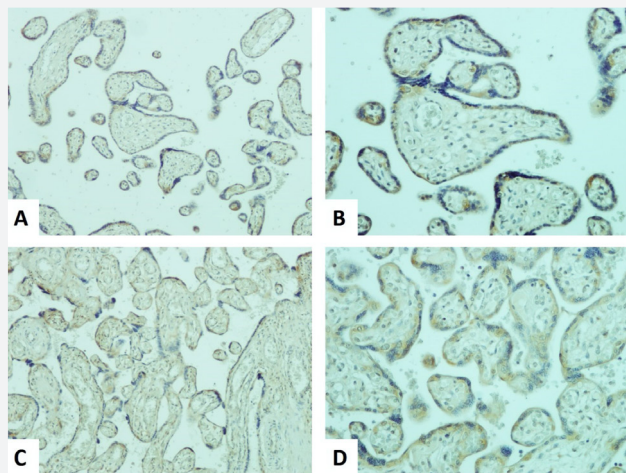
Figure 3. Anti-NRG-1 immunoreactivity. A) Group 1: Control, Normal placenta; x10. B) Group 1: Control; x20. C) Group 2: Preeclampsia; x10. D) Group 2: Preeclampsia; x20

NRG-1: Neuregulin-1

KİLİS et al. Placental NRG-1 Expression in Preeclampsia

Nam Kem Med J 2023;11(4):322-327

morbidity and affecting many systems in the body, including the kidney, liver, brain and lung, through endothelial damage[14]. In a study conducted to determine vascular endothelial growth factor (VEGF) protein levels in placenta biopsies of pregnant women with preeclampsia, Akercan et al.[15] revealed that in the preeclamptic group compared to normal pregnant placenta; the three layers in each villus, consisting of the outermost syncytial layer, intermediate cytotrophoblast, and inner fetal capillaries supported by mesenchyme, produce very strong VEGF immunoreactivity. In a study conducted on 34 pregnant women to demonstrate the relationship between NF-κB expression and trophoblastic cell apoptosis in pregnancies complicated by preeclampsia or intrauterine growth restriction; immunohistochemical examination of the placenta of pregnant women with preeclampsia showed that NF-κB expression increased, M30 expression increased, caspase-3 expression and bcl-2 expression decreased compared to normal pregnant women[16].

In the study by Shu et al.[17] where they investigated the clinical importance of apoptosis in the placenta with preeclampsia using the annexin V method; they found that annexin V was detected in trophoblasts in the placenta and that the staining intensity of annexin V in the placenta from these patients was reduced compared to normal placenta. Apoptosis is involved in every step of the pathogenesis of preeclampsia. Reduced apoptosis may induce a maternal immune response against the fetus, while increased apoptosis may influence the process of placental ischemia and subsequent systemic endothelial damage[18]. In our study, it was observed that, compared to the placentas of normal pregnancies, the villus lumen in the placentas of preeclamptic pregnancies was narrowed and the villi were fewer in number, and there was a significant increase in the number of nuclear chains around the syncytial nodes and villi. In addition, it was observed that the number of fetal capillaries and the number of fetomaternal barriers decreased in the placentas of preeclamptic pregnant women, and dilatation was observed in some of the veins. Terminal villous stroma was found to be rich in collagen. Intervillous and perivillous fibrin accumulation was clearly observed.

In recent studies; NRG-1 has been shown to be an important molecule for cardiovascular system development and maintenance of adult heart function and is a positive regulator of angiogenesis[19]. Russell et al.[20] reported that NRG-1 and ErbB molecules are expressed in vascular endothelial cells. However, in a study on myocardial cells carried out by Xu et al.[21] it has been shown that NRG-1 protects myocardial cells against oxidative damage by regulating endoplasmic reticulum stress. NRG-1 promotes trophoblast growth and differentiation[22]. Recent studies have shown that NRGs are endocrine regulators of metabolic events. NRG-1 plays an essential role in the growth and development of skeletal muscle, cardiac muscle and nervous tissue. The NRG-1/ErbB pathway is considered a potential target for the treatment of neuromuscular and cardiac disorders[23].

Preterm newborns with low NRG-1 levels have been found to be at higher risk of developing short-term morbidity[24]. It has been reported that NRG-1 may be a potential endogenous protector against perinatal brain white matter damage[25]. Fock et al.[22] showed that NRG-1 is also expressed by human decidual stromal cells and that the NRG-1-dependent ErbB2-ErbB3 signaling pathway is necessary for the survival of differentiated trophoblast cell populations. NRG-1 is also required for blastocyst implantation[26]. NRG-1 has been observed in the human placenta in both the maternal and fetal parts. It is thought that this may also be responsible for placental growth[11]. In our study, it was determined that the intensity of anti-NRG-1 staining around the villi in the preeclampsia group was significantly less than that of normal pregnant placentas in the control group (p<0.05).

Study Limitations

Although our study reveals the results we expected, it has some limitations. The most important limitation is that since preeclampsia is a multisystemic disorder, biochemical data should also have been available. In addition, data obtained from electron microscopy examinations can also make the results more reliable.

CONCLUSION

Preeclampsia, which may occur during pregnancy, may cause pathological changes in the placenta at the light microscopic level, as well as a decrease in NRG-1 expression. We think that NRG-1, which is important for angiogenesis, embryogenesis, myogenesis, gonadogenesis and nervous system development, may be an important marker for the preeclampsia process. This study is the first study to address the role of NRG-1, as well as showing the histopathological changes of the placenta in case of preeclampsia in pregnant women. In this respect, it has made a significant contribution to the literature at the basic knowledge level.

Ethics

Ethics Committee Approval: For this study, approval was received from Muğla Sıtkı Koçman University Clinical Research Ethics Committee with the decision dated 26.02.2020 and numbered 04/II, stating that it is ethically appropriate to conduct the study.

Patient Consent: Patient approval was obtained.

Peer-review: Externally peer-reviewed.

Nam Kem Med J 2023;11(4):322-327

KİLİS et al. Placental NRG-1 Expression in Preeclampsia

Authorship Contributions

Surgical and Medical Practices: B.K., Concept: H.E., S.K., M.T., Design: H.E., S.K., M.T., Data Collection or Processing: B.G., D.Ç., S.K., M.T., Analysis or Interpretation: H.E., D.Ç., S.K., M.T., Literature Search: D.Ç., S.K., M.T., Written by: D.Ç., H.E.

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ORIGINAL ARTICLE

Oxidized Low Density Lipoprotein Receptor 1 3'UTR 188C>T Gene Polymorphism in Patients with Coronary Artery Bypass Grafting

Koroner Arter Bypass Grefti Uygulanan Hastalarda Okside Düşük Yoğunluklu Lipoprotein Reseptör 1 3'UTR 188C>T Gen Polimorfizmi

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ABSTRACT

Aim: Coronary artery disease (CAD) is a pathological process characterized by atherosclerotic plaque accumulation in the epicardial arteries. Inflammation and high lipid levels play a role in pathological changes in atherosclerosis. Besides traditional risk factors, genetic factors such as single nucleotide polymorphism (SNPs) can be involved in disease process. In this study, we aimed to evaluate the effects of oxidized low density lipoprotein receptor 1 (OLR1) 3'UTR188C>T gene polymorphism, C-reactive protein (CRP), and lipid status in patients with coronary artery bypass grafting (CABG).

Materials and Methods: The study population consisted of 109 CAD patients who had undergone CABG, and 127 healthy controls. The OLR1 3'UTR188C>T polymorphism was genotyped using PCR-RFLP technique. Serum CRP, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels were measured with an automatic biochemistry analyzer.

Results: The distribution of the OLR1 3'UTR188C>T genotypes and alleles did not differ significantly between CAD patients with CABG and controls. Serum CRP levels were increased in patients compared to the control group (p<0.001), but HDL-C, and LDL-C levels were not different between two groups. Traditional risk factor such as cigarette smoking, alcohol use, family history, diabetes mellitus and hypertension were increased in patients compared to the control group (p<0.001, for each). The CRP levels were higher in patients with the TT, CT, and CC genotypes than in controls with the same genotypes (p<0.001, p<0.01, and p<0.05, respectively).

Conclusion: OLR1 3'UTR 188C>T polymorphism may not be involved in susceptibility to atherosclerosis. However, traditional risk factors in atherosclerosis such as smoking, alcohol consumption, family history, hypertension, diabetes mellitus, and circulating CRP levels were increased in our CABG population. The evaluation of OLR1 3'UTR188C>T and different OLR1 SNPs may be useful for their single and combined effects in atherosclerosis.

Keywords: Coronary artery bypass grafting, *OLR1* gene, 3'UTR188C>T polymorphism, CRP, lipid level

ÖZ

Amaç: Koroner arter hastalığı (KAH), epikardiyal arterlerde aterosklerotik plak birikimi ile karakterize patolojik bir süreçtir. Enflamasyon ve yüksek lipid düzeyleri aterosklerozdaki patolojik değişikliklerde rol oynar. Geleneksel risk faktörlerinin yanı sıra, tek nükleotid polimorfizmi (SNP'ler) gibi genetik faktörler de hastalık sürecine dahil olabilir. Bu çalışmada koroner arter bypass greft (KABG) uygulanan hastalarda okside düşük yoğunluklu lipoprotein reseptörü 1 (OLR1) 3'UTR188C>T gen polimorfizmi, C-reaktif protein (CRP) ve lipid durumunun etkilerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Çalışma popülasyonunu KABG geçirmiş 109 KAH hastası ve 127 sağlıklı kontrol oluşturdu. OLR1 3'UTR188C>T polimorfizmi, PCR-RFLP tekniği kullanılarak genotiplendi. Otomatik biyokimya analizörü ile serum CRP, yüksek yoğunluklu lipoprotein-kolesterol (HDL-K) ve düşük yoğunluklu lipoprotein-kolesterol (LDL-K) seviyeleri ölçüldü.

Bulgular: OLR1 3'UTR188C>T genotiplerinin ve alellerinin dağılımı, KABG'li KAH hastaları ve kontroller arasında anlamlı farklılık göstermedi. Kontrol grubu ile karşılaştırıldığında hastalarda serum CRP seviyeleri yüksekti (p<0,001), ancak HDL-K ve LDL-K seviyeleri iki grup arasında farklı değildi. Sigara kullanımı, alkol kullanımı, aile öyküsü, diabetes mellitus ve hipertansiyon gibi geleneksel risk faktörleri hastalarda kontrol grubuyla karşılaştırıldığında yüksekti (her biri için p<0,001). CRP seviyeleri, TT, CT ve CC genotiplerine sahip hastalarda aynı genotiplere sahip kontrollere göre daha yüksekti (sırasıyla p<0,001, p<0,01 ve p<0,05).

Sonuç: OLR1 3'UTR 188C>T polimorfizmi, ateroskleroz duyarlılığında yer almayabilir. Ancak sigara, alkol tüketimi, aile öyküsü, hipertansiyon, diabetes mellitus ve dolaşımdaki CRP seviyeleri gibi aterosklerozdaki geleneksel risk faktörleri CABG popülasyonumuzda artmıştır. OLR1 3'UTR188C>T ve farklı OLR1 SNP'lerin değerlendirilmesi, aterosklerozdaki tek ve birleşik etkileri açısından yararlı olabilir.

Anahtar Kelimeler: Koroner arter bypass greftleme, *OLR1* geni, 3'UTR188C>T polimorfizmi, CRP, lipid düzeyi

INTRODUCTION

Coronary artery bypass grafting (CABG) is a surgical procedure in which vessels are used as grafts to bypass coronary arteries that are partially or completely occluded by atherosclerotic plaque[1]. Atherosclerosis is a disease characterized by the accumulation of lipids, fibrous elements, and calcification in the arteries. This process starts with the activation of the endothelium, and then a series of events that occur with the activation of the inflammatory response lead to vasoconstriction and atheroma plaque formation[2].

Many studies have tried to explain the biological and genetic basis of atherosclerosis[3-6]. Although experimental studies are helping to unravel the pathophysiology of atherosclerosis, clinical gaps remain[5]. Various methods used to study the genetic factors involved in chronic diseases such as atherosclerosis have focused on the genetic basis in the development of the disease. However, in most chronic diseases, multiple genes acting under the influence of several environmental factors have been used to determine the development of diseases such as atherosclerosis. The identification of these genes essentially followed a candidate gene approach. Based on the understanding of the pathogenesis of atherosclerosis genes involved in lipid mobilization, for example, the association with inflammation and endothelial function, the presence of atheroma or cardiovascular events has been studied[7]. It has been reported that genes associated with the coronary artery disease (CAD) process can be divided into three categories: disease-causing genes, susceptibility genes, and disease-associated genes[8,9].

The human gene encoding the oxidized low density lipoprotein receptor 1 (OLR1) maps to chromosome 12p13.1-p12.3 and consists of 6 exons interrupted by 5 introns. The *OLR1* gene is a good candidate gene for cardiovascular disease because of its location in a chromosomal region often associated with cardiovascular disorders and the biochemical role of its product in lipid metabolism pathways[10,11]. Six non-coding single-nucleotide polymorphisms (SNPs) in the 3'-terminal portion of OLR1 have been reported to be involved in alternative splicing of exon 5 and expression of the LOXIN transcript variant lacking exon 5 of OLR1. These 6 noncoding polymorphisms, including in 3'UTR (188C>T), intron 5 (IVS5-27G>T and IVS5-70A>G) and intron 4 (IVS4-14A>G, IVS4+27G>C, IVS4-73C>T), are located in a haplotype block region and affect the risk of developing CAD by changing LOXIN expression[12,13]. Studies have reported that OLR1 may play an important role in the pathophysiology of atherosclerosis and thrombosis[13,14]. 188C>T, a polymorphism in the 3'-UTR of the *OLR1* gene, has been found to be associated with CAD[13,15], but there are also studies reporting that this polymorphism is not altered in vascular diseases[16-18]. It has also been reported that C-reactive protein (CRP), which has proinflammatory effects, may play a role in the pathogenesis of CAD by showing proatherogenic and prothrombotic effects on vascular cells[19]. Considering this information, we aimed to investigate the OLR1 188C>T polymorphism and serum CRP and lipid levels in patients who underwent CABG in the Turkish population.

MATERIALS AND METHODS

Subjects

This prospective case-control study included 109 patients (30 women, and 79 men) who had undergone the CABG surgery and 127 healthy controls (47 women, and 80 men). The median age of patients and controls was 58 and 50 years, respectively. Healthy persons without any history of cardiovascular events and without any symptoms of CAD were selected for the control group. The exclusion criteria included cancer, autoimmune,

BAHTİYAR et al. OLR1 3'UTR188C>T SNP in CABG

Nam Kem Med J 2023;11(4):328-333

kidney, or hepatic disease. All study subjects were of Turkish origin and provided signed informed consent before the sample and data collection. This study was approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Ethics Committee (no: 162, date: 20.05.2022). All procedures followed the ethical standards of the responsible committee on human experimentation (institutional and national) and/or the Helsinki Declaration of 1964 and later versions.

Blood Collection

Blood samples were collected in two tubes containing ethylenediaminetetraacetic acid (EDTA) after an overnight fast. One of the blood tubes containing EDTA was used for genotype analysis and stored as frozen at -80 °C until analysis. After centrifugation of the other tube at 400 x g for 10 minutes at 4 °C, plasma samples were separated into eppendorf tubes and frozen at -20°C until analysis.

Biochemical Measurements

The plasma concentrations of CRP were measured by nephelometric immunoassay using Dade Behring kits (BN II System Analyzer Dade Behring, Germany). In addition, plasma high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) levels were measured by automated colorimetric methods with commercially available kits (Cobas 8000, Roche Diagnostics GmbH, Mannheim, Germany).

DNA Isolation and Genotyping

Blood was drawn into EDTA-containing tubes for DNA isolation. The Roche DNA purification kit (Roche Diagnostics GmbH, Mannheim, Germany) was used to extract DNA from peripheral blood leukocytes in accordance with the manufacturer's instructions. Isolated DNA samples were kept frozen at -80 °C. The ORL1 188C>T polymorphism was genotyped using standard polymerase chain reaction (PCR) procedures and restriction enzyme digestion. There were two primer pairs used: F: 5'-TGTCAACATTTTTGATTCTAGCTA-3', and R: 5'-GTTCTCCATGTTCTGTCTTTCA-3'. The PCR mixture (20 μL total volume) consisted of 25 ng of gDNA, 10 pmol/μL of each primer, and 2 x PCR master mix solution (intron Biotechnology, Korea) which contents of 2.5 mM of each dNTPs, 2.5 U of i-Taq™ DNA polymerase, 1 x of PCR reaction buffer and 1 x of gel loading buffer. A Techne Thermal Cycler (Applied Biosystems Gene Amp PCR System 9700, Singapore) was used to perform the PCR. PCR conditions were as follows: initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 20 sec, annealing at 51 °C for 10 sec, and elongation at 72 °C for 30 sec. The final amplicon extension was performed at 72 °C for 5 min. PCR amplicons were separated by electrophoresis in 2% agarose gel and visualized by ethidium bromide staining. The

188C>T PCR product was 207 bp. The amplified PCR products were directly digested by the KpnI (Invitrogen Life Tecnologies Corporation, Carlsbad, CA, USA) restriction enzyme (10 units/μL) at 37 °C for overnight. After KpnI restriction, two fragments were obtained: 184 and 23 bp for the C allele and a single fragment of 207 bp for the T allele. The digested DNAs were separated on 3% agarose gel in 1 x Tris borate EDTA buffer followed by staining with ethidium bromide solution. The 188C>T genotypes were detected using a Polaroid camera and viewed under ultraviolet light.

Statistical Analysis

The Statistical Package for the Social Sciences statistic 21.0 program was used for the analyses of the patients and control values. Hardy-Weinberg equilibrium was tested by chi-square analysis. Genotype and allele frequencies were compared between cases and controls by chi-square analysis. Odds ratio and respective 95% confidence intervals evaluated the effects of any difference between the allelic and genotype distributions. The unpaired Student's t-test (normally distributed variables) or Mann-Whitney U test (not normally distributed variables) were used for the comparison of other parameters. A value of p<0.05 was considered the minimum statistical significance.

RESULTS

The characteristics of patients undergoing CABG and the control groups were summarized in Table 1. There was no significant difference in terms of age, gender, body mass index (BMI), HDL-C, LDL-C, and systolic blood pressure between the patient and control groups (p>0.05). Cigarette smoking, alcohol use, family history, diabetes mellitus, CRP, hypertension, and diastolic blood pressure were increased in patients compared to the control group (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, and p<0.05, respectively).

We investigated the OLR1 3'-UTR 188C>T gene polymorphism, the frequencies of the genotypes in CABG patients and control groups were shown in Table 2. The distribution of TT, CT, and CC genotypes was 53.21%, 26.60%, and 20.18%, respectively, in the cases, and 46.45% and 33.85%, and 19.69%, respectively, in the controls. No statistically significant difference was observed in the gene polymorphism between the two groups (p>0.05). Also, the distribution of T and C alleles was 66.51% and 33.48%, respectively, in the cases and 63.38 % and 36.61 %, respectively, in the controls (p>0.05) (Table 2).

To assess whether OLR1 3'-UTR 188C>T gene polymorphism had any effect on BMI, CRP, HDL-C, LDL-C, and blood pressure levels, we compared these parameters among the genotype groups in patients and controls (Table 3). The CRP levels were higher in patients with the TT, CT, and CC genotypes than in controls with the same genotypes (p<0.001, p<0.01 and p<0.05,

Nam Kem Med J 2023;11(4):328-333

BAHTİYAR et al. OLR1 3'UTR188C>T SNP in CABG

respectively). Also, the systolic blood pressure levels were higher in patients with the CC genotype than in controls with the same genotype (p<0.05). The diastolic blood pressure levels

Table 1. Clinical characteristics of patients undergoing coronary artery bypass grafting and healthy controls

Parameters	Patients (n=109)	Controls (n=127)	p
Age, median (min–max) (year)	58 (32–76)	50 (35–69)	NS
Gender (M/F)	79/30	80/47	NS
BMI (kg/m²)	27.66±5.15	27.01±4.05	NS
Cigarette smoking, n (%)	85 (77.98)	19 (14.96)	0.001
Alcohol use, n (%)	89 (81.65)	23 (18.11)	0.001
Family history, n (%)	75 (68.80)	59 (46.45)	0.001
Diabetes mellitus, n (%)	41 (37.61)	1 (0.79)	0.001
Hypertension, n (%)	68 (62.39)	1 (0.79)	0.001
LDL–C (mg/dL)	127.46±37.54	123.86±34.04	NS
HDL–C (mg/dL)	42.79±13.66	46.79±15.48	NS
CRP (mg/dL)	13.30±19.02	3.49±2.96	0.001
Systolic pressure (mmHg)	134.13±14.97	130.53±5.76	NS
Diastolic pressure (mmHg)	78.11±10.75	76.61±11.29	0.030

Data were presented as mean±SD, and n (%).

SD: Standard deviation, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol, CRP: C-reactive protein, NS: Not significant, min-max: Minimum-maximum, BMI: Body mass index, M/F: Male/Female

Table 2. Distribution of genotypes and allele frequencies of OLR1 3'UTR188C/T polymorphism in patients undergoing coronary artery bypass grafting and control groups

Gene	Patients n (%)	Controls n (%)	p	OR (CI 95%)
LOX-1 3'UTR188C/T polymorphism	109	127		
Genotypes				
TT	58 (53.21)	59 (46.45)		1
CT	29 (26.60)	43 (33.85)	0.276	1.45 (0.74 -2.82)
CC	22 (20.18)	25 (19.69)	0.156	0.523 (0.21-21.28)
Alleles				
T	145 (66.51)	161 (63.38)		1
C	73 (33.48)	93 (36.61)	0.357	1.20 (0.82-1.75)

Genotypes and allele frequencies were shown as n (%).

OR: Odds ratio, CI: Confidence interval

were higher in patients with the TT genotype than in controls with the same genotype (p<0.05). No significant difference was detected in the comparison of the same genotypes of other parameters between the patient and control groups.

DISCUSSION

It has been reported that the human gene encoding the lectin-like oxidized LDL receptor, also called OLR1, is a good candidate for cardiovascular disease and is located in a chromosomal region associated with cardiovascular disorders[10]. Also, SNPs in the *OLR1* gene have been reported to be associated with the risk of developing CAD[12]. OLR1 3' UTR 188C>T polymorphism can affect LOX-1 expression by altering its regulator binding site and thus modifying protein homeostasis[20]. Therefore, in our study, OLR1 3' UTR 188C>T polymorphism was investigated in patients who underwent CABG, and CRP, lipid, and blood pressure levels were evaluated in the same genotype distributions.

In the literature, studies investigating the OLR1 188C>T polymorphism in vascular diseases have reported conflicting findings[13,15,16,18,21]. Mango et al.[13] examined the 3' UTR 188C>T polymorphism in patients with acute myocardial infarction in the Italian population and they reported that there was a significant change between the patient and control groups, and T allele carriage increased the risk of cardiovascular events. Similarly, Guo et al.[21] observed that the frequency of the T allele in 188C>T polymorphism was significantly higher in patients with atherosclerotic cerebral infarction compared to healthy controls in the Chinese population. Novelli et al.[15] confirmed the association of the 3'UTR 188C>T SNP with myocardial infarction (p<0.003). Cheng et al.[22] studied a meta-analysis including 8 studies and reported that 3'UTR 188C>T increased CAD sensitivity. According to a meta-analysis study including findings of 11 researches suggested that variant genotype in the 188C>T polymorphism was associated with the increased risk of CAD[23].

However, there are also studies reporting any changes in OLR1 3'UTR 188C>T polymorphism in vascular diseases. Liu et al.[24] investigated the association between *LOX-1* gene polymorphism, 3'UTR 188C>T, and cerebral infarction in the northern Chinese Han population. However, they did not find an association between 3'UTR 188C>T and cerebral infarction risk. Sentinelli et al.[16] reported that there was no significant difference in the allele frequencies of the *OLR1* gene between CAD and controls in the Italian population, and that the 3' UTR 188C>T polymorphism was unlikely to play a role in the pathogenesis of CAD in the studied population. Similarly, Trabetti et al.[17] did not find a statistically significant difference in the allele or genotype distribution of 3'UTR SNPs in CAD in the Italian population when compared to subjects without CAD. Kurnaz et al.[18] reported that no correlation was observed between the 3'UTR 188C>T SNP and the presence of CAD in

BAHTİYAR et al. OLR1 3'UTR188C>T SNP in CABG

Nam Kem Med J 2023;11(4):328-333

Table 3. The levels of BMI, HDL–C, LDL–C, CRP, systolic blood pressure and diastolic blood pressure in patients undergoing coronary artery bypass grafting and controls according to OLR1 3′UTR188C/T polymorphism

Parameters	Patient group OLR1 3′UTR188C/T polymorphism			Control group OLR1 3′UTR188C/T polymorphism		
	TT	CT	CC	TT	CT	CC
BMI (kg/m²)	27.93±4.64	27.21±3.02	27.58±7.99	27.23±4.27	29.09±3.69	26.89±3.93
HDL-C (mg/dL)	41.47±12.97	46.07±16.31	42.62±11.97	44.55±8.78	52.80±25.28	41.50±3.53
LDL-C (mg/dL)	123.71±39.31	126.30±36.34	145.69±32.17	122.87±27.53	127.10±49.74	119.00±16.67
CRP (mg/dL)	**12.55±15.82[a]*****	**14.86±23.52[b]****	**13.01±20.16[c]***	3.52±2.39	3.44±3.83	3.57±2.32
Systolic blood pressure (mmHg)	134.68±14.86	133.00±17.07	**133.69±10.18[c]***	133.07±6.71	128.42±4.49	121.14±5.52
Diastolic blood pressure (mmHg)	**79.25±10.66[a]***	77.11±12.07	76.86±10.07	76.89±11.25	79.58±10.64	74.08±7.73

Data were presented as mean±SD. Bold values indicate statistical significance.

[a]TT genotypes in patient group vs. TT genotypes in controls, [b]CT genotypes in patient group vs. CT genotypes in controls, [c]CC genotypes in patient group vs. CC genotypes in controls, *p<0.05, **p<0.01, and ***p<0.001, (Student's t-test or Mann-Whitney U test).

BMI: Body mass index, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol, CRP: C-reactive protein

the Turkish population, and there was no difference in the comparison analysis between genotype groups and the mean values of cardiovascular risk factors. Tripathi et al.[25] reported that the 3′UTR 188C>T polymorphism in the North Indian population did not show a significant difference between CAD patients and healthy controls.

When the findings of our study were evaluated, it was observed that there was no significant change in the OLR1 3′UTR 188C>T genotype and allele distributions between CABG patients and healthy volunteers. The conflicting findings regarding the 3′UTR 188C>T polymorphism reported in the literature may be due to the limited size of the study populations, ethnic diversity of polymorphisms, and complex environmental factors.

In addition to genetic factors in the development of CAD, modifiable factors such as smoking, physical inactivity, overweight, uncontrolled stress, and unhealthy diet are also effective as well as non-modifiable factors such as advanced age, male gender, and race. Epidemiological studies report that risk factors such as high cholesterol level, hypertension, and diabetes mellitus also play a role in the development of atherosclerosis[26].

It is known that atherogenesis is promoted by high plasma and tissue levels of oxidized low-density lipoproteins (OxLDLs). Ox-LDLs increase the expression of proinflammatory genes, leading to monocyte recruitment to the vessel wall, and dysfunction of vascular endothelial cells. Ox-LDLs transform macrophages into foam cells that form atherosclerotic plaques[27].

Stancel et al.[19] suggested that CRP and LOX-1 constituted a cyclic mechanism with ox-LDL in atherogenesis. CRP is an acute phase protein primarily synthesized by hepatocytes. It has been reported that CRP may play a direct role in promoting the inflammatory component of atherosclerosis. In addition, an upregulation of CRP levels was detected in plaque tissues[2]. CRP is a ligand for OLR1, increases vascular permeability, impairs

endothelium-dependent vasodilator function, and plays a role in monocyte-endothelial cell adhesion[19,27].

When the findings of our study were evaluated, it was noted that smoking, alcohol consumption, family history, hypertension, diabetes mellitus, and CRP levels increased in the CABG group compared to the control (Table 1). These data are compatible with the findings on risk factors affecting the development of atherogenesis. We also investigated the effect of the same genotype on BMI, HDL-C, LDL-C, CRP, systolic blood pressure, and diastolic blood pressure in the patient and control groups. CRP values were increased in all 3 genotypes compared to the control group, and the systolic blood pressure value in the CC genotype and the diastolic blood pressure value in the TT genotype were lower in the patient group compared to the control group (Table 3).

Unlike many other inflammatory mediators, CRP is not subject to diurnal fluctuation or biological variance. Therefore, CRP concentration seems to be proportional to the severity of the disease. However, the main limitation of CRP is its elevation in systemic inflammation, which may limit its use as a prognostic marker in postoperative patients[28]. It is thought that the increase in CRP values in our patient group may be related to the CABG operation.

Study Limitations

It should be noted that the limitations of our study are its small sample size and its being a single-center study, which may have influenced the statistical power of our analysis.

CONCLUSION

In conclusion, the results of the study indicate that, for our Turkish sample, OLR1 3′UTR 188C>T polymorphism may not be involved in susceptibility to atherosclerosis but traditional risk factors in atherosclerosis such as smoking, alcohol consumption,

Nam Kem Med J 2023;11(4):328-333

BAHTİYAR et al. OLR1 3'UTR188C>T SNP in CABG

family history, hypertension, diabetes mellitus, and circulating CRP levels were increased in our CABG population. The OLR1 3'UTR188C>T and different OLR1 SNPs may need to be evaluated with regard to their single and combined effects at risk of atherosclerosis.

Ethics

Ethics Committee Approval: This study was approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Ethics Committee (no: 162, date: 20.05.2022).

Informed Consent: All study subjects provided signed informed consent before the sample and data collection.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.H., Concept: O.B., C.A., Ç.T., R.H., B.Ar., G.K-S., Design: N.B., T.Ö., F.B.C., B.A., İ.O., G.K-S., Data Collection or Processing: N.B., O.B., Y.H., T.Ö., F.B.C., B.A., İ.O., C.A., Ç.T., R.H., B.Ar., A.R.K., Analysis or Interpretation: N.B., Y.H., T.Ö., F.B.C., A.R.K., İ.O., Literature Search: N.B., O.B., Y.H., T.Ö., F.B.C., B.A., İ.O., C.A., Ç.T., R.H., A.R.K., Writing: N.B., O.B., F.B.C., B.A., İ.O., C.A., Ç.T., R.H., A.R.K., G.K-S.

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The Effect of Changes in Thyroid Hormone Levels on Learning and Memory in Middle Aged Rats

Orta Yaşlı Sıçanlarda Tiroid Hormon Seviyesindeki Değişikliklerin Öğrenme ve Bellek Üzerine Etkisi

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ABSTRACT

Aim: The increase in the frequency of thyroid diseases in old age makes it necessary to reveal the relationship between aging and irregularities in thyroid hormone levels. In the present study, the effects of experimental hypothyroidism and thyrotoxicosis induced in middle aged rats on spatial learning and memory performance were investigated.

Materials and Methods: In this study, 45 Wistar albino 12-month-old rats weighing 400-450 g were used. The rats were divided into three groups according to thyroid hormone levels; euthyroid (n=16), hyperthyroid (n=16) and hypothyroid (n=13). Thyrotoxicosis was induced by intraperitoneal (i.p.) administration of L-thyroxine at a dose of 0.2 mg/kg/day. Hypothyroid state was induced by daily administration of 0.05% 6-n-propyl-thiouracil (PTU) in the drinking water of rats. Spatial memory and learning performance of rats were evaluated by Morris water maze test. Thyroid hormone levels were determined with a commercial ELISA kit.

Results: One-way ANOVA test revealed that the mean distance to the platform (p<0.05), escape time (p<0.01), swimming speed (p<0.001) and distance traveled (p<0.05) values of the rats showed significant differences between the groups. According to these results; learning performance of the hypothyroidism group decreased compared to the control group, while learning and memory performance of the thyrotoxicosis group did not differ from the control group.

Conclusion: Hypothyroidism during aging has a negative effect on learning and memory performance compared to thyrotoxicosis. Revealing the changes in thyroid hormone metabolism in middle aged and determining the daily requirement will provide a different perspective on aging-related dementia-type diseases.

Keywords: Aging, hypothyroidism, learning and memory, thyrotoxicosis

ÖZ

Amaç: Yaşlılık döneminde tiroid hastalıklarının sıklığındaki artış, yaşlanma ve tiroid hormon (TH) seviyesindeki düzensizliklerin ilişkisinin ortaya konulmasını gerekli kılmaktadır. Bu çalışmada, orta yaşlı sıçanlarda indüklenen deneysel hipotiroidi ve tirotoksikozun uzamsal öğrenme ve bellek performansı üzerine etkisi araştırılmıştır.

Gereç ve Yöntem: Bu çalışmada 45 adet Wistar albino cinsi 400-450 gr ağırlığında 12 aylık sıçanlar kullanıldı. Sıçanlar TH seviyesi dikkate alınarak üç gruba ayrıldı; ötiroidi (n=16), tirotoksikoz (n=16) ve hipotiroidi (n=13). Tirotoksikoz durumu, her gün 0,2 mg/kg/gün dozda L-tiroksin intraperitoneal (i.p.) olarak uygulaması ile oluşturuldu. Hipotiroidi durumu günlük %0,05'lik 6-n-propil-tiyourasilin (PTU) sıçanların içme suyuna karıştırılması ile indüklendi. Sıçanların uzaysal hafıza ve öğrenme performansları Morris su tankı testi ile değerlendirildi. TH testleri ticari ELISA kiti ile çalışıldı.

Bulgular: Tek yönlü ANOVA testi, sıçanların platforma olan ortalama uzaklık (p<0,05), kaçış süresi (p<0,01), yüzme hızı (p<0,001) ve kat edilen mesafe (p<0,05) değerlerinin gruplar arasında anlamlı farklılık gösterdiğini ortaya koydu. Bu sonuçlara göre; hipotiroidi grubu öğrenme performansı kontrol grubuna göre azalırken tirotoksikoz grubunun öğrenme ve bellek performansı kontrol grubundan farklılık göstermedi.

Sonuç: Yaşlanma sürecinde ortaya çıkan hipotiroidi durumu tirotoksikoz durumuna kıyasla öğrenme ve bellek performansı üzerine olumsuz etki göstermektedir. Orta yaşlılık döneminde TH metabolizmasındaki değişimin açığa çıkarılması ve günlük ihtiyacın belirlenmesi, yaşlanmaya bağlı görülen demans tipi hastalıklara farklı bir bakış açısı sağlayacaktır.

Anahtar Kelimeler: Yaşlanma, hipotiroidi, öğrenme ve bellek, tirotoksikoz

INTRODUCTION

Biologically, aging is a term used to express the physiological changes that occur in the organism with the advancement of chronological age. Developments in the field of health and social welfare have resulted in an increase in life expectancy and therefore an increase in the proportion of the elderly population in the population. The ratio of the elderly population to the total population is increasing rapidly in our country, as in the rest of the world. An increase of 201% in the elderly population in Turkey between 2008 and 2040 has been reported[1]. While this phenomenon highlights the importance of healthy aging, it also creates the need to focus on the diagnosis and treatment of dementia-type diseases, the incidence of which increases in old age. Many studies on the aging brain have revealed pathological changes such as neuron loss, synaptic dystrophy and decrease in brain volume[2,3].

In various animal species, the aging process is associated with learning and memory disorders that correlate with the decrease in synaptic transmission and plasticity occurring in different regions of the brain[4]. Research on memory has revealed that the hippocampus is the structure that plays a fundamental role in the formation, storage and recall of memories[5]. Revealing the complex circuits between the hippocampus and the entorhinal cortex and other structures, damage to memory-related functions in hippocampal damage, and histological demonstration of place cells have provided further evidence on the role of the hippocampus in memory[6,7]. It is thought that the weakening of cognitive functions in older people will be due to the decrease in synaptic plasticity, which is defined as the ability of the individual to form and eliminate neural connections throughout his life[8]. Numerous data have revealed that granule cells in the hippocampus decrease radically with aging[9]. At the same time, the decrease in these cells with neurogenesis ability with aging emerges as one of the factors suggested for the deterioration of the cognitive process during aging[10].

Changes in thyroid hormone (TH) levels have dramatic effects on cortical functions and neuronal functions both in the intra-uterine and postnatal periods. While studies on the effects of THs on the central nervous system in the early period are widely available in the literature, studies on the irregularities in TH levels seen in adulthood are limited. In line with the roles of THs in growth and development, TH levels are closely monitored during pregnancy or in newborns. However, the frequency of thyroid diseases that begin in childhood and adulthood is equally high. The prevalence of hypothyroidism in children under the age of 10 in Turkey is reported to be approximately 21%[11]. In the general population, subclinical hypothyroidism is seen at a rate of 14-18%[12]. The prevalence of hypothyroidism in people over the age of 74 was found to

be 21% in women and 16% in men[13]. Therefore, considering the increase in the frequency of dementia-type diseases characterized by a decrease in cognitive performance and functional weakening with age, there is a need to reveal the relationship between TH and cognitive functions in old age. It has been reported that hypothyroidism and hyperthyroidism in adulthood increase the deterioration in electrophysiological and behavioral indicators that evaluate synaptic plasticity[14]. In this study, the effects of experimental hypothyroidism and thyrotoxicosis induced in old rats on spatial learning and memory performance were investigated. The increase in the incidence and prevalence of thyroid diseases in old age makes it necessary to reveal the relationship between old age and TH irregularities.

MATERIALS AND METHODS

Experimental Animals and Grouping

All experiments were performed on Wistar Albino rats. Forty-five 12-month-old rats weighing 400-450 g were obtained from Erciyes University Hakan Çetinsaya Experimental and Clinical Research Center and were housed in accordance with the National Health Institutes guide for humane animal care and use. The rats were subjected to a 14-hour light-10-hour dark cycle and were fed without water or feed restrictions. Ethics committee approval was received with the decision of Erciyes University Animal Experiments Ethics Committee dated 09.12.2015 and numbered 15/150. Rats; they were divided into three groups as thyrotoxicosis group (n=16), hypothyroid group (n=13), euthyroid (control) group (n=16). Thyrotoxicosis group: L-thyroxine was injected intraperitoneally (i.p.) at 0.2 mg/kg daily for 60 days to induce thyrotoxicosis in 16 12-month-old rats[15]. This induction method and drug dose was preferred because chronic L-thyroxine administration caused a high increase in serum free T3 and free T4 levels[16,17]. Hypothyroidism group: To create hypothyroidism in 13 12-month-old rats, 0.1 ml of 0.5% 6-n-propyl thiouracil (PTU) was added to their drinking water every day for 60 days.

Old age for Wistar albino rats; early old age (equivalent to 25-40 years in humans, 150-300 days for rats), middle old age (equivalent to 40-65 years in humans, 300-600 days for rats), old old age (equivalent to 65-75 years in humans, 600-600 days for rats) and late senility (its equivalent in humans is over 75 years of age, for rats it is 730 days and above)[18].

Measurement of Thyroid Hormone Levels

Rats were anesthetized by i.p. administration of a mixture of ketamine (100 mg/kg) and xylazine (2.5 mg/kg) and blood was collected into heparinized tubes. The obtained plasma was stored at -80 °C for measurement of T4 levels. Measurements were made using the ELISA method with the Multiskan™ FC

BABUR et al. The Effect of Dysthyroidism on Learning and Memory

Nam Kem Med J 2023;11(4):334-339

Microplate Photometer device at 450 nm, in accordance with the manufacturer's instructions.

Morris Water Tank Test Application

Morris Water Tank (MST) experiments were carried out with the video monitoring and recording system of the water tank (130 cm in diameter and 45 cm in height) located in Erciyes University Physiology Behavior Laboratory (n=16). An escape platform (diameter: 10 cm, height 22 cm) on which the rats could stand was placed in one of the four quadrants of the water tank area, and it was filled with water to a level of 1 cm above this platform. The water was dyed with a blue, non-toxic dye to prevent the rats from seeing the location of the hidden platform. The location of the platform was kept constant during all learning trials and care was taken to keep the water temperature between 20-22 °C. During each learning trial, the rat was placed in the water so that it could see the large clues around it from a quadrant other than the quadrant where the platform was located. The behavior to be learned in these trials is for the rat to find the platform and get out of the water by climbing on it within 2 minutes of swimming. Rats that could not find the platform during this period were helped to find the platform and were left on the platform for 15 seconds to observe the clues.

Learning trials were performed on each rat four times a day (half an hour apart) for 4 days. Twenty-four hours after the last learning trial (16th trial), the platform in the tank was removed and the same test was applied without the platform (Probe trial). While performing statistical analysis, the change in learning performance according to days and the change in learning performance according to trials were recorded, and it was investigated whether learning took place and whether there was a significant difference between the groups. In each trial, the behavior of the rats was recorded by the Noldus Ethovision video-monitoring and analysis system, and the time it took the rats to find the platform, swimming distance, time spent in the platform area, swimming speed and time in each quadrant area were recorded. Trials were carried out every day and at the same time of the day (10:00-14:00).

Statistical Analysis

Statistical analysis of behavioral experiments was performed with Statistical Package for the Social Sciences version 16 package program. Learning performances changing throughout the days were analyzed via factorial ANOVA with repeated measurements. Normality and sphericity evaluations of the data were made with Shapiro-Wilk and Mauchly tests. Since the data had equal variance, comparisons between groups were made with one-way ANOVA test. The probability level for statistical significance was accepted as $p<0.05$. Values are expressed as mean±standard error.

RESULTS

Thyroid Hormone Values

At the end of the behavioral experiments, after the rats were anesthetized with the ketamine+xylazine combination, plasma free T3 (fT3) and free T4 (fT4) levels were measured in the blood taken intracardially. Plasma fT3 (control: 11.48±5.09 pg/mL, hypothyroid: 6.10±1.16 pg/mL, thyrotoxicosis: 29.09±2.92 pg/mL) and fT4 (control: 34.44±6.55 ng/dL, hypothyroid: 23.68±3.67 ng/dL, thyrotoxicosis: 51.09±2.92 ng/dL) values were measured lower in the hypothyroid group than in the control group and the thyrotoxicosis group ($p<0.05$, n=6).

Morris Water Tank Test Results

The distance traveled decreased significantly throughout Day (F=41.076, $p<0.001$), Trial (F=48.26, $p<0.001$) and Day*Trial (F=2.491, p=0.009), showing that learning took place (Figure 1A). TH level status variable has shown a statistically significant effect on Day*Group (F=2.512, p=0.025), Trial*Group (F=2.673, p=0.18) and Day*Trial*Group (F=2.654, $p<0.001$). In the 1st and 2nd trial of the 2nd day, the 3rd trial of the 3rd day, and the 1st and 2nd trial of the 4th day, the hypothyroidism group covered more distance than the control and thyrotoxicosis groups, creating a statistically significant difference.

Escape time values are given in Figure 1B. The significance of Day (F=48.313, $p<0.001$), Trial (F=14.232, $p<0.001$) and Day*Trial (F=4.603, $p<0.001$) during the escape period showed that learning occurred in all groups. While the TH level variable did not show a statistically significant effect on Day*Group and Trial*Group, the Day*Trial*Group effect (F=2.266, p=0.002) was found to be significant. In the 1st trial and 4th trial of the 1st day, in the 1st trial and 2nd trial of the 2nd day, in the 2nd trial and 4th trial of the 4th day, the hypothyroidism group found the hidden platform in a longer time than the control and thyrotoxicosis groups, creating a statistically significant difference from them.

When the average distance values of euthyroid and experimental group rats to the learning platform were examined, a significant Day (F=42.913, $p<0.001$) and Trial (F=14.543, $p<0.001$) effects were observed (Figure 1C). Day*Group (F=2.315, $p<0.05$) and Day*Group*Trial (F=1.740, $p<0.05$) interactions were found to be statistically significant. The hypothyroid group performed worse than the control group in the 1st and 2nd trials of the 2nd day, the 4th trial of the 3rd day, and the 1st and 2nd trials of the 4th day.

Swimming speed values of the groups are given in Figure 1D. Day*Group (F=4.749, $p<0.001$), Trial*Group (F=6.970, $p<0.001$) and Day*Trial*Group (F=6.345, $p<0.001$) interactions showed a statistically significant difference. The groups showed different swimming speed performances on different days and trials.

The hypothyroid group had a lower swimming speed than the control group in the 1st, 3rd and 4th trial of the 1st day, the 3rd trial of the 2nd day, the 1st trial of the 3rd day, and the 2nd and 3rd trial of the 4th day. The thyrotoxicosis group had a higher swimming speed than the control group in all trials of the 3rd day, and in the 2nd and 4th trials of the 4th day.

The percentage values of time spent in the target area in the last trial in which reference memory was evaluated are shown in Figure 2. The time values spent in the target quadrant showed statistically significant differences between the groups. The percentage values of time spent in the target quadrant of the hypothyroidism group created a statistically significant difference compared to the control group. The percentage values of time spent in the target quadrant in the hypothyroid group decreased (p=0.002). This deterioration was not observed in the thyrotoxicosis group (p=0.328).

DISCUSSION

Spatial navigation requires memorizing places and routes so that the organism does not get lost by encoding environmental cues. Many living creatures need to leave their habitats and return safely to their homes in order to find food and water, to mate, and for other needs[19]. MST, one of the tests developed in this context, was developed to evaluate spatial learning and memory in rodents. MST is one of the most frequently used measures of spatial memory formation and retention related to hippocampal functions[20]. This test is based on the fact that rats quickly learn the location of the escape platform with their innate swimming ability and fear instinct. In this study, we evaluated learning performance based on escape time, average distance to the platform, distance traveled to find the platform, and time spent in the target quadrant in the probe trial. Swimming speed averages were also included in the evaluation as a factor affecting other parameters. In our study, the distance traveled by the middle-old euthyroid, middle-old hypothyroid and middle-old thyrotoxicosis groups to find the platform in the tank differed between groups. The distance traveled by hypothyroid rats to find the platform is greater than euthyroid animals. These results show the negative effect of hypothyroidism on spatial learning performance. When
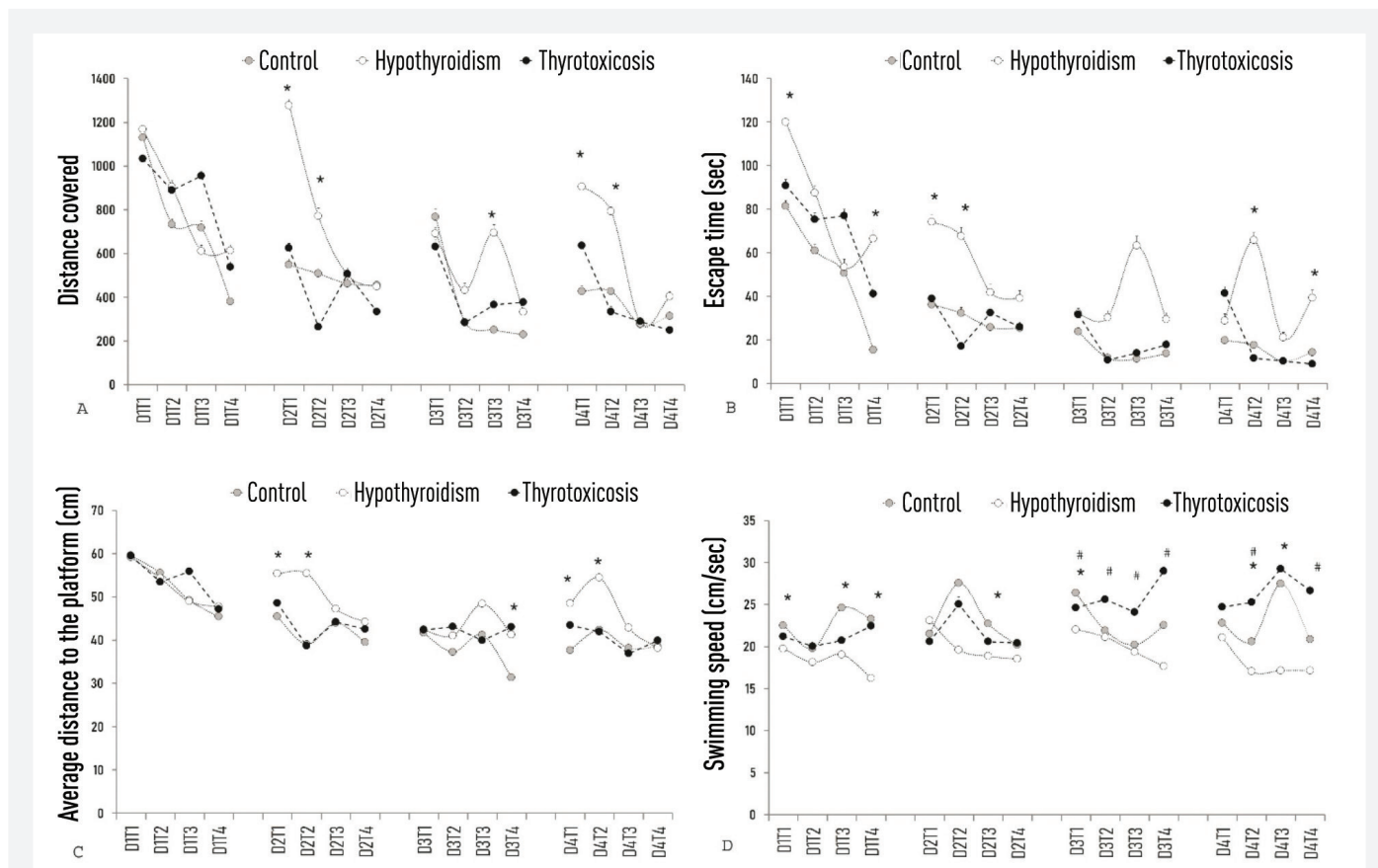
Figure 1. Change in distance covered (A), escape time (B), average distance to the platform (C) and swimming speed (D) during the learning period for the groups. Values are given as mean±standard error (D1: 1st day, T1: 1st trial)

*: Indicates a significant difference between the control and hypothyroid groups. #: Indicates a significant difference between the control and thyrotoxicosis groups

BABUR et al. The Effect of Dysthyroidism on Learning and Memory

Nam Kem Med J 2023;11(4):334-339

the effect of thyrotoxicosis on learning was examined, it was observed that it did not differ from the control group. Analysis of escape time values revealed similar results as the distance traveled parameter. While learning performance was impaired in the hypothyroidism group, learning performance was not affected in the thyrotoxicosis group.

Swimming speed evaluation showed that the groups had different swimming speeds on different days and trials. The hypothyroid group exhibited lower swimming speed values than the control group. This finding is consistent with Hosseini et al.'s[21] finding of low swimming speeds observed in rats with methimazole-induced hypothyroidism. These results reflect the necessity of TH for skeletal muscle. The fact that the enzymes that regulate energy production and glycogen accumulation, especially in type 1 skeletal muscle fibers, are affected by hypothyroidism seems to be the underlying reason for these results[22]. Thyrotoxicosis had the opposite effect, causing a higher rate than the control group. Hyperthyroidism above physiological doses and for a long time causes proteolysis in skeletal muscle, causing muscle weakness[23]. However, considering the duration of the experimental groups and the doses in this study, the high swimming speed values may be explained by the fact that the experiment was carried out during the period when catabolism in the muscles was not yet dominant and the positive effects of THs on skeletal muscle contraction were observed[24].

Average distance to the platform values provide more reliable information as it is a parameter that is not affected by

Figure 2. The ratio of the time spent by the groups in the quadrant where the platform was located in the last day's trial where memory was tested, to the total time (values are given as mean±standard error, significance level p<0.05 indicates statistical significance)

*: Indicates a significant difference compared to the control group

swimming speed, unlike the distance traveled and escape time. In this study, rats in the hypothyroid group swam farther from the platform than the other groups. At the same time, probe trials used to evaluate memory performance showed that the percentage values of time spent in the target quadrant of hypothyroid rats were lower than the control group. These results show that in addition to the impairment in learning performance in hypothyroidism, there is also impairment in memory consolidation processes. In the literature, the effect of hypothyroidism on learning has been mostly investigated on young rats, and studies on middle-aged hypothyroidism are limited. There are many studies in the literature showing that learning and memory performance is impaired in young hypothyroid rats[25,26].

Epidemiological studies reveal a striking increase in the incidence and prevalence of thyroid diseases in old age[27]. This relationship between the aging process and thyroid diseases requires further investigation of the hypothyroidism situation in the elderly. In terms of thyrotoxicosis, no effect of thyrotoxicosis on learning and memory was observed in middle-aged rats in this study. In addition to the studies in the literature showing that induced thyrotoxicosis in young rats causes learning impairment[15], there are also studies suggesting that it causes an increase in learning performance in mice administered postnatal L-thyroxine[28]. These differences may result from differences in the induction of thyrotoxicosis, the type of experimental animals selected, and the procedures for behavioral testing.

Study Limitations

In this study, hypothyroidism and thyrotoxicosis status were confirmed using the ELISA method at plasma free T3 and T4 levels. Plasma thyroid-stimulating hormone levels could not be measured. Another limitation of the study is that protein measurements were not made to show the molecular mechanisms of impaired learning and memory performance in hypothyroidism.

CONCLUSION

During the aging process, cognitive functions are more sensitive to the decrease in THs than to the increase in TH levels. Hypothyroidism in middle age has negative effects on learning and memory performance. It should be taken into consideration that diseases affecting TH metabolism may be involved in the etiology of dementia-type diseases seen in middle and late old age and should be included among the diagnostic tests. Conducting detailed studies at the molecular level about the effects of dysthyroidism on the central nervous system during the aging process will provide information about the functions of THs in the aging brain. In addition, the findings may make a significant contribution to determining

the factors that play a role in the development of Alzheimer's disease or other dementia-type diseases.

Ethics

Ethics Committee Approval: Ethics committee approval was received with the decision of Erciyes University Animal Experiments Ethics Committee dated 09.12.2015 and numbered 15/150.

Informed Consent: Animal experiment.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.K.T., B.T., Concept: R.K.T., N.D., Design: R.K.T., N.D., Data Collection or Processing: N.D., B.T., C.S., Analysis or Interpretation: E.B., B.T., C.S., Literature Search: E.B., C.S., Writing: E.B.

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Retrospective Evaluation of Stool *Helicobacter pylori* Antigen Test, Endoscopy and Histopathological Findings in Elderly Patients with Dyspepsia

Dispepsiyle Başvuran Yaşlı Hastalarda Dışkıda *Helicobacter pylori* Antijen Testi, Endoskopi ve Histopatolojik Bulguların Retrospektif Olarak Değerlendirilmesi

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ABSTRACT

Aim: The number of studies examining the frequency of *Helicobacter pylori* (Hp) infection, Hp antigen (Ag) test in the stool together with endoscopy and pathology findings in the elderly is limited in the literature. The aim of this study was to investigate the frequency of Hp Ag positivity in the stool samples of elderly patients who checked in to the internal medicine clinics of our hospital with dyspepsia and its relationship with age, gender, seasonal changes, endoscopy and pathology findings.

Materials and Methods: The data of the geriatric patients whose stool samples were investigated for the presence of Hp Ag who applied to the Internal Medicine Clinics of our university with dyspepsia between January 1, 2018 and January 1, 2023 were retrospectively analyzed. Besides demographic and clinical data, endoscopy and pathology reports were recorded.

Results: A total of 2276 patients were included in the study. 60.3% of the patients who requested Hp Ag test were female. Of the total 592 stool samples tested, 20.3% were positive. Hp positivity was highest in the young-old group with a rate of 81.7% (p<0.01). In the distribution of Hp positivity, according to the seasons, it was observed that the highest positivity rate was in winter with 44.2% (p<0.001). It was found that endoscopy was performed in 11.4% of the patients, and the most common findings were gastritis in 76.9%, and duodenitis in 38.5% in patients whose Hp Ag test positive and undervent endoscopy.

Conclusion: Hp infection appears to be more common in the young-old group and in the winter months. Although in the guidelines endoscopy is recommended for elderly patients presenting with dyspepsia, it has been observed that endoscopy was performed at a low rate in elderly patients. Endoscopy should not be avoided in elderly patients presenting with dyspepsia.

Keywords: *Helicobacter pylori*, stool antigen test, elderly, endoscopy, pathology

ÖZ

Amaç: Literatürde yaşlılarda *Helicobacter pylori* (Hp) enfeksiyonu sıklığını, dışkıda Hp antijen (Ag) testi ile endoskopi ve patoloji bulgularını birlikte inceleyen çalışma sayısı kısıtlıdır. Bu çalışmada; dispeptik yakınmalar ile hastanemiz iç hastalıkları kliniklerine başvuran yaşlı hastaların dışkı örneklerinde Hp Ag pozitifliğinin sıklığı ve yaş, cinsiyet, mevsimsel değişiklikler, endoskopi ve patoloji bulguları ile olan ilişkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Üniversitemiz iç hastalıkları kliniklerine 1 Ocak 2018-1 Ocak 2023 tarihleri arasında dispepsi ile başvuran geriatrik hastalarda dışkı örneklerinde Hp Ag varlığı araştırılan hastaların verileri retrospektif olarak incelendi. Demografik ve klinik verilerin yanı sıra, endoskopi ve patoloji raporları kaydedildi.

Bulgular: Çalışmamıza toplam 2.276 hasta dahil edildi. Hp Ag testi istenen hastaların %60,3'ü kadın idi. Test edilen toplam 592 dışkı örneğinden %20,3'ü pozitif saptandı. Hp pozitifliği en yüksek %81,7 oranında genç yaşlı gruptaydı (p<0,01). Hp pozitifliğinin mevsimlere göre dağılımında en yüksek pozitiflik oranının %44,2 ile kış mevsiminde olduğu görüldü (p<0,001). Hastaların %11,4'üne endoskopi yapıldığı, Hp Ag testi pozitif olup endoskopi yapılan hastalarda en sık %76,9 gastrit ve %38,5 duodenit olduğu saptandı.

Sonuç: Hp enfeksiyonu genç yaşlı grupta ve kış aylarında daha sık gözükmektedir. Kılavuzlarda dispepsi yakınması ile başvuran yaşlı hastalara endoskopi önerilmesine rağmen yaşlı hastalara düşük oranda endoskopi yapıldığı görülmüştür. Dispepsi ile başvuran yaşlı hastalarda endoskopi yapılmasından çekinilmemelidir.

Anahtar Kelimeler: *Helicobacter pylori*, dışkı antijen testi, yaşlı, endoskopi, patoloji

INTRODUCTION

Dyspepsia, defined as pain and discomfort in the upper abdominal region, is a common symptom with a comprehensive differential diagnosis and heterogeneous pathophysiology[1]. Although its prevalence is 20% worldwide, it is more common in the geriatric population due to the increased frequency of chronic diseases and drug use[2]. The 75-80% of dyspepsia for which no organic cause can be identified is called functional or non-ulcer dyspepsia. Dyspepsia can be observed due to many organic causes, especially peptic ulcer, gastroesophageal reflux, pathologies related to *Helicobacter pylori* (Hp) infection, drugs and gastric malignancies[3].

Hp is a spiral-shaped, microaerophilic, Gram-negative, flagellated and motile bacterium[4]. The prevalence of Hp infection increases with age worldwide, being 10% between the ages of 18 and 30, rising to 40-60% in asymptomatic elderly individuals and over 70% in elderly patients with gastroduodenal disease[5,6]. However, the percentage of Hp-positive elderly patients treated for their infections is very low[7]. The elderly population is rapidly increasing in Turkey and around the world[8]. With the increasing elderly population, the number of elderly patients admitted to hospitals with gastrointestinal system (GIS) problems is also increasing. The abundance of comorbid chronic diseases, polypharmacy and drug interactions, and the increased frequency of GIS diseases in the elderly make the differential diagnosis and treatment of dyspepsia difficult[9]. Guidelines recommend testing and treatment for Hp for those presenting with dyspepsia and that anyone over the age of 60 with symptoms of dyspepsia should undergo endoscopy. Among the non-invasive tests, active infection tests (urea breath test or stool antigen test) are recommended for patients, but serological tests are not recommended due to their low positive predictive values[10].

In our literature review, to the best of our knowledge, the number of studies examining the prevalence of Hp in the elderly and examining fecal Hp antigen (Ag) test and endoscopy and pathology findings together is limited. Our aim in this study is to investigate the frequency of Hp infection in elderly patients and to examine its relationship with endoscopy and pathology findings.

MATERIALS AND METHODS

Our study is a descriptive cross-sectional study conducted among patients who applied to our hospital's internal medicine clinics with dyspeptic complaints between 01 January 2018 and 01 January 2023.

Inclusion criteria were determined as:

- Presence of Hp Ag in their fresh stool samples is investigated,

- Patients aged 65 and over with complaints of dyspepsia.

Exclusion criteria were determined as:

- Patients known to have had any previous Hp test positivity,

- Patients younger than 65 years of age.

Demographic data of the patients; endoscopy and pathology reports, along with the localization of findings, were scanned from patient files and the hospital information system. Demographic data and Hp frequencies of the patients were divided into general, gender and age groups. Patients were grouped as youngest-old (65-74), middle-old (75-84) and oldest-old (≥85)[11]. In the endoscopy reports, the esophagus, Z line, cardia, fundus, corpus, antrum, pylorus, bulbus and the second part of the duodenum were examined.

The study was carried out by obtaining the necessary permissions from the Atatürk University Faculty of Medicine Clinical Research Ethics Committee (decision no: 435, dated: 01.06.2023).

Statistical Analysis

The data were recorded in the Statistical Package for the Social Sciences-23.0 package program and analyzes were conducted using the same program again. Demographic data were presented as number (n), percentage (%) and median (minimum-maximum). Pearson chi-square test was used to compare categorical variables, and Mann-Whitney U test was used to compare the numerical values of two independent groups whose data were distributed nonparametrically. Results were accepted as statistical significance p<0.05 within the 95% confidence interval.

RESULTS

In our study, 2,276 patients whose fresh stool samples were investigated for the presence of Hp Ag after presenting with complaints of dyspepsia were examined. 1,640 (72.1%) of the patients did not provide a sample, 44 (1.9%) of the patients could not be tested due to lack of a kit, 472 (20.7%) patients were Hp Ag negative, and 120 (5.3%) patients were Hp Ag positive. Of the patients for whom Hp Ag testing was requested, 1,372 (60.3%) were women and 904 (39.7%) were men. Their mean age was 71.76±6.03 and the median was 70.0 (minimum-oldest: 65-99) (Table 1). Of the 592 patients who underwent Hp Ag testing, 356 (60.1%) were female and 236 (39.9%) were male. Hp Ag positivity was found to be 20.3% on average. This rate was found to be 20.8% in men and 19.9% in women. Of the Hp Ag positive patients, 71 (59.2%) were female and 49 (40.8%) were male. No statistically significant difference was found between genders (p=0.83) (Table 2). When Hp Ag test positive and negative patients were compared in terms of age without grouping, no statistically significant difference was detected (p=0.11). Of the 592 patients for whom the Hp Ag test was studied, 445 (75.2%) were in the youngest-old group (Table 3) and the highest Hp Ag positivity was detected in the young-old group (81.7%) (Table 4). There was statistical significance in terms of Hp Ag positivity between age groups (p<0.01). When Hp Ag positivity was compared according to seasons, it was determined that it was most common in winter

months with 44.2%. The relationship between Hp frequency and seasons was statistically significant (p<0.001) (Table 5).

It was determined that endoscopy was performed in 259 (11.4%) of 2,276 patients for whom Hp Ag test was requested, and 86 (33.2%) patients who underwent endoscopy had a pathology report; 13 (10.8%) of 120 patients with Hp Ag (+) underwent endoscopy, 2 (15.4%) patients who underwent endoscopy had a pathology report, 31 (6.6%) of 472 Hp Ag (-) patients underwent endoscopy, and 8 (25.8%) patients who underwent endoscopy had a pathology report. In 13 patients with Hp Ag (+) who underwent endoscopy, the most common was gastritis (76.9%), and the second most common was duodenitis (38.5%). In terms of localization, 70% of gastritis is pangastritis, 30% is antral gastritis; 60% of duodenitis was detected as only the bulbus, and 40% was detected as both the bulbus and the second part of the duodenum. Endoscopy findings of patients with Hp Ag (+) are shown in Table 6. The number of patients was insufficient to statistically evaluate the difference between endoscopy and pathology findings of patients with Hp Ag (+) and (-).

DISCUSSION

Hp infection is the most common chronic bacterial infection worldwide[12] and has a role in the etiology of chronic gastritis, non-ulcer dyspepsia, most duodenal and gastric ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue-lymphoma[13-16]. The main reservoir of HP is humans and it colonizes the corpus, cardia and distal antrum of the stomach.

Table 1. Gender distribution of patients for whom Hp Ag testing was requested		
Gender	Number	Percentage (%)
Female	1,372	60.3
Male	904	39.7
Total	2,276	100
Hp: *Helicobacter pylori*, Ag: Antigen		

Table 2. Comparison of Hp Ag positivity according to gender				
Gender	Antigen (–) n (%)	Antigen (+) n (%)	Total n (%)	p
Female	285 (80,1)	71 (19.9)	356 (60.1)	0.83
Male	187 (79,2)	49 (20.8)	236 (39.9)	
Total	472 (79,7)	120 (20.3)	592 (100)	
Hp: *Helicobacter pylori*, Ag: Antigen				

Table 3. Comparison of Hp Ag test results by age				
Age Group	Antigen (–) n (%)	Antigen (+) n (%)	Total n (%)	p
65-74	347 (78.0)	98 (22.0)	445 (78.0)	0.15
75-84	109 (85.8)	18 (14.2)	127 (21.4)	
≥85	16 (80.0)	4 (20.0)	20 (3.4)	
Total	472 (79.7)	120 (20.3)	592 (100)	
Hp: *Helicobacter pylori*, Ag: Antigen				

Table 4. Comparison of Hp Ag positivity according to age groups			
Age Group	Hp antigen positive		p
	n	%	
65-74	98	81.7	<0.01
75-84	18	15.0	
≥85	4	3.3	
Toplam	120	100	
Hp: *Helicobacter pylori*, Ag: Antigen			

Table 5. Comparison of Hp Ag positivity according to seasons			
Season (months)	Hp antigen positive		p
	n	%	
Winter (December-January-February)	53	44.2	<0.001
Spring (March-April-May)	18	15.0	
Summer (June-July-August)	17	14.2	
Autumn (September-October-November)	32	26.7	
Total	120	100	
Hp: *Helicobacter pylori*, Ag: Antigen			

Although the exact mode of transmission of the infection is not known, fecal-oral or oral-oral routes through water or food consumption are thought to be a very common cause[17].

In our study, consistent with the literature, the majority (60.3%) of the patients who were asked for Hp Ag test after presenting with complaints of dyspepsia were female patients[18-21]. In the meta-analysis conducted by Ford et al.[2], it was reported that dyspepsia was more common in women, and in the study conducted by Bektaş et al.[22], it was reported to be seen in 41.1% of women and 22.1% of men. We think that the reason why the Hp Ag test is requested more in women in our study is that dyspepsia is more common in female patients. In our study, Hp positivity was detected in 120 of 592 cases (20.3%). The prevalence of Hp varies according to the social and economic status of the society in different geographical regions and ethnic groups. The prevalence is higher in low socioeconomic status groups and developing countries[23]. While it is observed between 10-50% in developed countries, it is more common than 80% in developing countries[5]. The prevalence of Hp in the geography including our country is higher than in western societies. In the study conducted by Vilaichone et al.[24], it was

shown that the prevalence of Hp varies not only from country to country but also in different regions of the same country. The fact that Hp infection is more common in individuals with low socioeconomic status and living in crowded environments supports that the mode of transmission may be fecal-oral. Studies conducted in our country have reported that the prevalence of Hp varies according to regions and age groups (20.3-89.8%), increases with age, and has tended to decrease in recent years[18,19,25-29]. Demir et al.[26] reported the prevalence of Hp as 25.2% in their study, while Selek et al.[25] reported that it was 20.3%, similar to our study (20.3%), which was low compared to other studies. The frequency of HP infection decreases after eradication treatments in line with the 'test and treat' recommendations of the guidelines. A study conducted in Sweden showed that the prevalence of Hp in the 56-80 age group, which was 64% in 1989, decreased to 22% in 2012[30]. The decline in Hp prevalence in a country is associated with economic recovery and improvement in healthcare services. In Japan, the prevalence of Hp was found to be 70-80% in adults born before 1950, 45% in those born between 1950 and 1960, and 25% in those born between 1960 and 1970, and this rapid decline was attributed to economic progress and improvement in sanitation[31]. Our study supports the decreasing trend in prevalence seen in the data obtained in recent publications. In our country, this decrease in recent years is may be due to the improvement in hygiene conditions along with the progress in socioeconomic status, changes in health policies, the prevention of Hp infection by non-invasive methods and more frequent application of eradication treatments, either through testing or empirically.

Table 6. Endoscopy findings of Hp Ag positive patients		
Endoscopic finding (n=13)	n	%
Normal	1	7.7
Tracheoesophageal fistula	-	-
Inlet patch	-	-
Esophageal stenosis	-	-
Esophagitis	-	-
Esophageal candidiasis	-	-
Esophageal varicose veins	1	7.7
Esophageal mass	-	-
Gastroesophageal reflux disease	-	-
Lower esophageal sphincter insufficiency	1	7.7
Esophageal ulcer	-	-
Hiatal hernia	-	-
Previous stomach surgery	-	-
Gastritis	10	76.9
Gastric erosion	2	15.4
Gastric ulcer	-	-
Gastric polyp	-	-
Gastric mass	-	-
Enterogastric bile reflux	2	15.4
Duodenal erosion	1	7.7
Duodenal ulcer	-	-
Duodenitis	5	38.5
Duodenal polyp	-	-
Duodenal diverticulum		
Hp: *Helicobacter pylori*, Ag: Antigen		

Although there is no difference in Hp positivity between genders in many studies, there are also studies reporting that positivity is higher in men than in women[19,21]. As in similar studies, Hp positivity was found to be higher in male patients in our study, although it was not statistically significant. In the study carried out by Demirtaş et al.[18], in which the prevalence of Hp was investigated by including 1,405 patients, no significant difference was found between the geriatric age groups in terms of the frequency of Hp. In the study conducted by Uyanıkoğlu et al.[19] in which 1,298 endoscopic antrum biopsies were examined histopathologically, it was shown that Hp positivity was not related to age. Again, in the study conducted by Şengül and Şengül[29] in which the prevalence of Hp was investigated in 373 patients, no difference was found between Hp frequency and age groups. In our study, similar to the literature, no significant difference was found in terms of Hp positivity between age groups in the geriatric population. However, when we compare Hp (+) patients according to age groups, the highest number of Hp (+) patients was in the youngest-old group. We think that this may be due to the high ratio of youngest-old people in the elderly population (64.5%)[8].

Moshkowitz et al.[32] and Selek et al.[25] found in their study that Hp positivity was statistically significantly higher in the winter season, similar to our study. The higher frequency of Hp positivity in winter may be due to immunosuppression and increased social life.

In our study, it was shown that endoscopy was performed at a low rate in elderly patients who applied with complaints of dyspepsia (11.4%) and biopsies were taken at a low rate in patients who underwent endoscopy (33.2%). The low rate of procedures performed may be due to the fact that elderly patients are considered to have a high probability of complications due to the presence of increased comorbid diseases, especially cardiac and pulmonary diseases, increased use of medications including anticoagulants and antiaggregants, fragility that occurs in older ages, and problems such as low performance status. Guidelines recommend that all patients aged 60 and over who present with dyspepsia undergo upper GI endoscopy and take five biopsies: two from the antrum, two from the corpus, and one from the incisura angularis[33]. It has been shown in the literature that upper GI endoscopy is well tolerated in elderly patients and there is no increase in the risk of complications[34]. In our study, the most common endoscopy findings of Hp (+) patients were found to be gastritis and duodenitis, consistent with the literature[20,35]. This finding is an expected finding considering the pathophysiology of Hp, especially since it colonizes the gastric type epithelium and settles in the stomach, causing inflammation and acid hypersecretion.

Study Limitations

Our study has some limitations that should be mentioned. The main limitations of our study are that the data were collected from patient records, that the data were collected from patient records, that the cases were sourced from a single center, that they were not community-based and that they were only cases that could reach tertiary care.

CONCLUSION

As a result, although dyspeptic complaints are more common in women in the geriatric population, there is no difference between genders in terms of Hp infection. The prevalence of Hp in our country is decreasing compared to previous years. Hp infection seems to be more common in youngest-old groups and in winter months. Although endoscopy is recommended in the guidelines for elderly patients presenting with dyspepsia, it has been observed that endoscopy is performed at a low rate in elderly patients. In elderly patients, if clinically necessary, upper GIS endoscopy, which is a reliable procedure with a low risk of complications, should not be avoided.

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How Did the Pandemic Affect Autistic Children and Their Families? Challenges and Psychosocial Impacts

Otizmli Çocuklar ve Aileleri için Pandemi Dönemi Nasıl Geçti? Yaşanan Zorluklar ve Psikososyal Etkiler

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ABSTRACT

Aim: Children with autism spectrum disorder (ASD) are especially vulnerable to the pandemic due to their need for routine and difficulty in adapting to change. The aim of this study was to evaluate the psychosocial impact of the Coronavirus disease-2019 (COVID-19) pandemic on children diagnosed with ASD and their families and the challenges they faced during this period.

Materials and Methods: We surveyed 85 parents of children with ASD aged 3-18 years, who were followed up in our clinic between the years of 2020 and 2021. We assessed ASD families' pandemic impact with the "Child and Adolescent Reactions to Stressful Situations-Autism Family Adaptation and Resilience" items. Parents answered the questionnaire online or by phone.

Results: The mean age of the children was 10.3±4.1 years, 65 were boys and 20 were girls. 10.6% of the families had a medical emergency during the pandemic. The most common behavioral problems were anger, difficulty in concentrating, and hyperactivity. 37.6% of the children could not receive educational support, 74.1% could not receive treatment, 31.1% had difficulty in reaching a doctor, and 7.8% had difficulty in affording treatment. Furthermore, 33% of the children spent more than 4 hours a day on screens. 61.2% of parents rated the pandemic quarantine process as more difficult than before.

Conclusion: The pandemic disrupted the daily routines, special education, rehabilitation, and health care services of children with ASD. It also increased their behavioral problems. Therefore, recognizing and addressing the psychological needs of children with special needs, such as ASD, will be an important part of the response to future disasters.

Keywords: Autism spectrum disorder, pandemic, COVID-19, child psychiatry, psychosocial

ÖZ

Amaç: Otizm spektrum bozukluğu (OSB) olan çocuklar, rutin ihtiyaçları ve değişime uyum sağlamadaki zorlukları nedeniyle pandemiye karşı özellikle savunmasızdır. Bu çalışmanın amacı, Koronavirüs hastalığı-2019 (COVID-19) pandemisinin OSB tanılı çocuklar ve aileleri üzerindeki psikososyal etkisini ve bu dönemde karşılaştıkları zorlukları değerlendirmektir.

Gereç ve Yöntem: 2020-2021 yılları arasında kliniğimizde takip edilen 3-18 yaş arası 85 OSB'li çocuğun ebeveynine, araştırmacılar tarafından hazırlanan anket formu uygulandı. OSB tanılı çocuğu olan ailelerin pandemiden etkilenme durumları "Stresli Durumlara Çocuk ve Ergen Tepkileri-Otizm Aile Adaptasyonu ve Dayanıklılığı" maddeleri ile değerlendirildi. Ebeveynler anketi çevrimiçi olarak veya telefonla yanıtladı.

Bulgular: Çocukların yaş ortalaması 10,3±4,1 yıl, 65'i erkek ve 20'si kızdı. Ailelerin %10,6'sı pandemi sırasında tıbbi bir acil durum yaşadığını bildirdi. En sık görülen davranış sorunları öfke, konsantrasyon güçlüğü ve hiperaktivite idi. Çocukların %37,6'sının eğitim desteği alamadığı, %74,1'inin tedavi göremediği, %31,1'inin doktora ulaşmakta zorluk çektiği ve %7,8'inin tedaviyi karşılamakta güçlük çektiği bildirildi. Ayrıca çocukların %33'ü günde 4 saatten fazla ekran başında vakit geçiriyordu. Ebeveynlerin %61,2'si pandemi karantina sürecini eskisinden daha zor olarak değerlendirdi.

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Sonuç: Pandemi, OSB'li çocukların günlük rutinlerini, özel eğitim, rehabilitasyon ve sağlık hizmetlerini sekteye uğratmış, ayrıca davranış sorunlarını da arttırmıştır. Bu nedenle, OSB gibi özel gereksinimi olan çocukların ruhsal ihtiyaçlarını bilmek ve karşılayabilmek, gelecek afet durumlarında yapılacak yardımların önemli bir parçası olacaktır.

Anahtar Kelimeler: Otizm spektrum bozukluğu, pandemi, COVID-19, çocuk psikiyatrisi, psikososyal

INTRODUCTION

In late 2019, a novel coronavirus was identified as the cause of pneumonia cases in Wuhan, China. The outbreak rapidly spread throughout China, and subsequently throughout the world, causing a rapid increase in the number of cases[1]. On March 11, 2020, upon the detection of the first case in our country, the World Health Organization declared the onset of the Coronavirus disease-2019 (COVID-19) pandemic[2].

To date, the virus has infected more than 370 million people worldwide and caused 5.65 million deaths[3]. The high number of asymptomatic infections has made it difficult to control the epidemic and serious measures have been taken worldwide to prevent transmission[4]. During this time, the government implemented several regulations, including the halting of education in accordance with the principles of controlled social life, a curfew for individuals under the age of 20 years, and periodic curfews throughout the nation[5].

These social measures and restrictions have significantly hampered the lives of people of all ages. According to reports, individuals under the age of 18 years, who are still in their developmental stage and unable to meet their needs independently, are at risk of developing mental health issues. Additionally, they have been greatly affected by the pandemic due to the transition to remote learning and curfews, which has kept them away from their peers[4,6].

It is known that crisis events negatively affect psychological well-being. Imran et al.[7] stated that anxiety, depression, sleep and appetite disorders, and impaired social interactions were the most common responses to crisis in children. Jiao et al.[8] (2020) conducted a study examining the behavioral and emotional distress experienced by children and adolescents following the COVID-19 outbreak. The study found that attachment to parents, distraction, irritability, and fear of family members contracting the disease were the most commonly reported problems.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that presents significant challenges related to social communication and interaction, restricted interests, and stereotyped behaviors. According to Diagnostic and Statistical Manual of Mental Disorders-5, children diagnosed with ASD experience difficulties with both verbal and nonverbal forms of communication, developing social relationships and interpreting social cues, showing social-emotional reciprocity,

and adapting to changes in social environments[9]. Additionally, research shows that children with special needs are at a higher risk for negative psychological effects in the aftermath of natural disasters[7]. The pandemic affects these children and their families more than others for several reasons. They have to stay indoors for long periods, their daily routines are disrupted, and the specialized education and rehabilitation centers they need are closed. Moreover, these children struggle with online education, lack the necessary equipment, and face challenges in accessing basic health services[10].

Children with autism thrive on structure, and unexpected changes can cause them added stress. It is important to maintain a predictable routine to help them feel more secure and stable[11]. Since the changes in their daily routines during quarantine are difficult for these individuals and their families, they are at risk for developing or worsening mental health disorders, particularly anxiety disorders during the pandemic[12]. Furthermore, as these children face challenges in social communication, they require settings that can facilitate their social growth, such as school and peers[13]. The pandemic has made it harder for people with ASD to develop their social skills due to limited access to social environments, school closures, and disrupted special education. This places them at risk for negative psychosocial consequences related to the pandemic[14]. The pandemic has been a struggle for children with communication difficulties. Social isolation, disrupted therapies, and interrupted special education have all compounded the problem, making life especially challenging for them[15].

The pandemic continues to have significant ongoing impacts, including social isolation, restricted access to healthcare and education services, and decreased financial gains[16]. Although there are numerous ongoing studies regarding COVID-19 and its impact, research on the effects of this pandemic on children with ASD and their families in our country remains scarce. Limited studies have examined the effects of pandemic-induced changes in physical activity and daily routine on this population. It has been noted that various factors, including increased behavioral problems in these children, caregiving needs, and finances, increase the stress on the family. The pandemic has highlighted the added stress of parents having to take on the role of teacher for their children's education and changes in parenting roles. Furthermore, research indicates that the pandemic may have led to heightened anxiety levels in families regarding the potential impact on the physical and mental well-being of children[17-19].

AYKUTLU et al. Effects of COVID-19 Pandemic on Autistic Children

Nam Kem Med J 2023;11(4):346-353

The problems faced by children diagnosed with ASD during the pandemic process have been the subject of many international studies[11-15]. However, research on this topic is limited in our country[20]. Therefore, we assessed the pandemic's psychosocial impacts on children with ASD and their families using a comprehensive questionnaire. Our aim was to explore the challenges they encountered and to devise strategies for future disasters.

MATERIALS AND METHODS

This study investigates the psychosocial impact of the COVID-19 pandemic on children with ASD and their families, including the challenges encountered during this period.

The study follows a descriptive research design, and ethical approval was obtained from the Trakya University Faculty of Medicine Hospital Medical Research Ethics Committee on October 4th, 2021, with decision number 19/13. Caregivers who participated in the study received comprehensive information regarding the research and provided written authorization online for the publication of their medical data.

The study sample was comprised of children and their families diagnosed with ASD, who were followed up at the outpatient clinic of the Trakya University, Department of Child and Adolescent Psychiatry and who agreed to participate in the study. From our database, our study included 165 patients who were between 3 and 18 years old, applied to our outpatient clinic during the period of 2020-2021, and were diagnosed with ASD. We contacted the families of these patients over the phone and informed them about our study. We sent a link for our online questionnaire to the 85 families who volunteered to participate in the study and requested them to fill it out. Questionnaires were filled out via phone calls by families without online accessibility.

During the interviews, participants who consented to take part in the study were administered prepared questionnaires to assess their COVID-19 pandemic adaptation process and investigate its psychosocial impacts. Both patients and their families were included in the study. In the questionnaire form designed for parental response in our study, the questions developed as a result of the literature review and CRISIS-AFAR (The CoRonavIruS Health Impact Survey-Adapted for Autism and Related Neurodevelopmental Conditions; Child and Adolescent Responses to Stressful Situations-Autism Family Adaptation and Resilience) were included[21]. Some questionnaire questions were translated into Turkish for this purpose. CRISIS-AFAR was developed by Vibert et al.[21] and does not currently have an official Turkish translation, nor has validity and reliability been established through study. The objective of the questionnaire is to evaluate the coronavirus crisis-specific requirements and modifications in individuals with ASD and related neurodevelopmental disorders. The questionnaire includes parent notification forms for individuals aged 3-21 years, a self-report form for individuals aged 14 years and older, and an additional parent notification form for adults with autism or other neurodevelopmental disorders.

In the initial section of our four-part survey, we gathered sociodemographic data regarding the patient and their family, the pre-COVID-19 employment status of the parents, any history of psychiatric illness among family members, the patient's language proficiency, and any comorbidities aside from autism. In the second section, participants were queried on whether they had encountered any event warranting immediate communication with their psychiatrists during the pandemic, whether they obtained treatment or educational assistance during this period, and how they perceived the pandemic in comparison to pre-pandemic times. The subsequent section delved into the educational experiences of children during the pandemic. This section covers inquiries on the effects of educational services, access to distance learning, access to healthcare services, and the efficacy of such services. The last section delves into challenges faced during the pandemic, obstacles encountered, and advantageous outcomes resulting from the pandemic.

Respondents completed our online questionnaire, which is a parent notification survey consisting of closed-ended and Likert-type items with 2 or more pre-coded responses (Figure 1).

Statistical Analysis

The study's research data were analyzed using the Statistical Package for the Social Sciences 23.0 program. Categorical data were expressed as frequency (percentage), and age was expressed as mean±standard deviation. For data evaluation, descriptive statistical methods including number, percentage, arithmetic mean, and standard deviation were utilized.

RESULTS

Of the 165 families with children having ASD, who were included in the study, 85 completed our questionnaire. The participation rate was 51.5%. The mean age of the participants was 10.3±4.1 years. 20 patients were female and 65 were male. As shown in the table, 41.2% (n=35) of the patients could not speak at all, 38.8% (n=33) could speak on a word/sentence level, and 20% (n=17) could speak fluently (Table 1).

Mothers and fathers were asked about their educational level and employment status; the majority of mothers had completed primary school (43.5%, n=37%) and had not worked since pre-COVID period (83.5%, n=71). The majority of fathers had a high school education (37.6%, n=32) and were employed (88.2%, n=75). The majority of families lived in the

Figure 1. Survey question examples

COVID-19: Coronavirus disease-2019

Table 1. Sociodemographic characteristics of children diagnosed with ASD and their families		
Cases diagnosed with ASD (n=85)	Frequency	(%)
Age, mean–SD	10.3-4.1	
Sex		
Boy	65	76.5
Girl	20	23.5
Language skills		
No conversation	35	41.2
Word-level speech	16	18.8
Sentence-level speech	17	20
Fluent speech	17	20
Mother's education level		
Primary	37	43.5
High school	27	31.8
License	17	20
Master	4	4.7

Table 1. Continued		
Cases diagnosed with ASD (n=85)	Frequency	(%)
Maternal employment status		
Those who have not worked since pre-COVID-19	71	83.5
Employees	13	15.3
Those who have not worked since COVID-19	1	1.2
Father's education level		
Primary	29	34.1
High school	32	37.6
Undergraduate degree	20	23.5
Graduate degree	4	4.7
Father's employment status		
Those who have not worked since pre-COVID-19	7	8.2
Employees	75	88.2
Those who have not worked since COVID-19	3	3.5
Placement		
Province	28	32.9
County	48	56.5
Town	2	2.4
Village	7	8.2
Income level		
Low	40	47.1
Middle	43	50.6
High	2	2.4
ASD: Autism spectrum disorder, SD: Standard deviation, COVID-19: Coronavirus disease-2019		

district (56.5%, n=48) and had a moderate income (50.6%, n=43). Sociodemographic data are presented in Table 1.

10.6% (n=9) of families reported experiencing an emergency during the pandemic. The most common emergency experienced by the 9 reporting families was a temper tantrum (66.6%, n=6). While 8.2% (n=7) of the parents rated the parenting process during the pandemic as easier than before, 30.6% (n=26) rated it as the same, and 61.2% (n=52) rated it as more difficult (Table 2).

During the pandemic process, educational support and health services were affected to a large extent. 37.6% (n=32) of the patients could not receive educational support during the pandemic process, 43 patients (37.1%) had to continue their education at home, 63 patients (74.1%) reported that they could not receive treatment (Table 2).

When families were asked about health care problems experienced during the COVID-19 pandemic, routine appointments and treatments were canceled/delayed in 52.4% (n=54), difficulty in reaching a doctor in 31.1% (n=32),

AYKUTLU et al. Effects of COVID-19 Pandemic on Autistic Children

Nam Kem Med J 2023;11(4):346-353

Table 2. Pandemic events and services affected		
Pandemic emergency (n=85)	**Frequency**	**(%)**
Those who report an emergency	9	10.6
Non-reporters	76	89.4
Emergencies* (among reporters, n=9)		
Epileptic seizure	1	11.1
Self-injurious behavior	2	22.2
Excessive mobility	1	11.1
Emerging movement (Korea)	1	11.1
Tantrum	6	66.6
New-onset toothache	1	11.1
Difficulty level of closure period (n=85)		
Easy	7	8.2
Same	26	30.6
Difficult	52	61.2
Difficulty in performing daily activities (n=85)		
No	15	17.6
Partly	34	40
Yes	36	42.4
Education and other services affected since the COVID-19 crisis began (among reporters, n=81)		
Not affected	7	6
Those whose schools are closed	41	35.3
Those who continue their education at home	43	37.1
Those who have lost access to education and health care	25	21.6
Educational support intake (n=85)		
Those who do not receive support	32	37.6
Recipients of support	53	62.4
Treatment (n=85)		
Non-recipients	63	74.1
Fields	22	25.9
Benefiting from the services received (among the reporters, n=69)		
Never	15	17.6
Medium	43	50.6
High	11	12.9
Missing data	16	18.8
Health service problems in the COVID-19 outbreak (n=85)		
Cancellation or postponement of routine appointments and treatments	54	52.4
Difficulty reaching a doctor	32	31.1
Difficulty accessing or managing medications	9	8.8
Difficulty affording medications or treatment	8	7.8
Daily screen time (n=85)		
Never	8	9.4
0-3 hours	49	57.6
4-6 hours	18	21.2
More than 6 hours	10	11.8
Experiencing a positive change in the pandemic (n=85)		
No	71	83.5
Yes	14	16.5
COVID-19: Coronavirus disease-2019		

difficulty in accessing or managing medications in 8.8% (n=9), and difficulty in adhering to medications and treatments in 7.8% (n=8) (Table 2).

Screen time for children diagnosed with ASD was questioned and found to be higher than it should be for most patients. It was found that 33% (n=28) of the patients were exposed to more than 4 hours of screen time per day (Table 2).

When families were asked about the problems they experienced during the pandemic, 83.5% (n=71) reported behavioral problems. The most common problems reported were anger, concentration difficulties, hyperactivity, crying easily, excessive anxiety, and increased aggression (Figure 2). When families were asked if there had been a positive change in their lives, only 16.5% (n=14) reported a positive change.

DISCUSSION

Our study found that children with ASD experienced significant problems during the pandemic period, as reported by their parents. During the pandemic period, 10.6% of children with ASD experienced a medical emergency and 74.1% were unable to maintain their current treatment. In addition, 83.5% of children had at least one behavioral problem (e.g., anger, concentration difficulties), and 33% spent more than 4 hours per day in front of a screen. At the same time, 37.6% of children with ASD were not receiving any educational services.

To date, COVID-19 has infected more than 676 million people and killed more than 6.8 million people worldwide[3]. While the pandemic is a life-threatening threat, it has also brought with it factors that will adversely affect the mental health of society. In addition to the threat of disease, the social isolation and restrictions imposed by the closure under the controlled social life principles have been a source of stress for people from all walks of life. In addition to the current effects of this
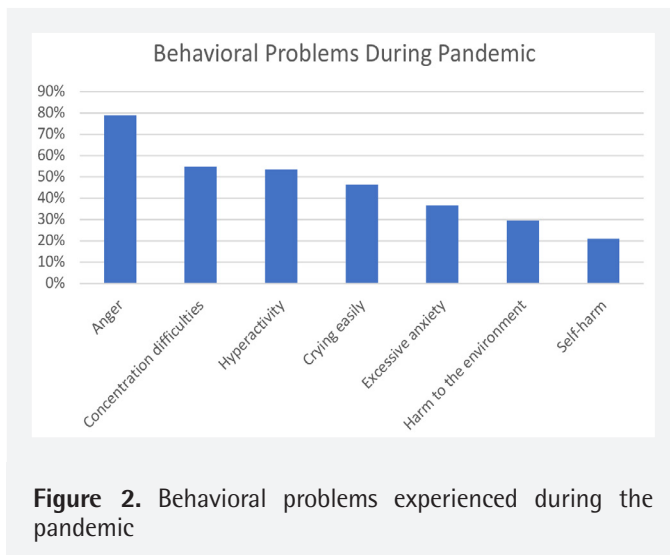

Figure 2. Behavioral problems experienced during the pandemic

Nam Kem Med J 2023;11(4):346-353

AYKUTLU et al. Effects of COVID-19 Pandemic on Autistic Children

epidemic, its long-term effects will only be seen in the coming years[22].

Public health emergencies, such as pandemics, negatively impact both physical and mental health. Children's limitations in understanding events make them more vulnerable than adults. Because their coping strategies are limited, they are physically and mentally unable to escape the harms of the situation. They may not be able to express their emotions like adults. In addition, school closures and separation from friends can cause stress and anxiety in children. The coverage of the crisis event in the mass media and unverified information circulating on social media can exacerbate mental distress[7,23].

The COVID-19 pandemic has caused significant challenges for all age groups. Changes in routines, social isolation, anxiety about illness, and lack of access to health services have contributed to these difficulties. However, children with special needs have faced particularly strenuous circumstances and have experienced immense stress. Many studies indicate that children with psychiatric illnesses are particularly susceptible to the pandemic's risks and the associated challenges[11,12].

According to parent surveys in previous studies, parents of individuals with ASD have described pandemic restrictions as a challenging process that requires more responsibility than before[10]. High levels of parental stress, as well as difficulty in understanding the emotions, reactions, and needs of these children, are important risk factors. In our study, 52 parents of children with ASD (61.2%) perceived the pandemic closure process as more challenging than before. This finding indicates that children with ASD are a part of a significant risk group.

Disruptions to daily routines, like school closures, can impede children with ASD and neurocognitive disabilities. This interruption may lead to problematic behaviors such as irritability, aggression, and social withdrawal, and can also cause a disruption in regular treatment sessions[6]. Healthcare and educational assistance received during the pandemic were significantly disrupted, and our study reported that 37.6% (n=32) of patients were unable to receive educational assistance, 74.1% (n=63) were unable to receive treatment, and 52.4% (n=54) had appointments and treatments canceled.

Individuals with ASD are more susceptible to routine disruptions due to deficits in executive functions. According to parental reports, managing daily activities, specifically leisure time and structured activities, posed challenges. It is important to consider the influence of the environment on behavior despite the complex genetic nature of ASD. Emotional changes resulting from the COVID-19 pandemic have been associated with heightened maladaptive behavior in individuals with ASD over a prolonged duration[11].

According to a study evaluating the effects of the pandemic on children with ASD through an online form, individuals who previously had behavioral issues experienced twice as many and more intense behavioral problems since the outbreak began[11]. In our study, 78.9% (n=56) of children with ASD had easy anger and 29.6% (n=21) had physical aggression. In addition, tantrums were reported as the most common emergency (66.6%, n=6).

Nearly 50% of the participants stated requiring assistance in healthcare, with household services being emphasized in previous studies. One in five parents mentioned that lifting restrictions or ending quarantine would be beneficial. Following the COVID-19 outbreak, persons with ASD lacking school support displayed heightened behavioral problems, underscoring the necessity of maintaining communication with educational institutions during crises[7].

In our research, 16.5% of families (n=14) reported experiencing a positive impact on their lives during the pandemic. One commonly reported aspect of these changes is the ability to spend more time with family. While social isolation has been a well-documented effect of lockdowns, the positive effects on family communication are worth exploring further.

In our study, it was observed that 33% (n=28) of the cases had more than 4 hours of daily screen use. In Guo et al.'s[24] large-sample survey study, in which they evaluated 10,933 children during the pandemic period, 44.6% (n=4,649) of the participants reported that screen exposure was over 5 hours. In a study examining the relationship between heavy screen use and mental health symptoms during the pandemic period, an increase in symptoms of depression, anxiety, behavioral problems, irritability, and hyperactivity/inattention has been reported[25]. The importance of the harmful effects of uncontrolled screen and technology use during the pandemic period is frequently emphasized, especially for risky groups such as children in developmental age and children with autism[26,27]. Children should be prevented from being exposed to panic-inducing news in the media and positive use of social media should be encouraged. In addition, children's screen time should be monitored[28].

Study Limitations

The key strength of our research is the use of telemedicine to reach families with ASD, enabling us to gather information swiftly and clarify the issues faced by ASD children during the pandemic. However, it is important to note that our study had a limited sample size (85 out of 165 patients, or 51.5%) and relied solely on parental feedback, using a descriptive approach. To better understand the experiences of children with ASD and their families during the pandemic and develop

AYKUTLU et al. Effects of COVID-19 Pandemic on Autistic Children

Nam Kem Med J 2023;11(4):346-353

effective solutions to address their challenges, it is necessary to conduct research that evaluates causal relationships and sheds light on this issue.

CONCLUSION

Our study reveals that the pandemic has presented significant challenges for both children with autism and their families. The education and treatment requirements, which are already difficult to meet, have been even more challenging to address[29,30]. The majority of parents reported experiencing many negative events during the pandemic and various behavioral disturbances in their children (hyperactivity, difficulty concentrating, increased anger and aggression, crying easily, and excessive anxiety). During the period of isolation, the decrease in social interaction and the increased time spent at home have led to uncontrolled growth in children's screen time. Along with negative feedback, positive responses from families suggest that the closure period has benefitted family communication and bonding, due to the increase in family time. Future studies are necessary to assess the enduring effects of the COVID-19 pandemic, which have significantly impacted this vulnerable population.

Ethics

Ethics Committee Approval: The study follows a descriptive research design, and ethical approval was obtained from the Trakya University Faculty of Medicine Hospital Medical Research Ethics Committee on October 4th, 2021, with decision number: 19/13.

Informed Consent: Caregivers who participated in the study received comprehensive information regarding the research and provided written authorization online for the publication of their medical data.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.C.A., E.B.K., Concept: H.C.A., B.G.Y., L.B., I.G., Design: H.C.A., B.G.Y., L.B., I.G., Data Collection or Processing: H.C.A., L.B., E.B.K., Analysis or Interpretation: H.C.A., B.G.Y., L.B., I.G., Literature Search: H.C.A., B.G.Y., L.B., E.B.K., Writing: H.C.A., B.G.Y., I.G.

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An Assessment of COVID-19 Infectivity and Fatality: Meta-analysis Study

COVID-19 Enfektivitesi ve Fatalitesi Üzerine Bir Değerlendirme: Meta-analiz Çalışması

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ABSTRACT

Aim: The effect size of the infectivity and fatality of the Coronavirus disease-2019 (COVID-19) virus, which shook the whole world, was examined using the meta-analysis method, which is considered to have high evidential value.

Materials and Methods: A comprehensive literature review (PubMed, Medline, Cochrane Library, Science Direct, ProQuest, Ulakbim, Ministry of Health, YÖK, WHO Global Index) was conducted between December 2019, the date of COVID-19 virus's first appearance in the world, and December 2020. Selection criteria for the study were determined. Twenty-one studies that met the criteria were included. The analyzed articles were coded by two independent coders, and the methodological quality of the studies to be included in the research was evaluated using the "Jadad score" and the "Newcastle Ottawa Criterion". Studies of medium and high quality were included in the study. Three versions of the Comprehensive Meta-Analysis program were used to analyze the data.

Results: The effect size on COVID-19 transmission and mortality was calculated as d=0.092 (p=0.000). According to Cohen (1988), studies have high effect sizes and are heterogeneous. According to the results of the moderator analysis investigating the heterogeneity of subgroup data, age, gender, clinical findings and comorbidity were found to be moderators for the mean effect size (p<0.05). In this context, demographic characteristics, clinical picture and comorbidity, as well as COVID-19 transmissibility and mortality rate, were determined to be significant and effective.

Conclusion: Due to the fact that it is a study covering the first year of the pandemic using the meta-analysis method in the field of nursing in global public health problems such as pandemics, it is thought that it will guide the studies to be done by using a wider time period and adding studies conducted in wider age categories and in different countries.

Keywords: COVID-19, infectivity, fatality, Coronavirus, meta-analysis

ÖZ

Amaç: Kanıta dayalı çalışmalar arasında en yüksek seviyede yer alan meta analiz yöntemi kullanılarak yürütülen bu çalışma, tüm dünyayı sarsan Koronavirüs hastalığı-2019 (COVID-19) virüsünün enfektivitesi ve fatalitesinin etki büyüklüğünü incelemektir.

Gereç ve Yöntem: COVID-19'un dünyada ilk görüldüğü tarih olarak ifade edilen Aralık 2019 ile Aralık 2020 zaman dilimleri arasında yapılan kapsamlı bir literatür taraması (PubMed, Medline, Cochrane Library, Science Direct, ProQuest, Ulakbim, Sağlık Bakanlığı, YÖK, WHO Global İndex) gerçekleştirildi. Çalışma için seçme kriterleri belirlendi. Çalışmaya seçme kriterlerine uyan 21 çalışma dahil edildi. Araştırmada analiz edilen makaleler, birbirinden bağımsız iki kodlayıcı tarafından kodlanarak, araştırmaya dahil edilecek çalışmaların metodolojik kalitesi "Jadad skoru" ve "Newcastle Ottawa Ölçütü" kullanılarak değerlendirildi. Araştırmaya orta ve yüksek kalitedeki çalışmalar dahil edildi. Verileri analiz etmek için Comprehensive Meta Analysis programının üç sürümü kullanıldı.

Bulgular: COVID-19 enfektivite ve fatalitesi üzerine yapılan çalışmamızın etki büyüklüğü d=0,092 (p=0,000) olarak hesaplandı. Cohen'e (1988) göre araştırmalar yüksek etki büyüklüğüne sahip ve heterojen yapıda bulundu. Heterojeniteyi araştırmak için yapılan alt grup verilerine ait moderatör analizi sonucuna göre, yaş, cinsiyet, klinik bulgu ve komorbiditenin ortalama etki büyüklüğü için bir moderatör olduğu (p<0,05) saptandı. Bu bağlamda COVID-19 enfektivite ve fatalitesinin demografik özellikler, klinik tablo ve komorbidite ile anlamlı ve etkili olduğu saptandı.

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Sonuç: Pandemi gibi global halk sağlığı sorunlarında hemşirelik alanında meta-analiz yöntemi kullanılarak yapılan ve pandeminin ilk yılını kapsayan bir çalışma olması nedeniyle, daha geniş bir zaman dilimi kullanılarak, geniş yaş kategorilerinde ve daha farklı ülkelerde yapılan çalışmaların da eklenmesiyle yapılacak çalışmalar için yol gösterici olacağı düşünülmektedir.

Anahtar Kelimeler: COVID-19, enfektivite, fatalite, Koronavirüs, meta-analiz

INTRODUCTION

The struggle with the fluctuations of the coronavirus disease-2019 (COVID-19), which has affected the whole world and has come to the fore with its viral variants, continues. Considering the frequent occurrence of COVID-19, which entered our lives as a pandemic, the increase in hospitalization rates due to the need for medical care, and its fatality, it has been determined to be a global public health problem. In the statement made by World Health Organization (WHO) on December 31st, 2019, it was announced that there was a cluster of cases belonging to pneumonia cases of unknown cause. As a result of the investigations, it was announced that these clusters of cases were caused by viruses, this virus was named Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) and the disease was named COVID-19. COVID-19 has existed in 210 countries, regardless of continent, in a one-year period since its official recognition, causing more than 1.7 million deaths and over 79 million positive cases[1].

The virus, which enters the body by respiratory or mucosal route after contact with infected areas, presents with a wide range of clinical manifestations, from mild infection to life-threatening severe clinical manifestations. This virus, which basically causes respiratory system infection, has both acute and long-term effects on mental health, as well as threatening physical health in humans. Although it is currently in the normalization process with the epidemic, the virus, which has survived by undergoing many mutations over time, continues to arouse feelings of fear, stress and anxiety in individuals[2-4].

The rapid spread of the virus all over the world after its appearance shows that the rate of contagion is very high. Moreover, Considering the increased fatality by passing a large number of mutations, it was declared as a COVID-19 pandemic by the WHO on March 11, 2020[5].

From the time the virus started the pandemic until December 2022, that is, in a period of about 3 years, it infected more than 655 million people in the world and caused the death of more than 6.6 million people, creating a public health problem at the international level. It has been observed that the numbers of COVID-19 cases and deaths have increased due to reasons such as the virus, which transmits rapidly and causes death by disease, frequently mutates, there is no proven definitive treatment yet, individuals do not show sufficient sensitivity in vaccination, and individuals do not use personal protectors in social areas. The biggest risk groups for this disease consisted of individuals over 65 years of age, those with comorbid diseases, healthcare workers, pregnant women, and children[1,2,6-8].

In this study, it is aimed to evaluate the infectivity and fatality of the COVID-19 pandemic in a one-year period from the moment it emerged, using the meta-analysis method, and to determine the public health approaches for prevention.

Purpose of the Research

To evaluate the infectivity and fatality of the COVID-19 pandemic in a one-year period from the time of its emergence, to summarize the prevalence and fatality of COVID-19, to evaluate the fatality in infected cases.

MATERIALS AND METHODS

Research Protocol and Registration

As a research protocol, it was created by the PRISMA steps, consisting of evidence-based items including reporting items used for systematic review and meta-analysis studies, and reported in the International Prospective Systematic Review Registry database (ID: CRD 42021255449).

Eligibility Criteria

In our study, studies within a period of 1 year from the date of first appearance of the cases were included by making a time limit. Retrospective descriptive and cohort studies were evaluated in the study. Moreover, observational studies were included to evaluate infectivity and fatality. The language of the article was determined as English and Turkish. We included posts from December 1st, 2019 to December 31st, 2020. Studies conducted in languages other than those specified, articles that did not specify specific data, articles and letters containing opinions, and studies that reported cases with missing data were excluded.

Data Sources and Search Strategy

PubMed, Medline, Cochrane Library, Science Direct, YÖK Thesis, Ulakbim, Ministry of Health, Who Global Index were used as databases. MeSH (Medical Subjects Headings) content was used in structuring the keywords in the search. In databases; "Covid-19 and infectivity", "Covid-19 and fatality", "New type coronavirus and infectivity", "New type coronavirus and fatality", "SARS-Cov-2 and infectivity", "SARS-Cov-2 and

fatality" ", "Coronavirus and infectivity", "Coronavirus and fatality" structures were scanned in both languages.

Study Selection

In the first search, the title and summary of the studies were scanned first. The full texts of the articles to be evaluated were examined, taking into account the determined criteria (inclusion and exclusion) (PRISMA flow diagram) (Figure 1). Only one study was counted from the same studies. Studies without numerical data were not included in the study.

Data Collection Process

A coding form created by the researchers was developed. This form includes three main headings. These are the identity of the study (type of study reviewed, country of study, year and date of publication, author(s), and sample size of the study), characteristics of the study (clinical characteristics (e.g., high fever, cough), number of deaths, comorbidities), and study data (effect size, Sd, Q, N, P, T2, I2, and Z.), which were filled in independently by two researchers. The two encoders were then brought together to perform cross-checks. Numerical data were reviewed at least twice in order to avoid duplicate articles or duplicate information and to avoid errors during coding. In this way, coding reliability was ensured, and intercoder compliance reliability was also calculated using the Cohen's Kappa statistical method. The result of Cohen's Kappa statistic was obtained as confidence ($\kappa=0.95$). Jadad score and Newcastle Ottawa Criterion were used to evaluate the quality of the studies determined to be used in the research.
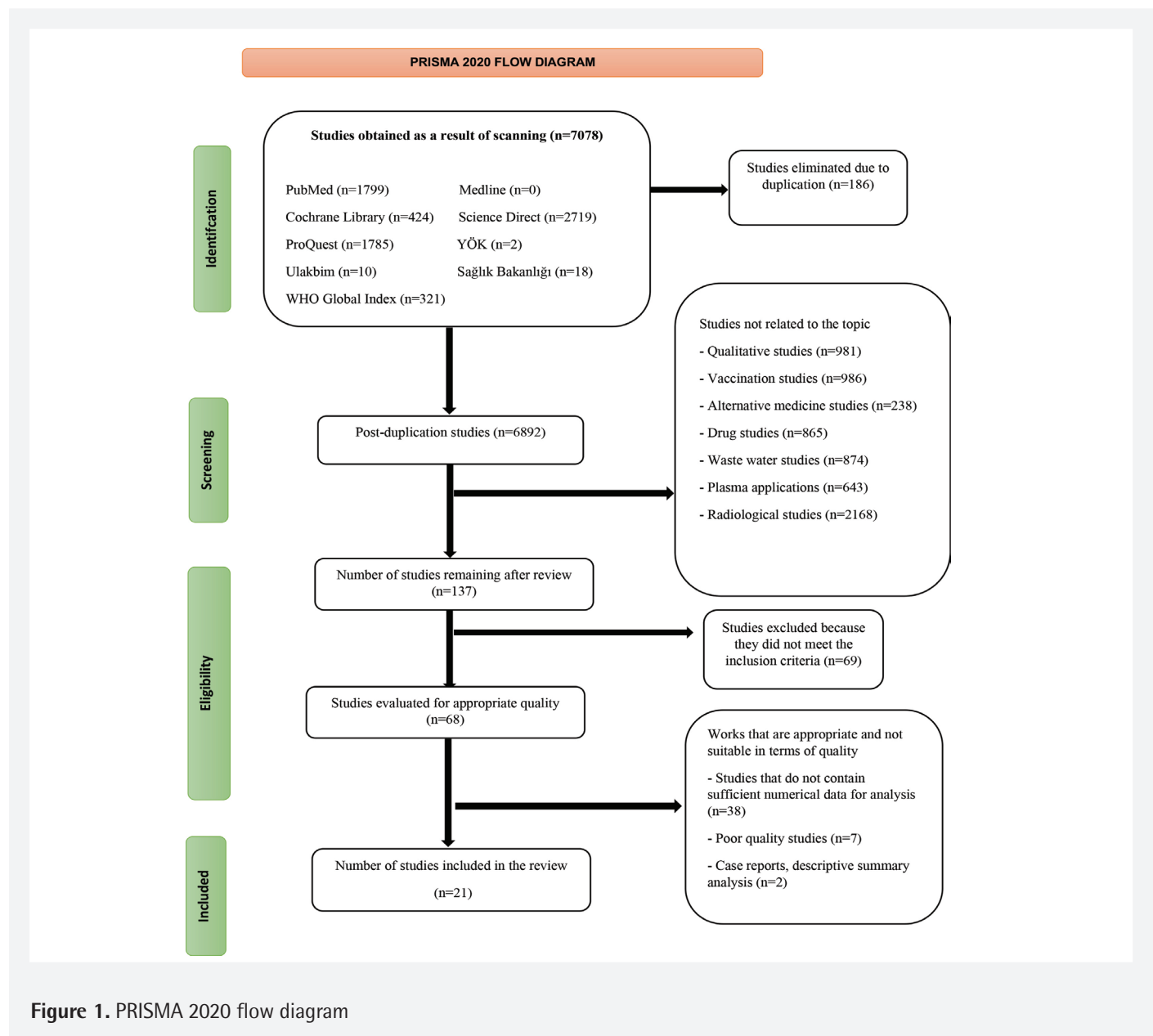

Figure 1. PRISMA 2020 flow diagram

Publication bias was evaluated using the funnel plot graph, as well as Fail-Safe N and Begg and Mazumdar's Kendall's Tau coefficient (p>0.05). A random effects model was used to calculate the 95% confidence interval (CI).

Statistical Analysis

Due to the unit mismatch of the studies accepted for the study, all data were converted into a standard measurement for the relevant variable and a common language was used. Percentages and means±standard deviations (SD) were calculated to describe the distributions of the categorical and continuous variables, respectively. Weighted averages and SDs were studied, since information on all cases included in the analysis was not included. Descriptive data were calculated using licensed Statistical Package for the Social Sciences statistics version 24. Microsoft Office Excel program was used to create the coding tables of the data. For statistical analysis, the Comprehensive Meta-Analysis (CMA) licensed version (CMA version 3) package program used in meta-analysis studies was utilized. The pooled prevalences with 95% CI were attempted to be summarized using weighted effect sizes for each grouped study variable using a random effects model. Weighting was done by considering sample sizes of systematic studies and meta-analysis studies.

Cochran's heterogeneity measures including Q statistics, I^2 index, p value, Tau and Fail-Safe N tests were calculated and reported. We conducted moderator analyses of data on demographic variables (age and gender), clinical findings, and comorbidity.

RESULTS

Selection of Evaluated Studies

A total of 7078 studies were found in the literature review using the search strategy. Considering the exclusion criteria, a total of 21 articles were included in the study. Considering the research design of these studies, 13 of them were in descriptive analysis and 8 of them were in cohort structure. The characteristics and methodological quality scores of the included studies are shown in Table 1.

In our research, the studies published between December 2019, when the COVID-19 infection emerged, and December 2020 were handled with a time limit. Most of the studies evaluated were performed in China (95.0%). One of them took place in Italy. Studies with moderate and high methodological quality assessments were included in the study (Jadad >2, Newcastle Ottawa Criterion ≥5). The characteristics of the included studies, the first authors, and the findings of the methodological

Table 1. Characteristics of studies included in the analysis and their methodological quality scores

First author	Release date (D/M)	Data source country	Research design	Size of study (n)	Jadad score (0–5)	Newcastle Ottawa Criterion (0–9)
Du et al.[13]	01/06	China	Descriptive research	85	2	–
Chen et al.[11]	21/02	China	Descriptive research	99	2	–
Zhou et al.[29]	28/03	China	Cohort study	191	–	7
Wang et al.[21]	17/03	China	Descriptive research	138	3	–
Onder et al.[20]	23/03	Italy	Descriptive research	355	2	–
Yang et al.[25]	01/07	China	Cohort study	205	–	8
Hua et al.[18]	20/02	China	Descriptive research	44672	2	–
Wan et al.[22]	01/04	China	Descriptive research	135	2	–
Huang et al.[17]	15/02	China	Descriptive research	41	3	–
Liu et al.[19]	05/05	China	Descriptive research	137	2	–
Guan et al.[16]	28/02	China	Descriptive research	1099	2	–
Chu et al.[12]	06/04	China	Cohort study	54	–	7
Feng et al.[14]	01/06	China	Cohort study	476	–	8
Gao et al.[15]	10/04	China	Cohort study	43	–	5
Xu et al.[24]	01/04	China	Cohort study	50	–	5
Zhen et al.[28]	01/03	China	Cohort study	161	–	5
Xu et al.23	19/02	China	Descriptive research	62	2	–
Chen et al.[10]	01/05	China	Descriptive research	21	2	–
Zhang et al.[27]	01/07	China	Cohort study	140	–	8
Chang et al.[9]	01/03	China	Descriptive research	13	2	–
Yang et al.[26]	01/04	China	Descriptive research	52	2	–

quality score were given in detail. The descriptive data of the studies included in the research are shown in Table 1.

Findings of Descriptive Data

A total of 48,229 people, 51.9% of whom were targets, participated in 21 studies. In the evaluated studies, individuals over the age of 65 years constituted the majority of the participation (90%). The most common clinical findings were high fever (88.0%) and cough (64.5%). These are followed by other findings. The comorbidity status was evaluated, while hypertension was the first rank for chronic diseases, followed by liver diseases (3.8%) and diabetes (3.2%) (Table 2).

Findings of Demographic Variables

In 20 of the evaluated studies, only the mean age of the patients was given, and the mean age was found to be 52.4 years (95% CI: 0.52-0.19), like the mean age in the other study. In terms of gender distribution, 51.9% were male (95% CI: 0.04-0.18) (Table 2).

Findings of Clinical Data

There were 3 studies that did not include clinical findings. Studies that included clinical findings were analyzed among themselves. The most common findings were reported to be fever (88.0%, 95% CI: 0.43-0.16), cough (64.5%, 95% CI: 0.43-0.16) and burnout (40.0%, 95% CI: 0.04-0.23). Since all of the studies included in the study did not contain the same clinical findings, common clinical findings were evaluated (Table 3).

Findings of Comorbidity

Three studies were exempted from comorbidity assessment due to lack of data. Available data were analyzed among themselves. The most common chronic diseases were identified to be hypertension (6.9%, 95% CI: 0.03-0.14), chronic liver disease (3.8%, 95% CI: 0.02-0.19) and diabetes (3.2%, 95% CI: 0.05-0.23) (Table 4). Considering the degrees of freedom, when the evaluations made at the CI of 0.05 from the χ^2 critical values, the table showed that the study was heterogeneous; when compared to the Q statistics, the I^2 index also revealed the level of heterogeneity.

DISCUSSION

There are 21 studies with a sample size of 13-44,672 in the research. When the sample size of these studies included in the analysis was evaluated, it was seen that 52.4% of them studied with 100 people or more (n>100). There is information that the large sample size of the individual studies included in the meta-analysis narrows the CI. This subject is also examined in the funnel plot graph. The number of samples is important for the generalization of the results of meta-analysis studies and for guiding future studies. It is possible to say that the research subject is COVID-19, which creates a worldwide pandemic, and that the period determined for the study (studies within 1 year from the day of the pandemic) gives priority to treatment and prevention methods. For this reason, it is considered normal to have a limited number of studies in the relevant period[36,37].

Table 2. Findings of the descriptive data of the studies included in the analysis					
Variable	Frequency	Percentage	Variable	Frequency	Percentage
Research design			Sample size		
Descriptive	13	61.9	n<100	10	47.6
Cohort	8	38.1	100<n<1,000	9	42.9
			n>1,000	2	9.5
Age			Gender		
n≤65	18	90.0	Man	25,062	51.9
n>65	2	10.0	Woman	23,167	48.1
Clinical findings*			Comorbid diseases*		
Fire	18	88.0	Other diseases	10	8.7
Cough	18	64.5	Hypertension	15	6.9
Burnout	12	40.0	Chronic liver diseases	9	3.8
Anorexia	4	25.3	Diabetes	18	3.2
Dyspnea	15	23.3	Cardiovascular disease	17	2.6
Myalgia	16	19.2	Chronic kidney diseases	8	1.3
Headache	14	12.2	COPD	16	1.2
Diarrhea	17	7.5	Malignancies	13	0.9
Vomiting	10	4.6			
*It was determined that the criteria determined in the patients included in the studies were different from each other, and the criteria commonly used in the study were analyzed.					
COPD: Chronic obstructive pulmonary disease					

It was observed that the researchers used descriptive and cohort research techniques obtained by using existing registration systems. All data were quantitative as it was a retrospective study. No qualitative study was found on the specified dates. All studies included in the study were published in 2021 due to the time limitation. For this reason, we can say that the fact that the studies (20 studies) were carried out in China, the country where the pandemic was first seen, brings regional limitations to our research, since it will take time to see cases in other countries and to carry out studies in a limited time[9-29].

In the study, it was observed that the transmission was in both genders, while the rate of infection was higher in men than in women. The fact that the majority of the cases (90.0%) covered in the study were under 65 years of age indicates that the active population has a high spread of contagiousness, while it is known that most of the patients who died in the studies were men aged 60 years and over. However, there is a need for a detailed examination of the causes of death, taking into account all ages, genders and underlying comorbidities, and more studies with high levels of evidence.

While evaluating effect sizes in meta-analysis studies, many tests are used to test publication bias. The most used of these tests is the funnel plot chart. Since the funnel plot is a subjective evaluation, it is not considered sufficient on its own when evaluating publication bias. While evaluating publication bias in the study, other statistics were also evaluated.

When the fail-safe N statistics are examined, a total of 1482 reverse studies are needed in order to invalidate the meta-analysis results of the 21 studies included in the study. Since the number 1482 is very far from 21 and is a large number, we can say that there is no publication bias in our study based on

Table 3. Clinical characteristics of the study subjects

Variable	Number of studies (n)	Mean/prevalence (%)	95% CI[a]	n	SD	Q[b]	I[2c]	t[2d]	p
Fever	18	88.0	0.43-0.16	3,159	17	277,41	93.9	1,727	<0.001
Cough	18	64.5	0.43-0.16	3,159	17	277,41	93.9	1,727	<0.001
Burnout	12	40.0	0.04-0.23	2,333	11	242,955	95,472	2,341	<0.001
Anorexia	4	25.3	0.01-0.08	498	3	40,028	92.5	11,314	<0.001
Dyspnea	15	23.3	0.04-0.17	2,893	14	232,47	93.9	1,907	<0.001
Myalgia	16	19.3	0.04-0.17	2,814	15	263,49	94.3	2,080	<0.001
Headache	14	12.2	0.04-0.18	2,569	13	208,466	93.8	2,287	<0.001
Diarrhea	17	7.5	0.04-0.13	3,107	16	206,76	92.3	1,388	<0.001
Vomiting	10	4.6	0.04-0.27	2,224	9	238.118	96.2	2,540	<0.001

[a]95% CI=95% confidence interval.
[b]Cochran's Q statistic for heterogeneity.
[c]I2 index for the degree of heterogeneity.
[d]Tau-squared measure of heterogeneity.
CI: Confidence interval, SD: Standard deviation, COPD: Chronic obstructive pulmonary disease

Table 4. Comorbidities of the study subjects

Variable	Number of studies (n)	Mean/ prevalence (%)	95% CI[a]	n	SD	Q[b]	I[2c]	t[2d]	p
Other diseases	10	8.72	0.05-0.27	2,363	9	243,890	96.3	2,196	<0.001
Hypertension	15	6.9	0.03-0.14	4,7606	14	612,203	97.7	2,117	<0.001
Chronic liver diseases	9	3.8	0.02-0.19	2,030	8	130,656	93.9	2,444	<0.001
Diabetes	18	3.2	0.05-0.23	48,112	17	875,243	98.1	2,668	<0.001
Cardiovascular disease	17	2.6	0.05-0.22	48,091	16	862,062	98.1	2,676	<0.001
Chronic kidney diseases	8	1.3	0.04-0.23	2,396	7	182,104	96.2	1,795	<0.001
COPD	16	1.2	0.03-0.16	47,736	15	811,771	98.2	2,527	<0.001
Malignancies	13	0.9	0.08-0.36	47,685	12	855,631	98.6	2,698	<0.001

[a]95% CI=95% confidence interval.
[b]Cochran's Q statistic for heterogeneity.
[c]I2 index for the degree of heterogeneity.
[d]Tau-squared measure of heterogeneity.
CI: Confidence interval, SD: Standard deviation, COPD: Chronic obstructive pulmonary disease

the fail-safe N value. Publication bias was analyzed using Duval & Tweedie's trim and fill and Begg and Mazlumdar statistics. The value in Kendall's Tau diagram was found to be 0.18396, and the fact that this value is greater than 0.05 is another indication that there is no publication bias in our study. All statistics were aimed to obtain reliable results in the meta-analysis study. In addition to the fact that the results in meta-analysis studies are reliable, the number of publications to be included in the research is high, publication bias is the focus of the study in order to prevent only studies with statistically significant results from the analysis[30].

In this context, trying to reach all studies that meet the inclusion-exclusion criteria within the specified period of time by restricting the time of the research in order to exclude only the studies containing the desired results from the meta-analysis also explains the heterogeneity. The effect size (d=0.092, p=0.000) in the analysis of the studies included in the study by applying the random effects model, and the studies included in the meta-analysis according to Cohen's (1998) were found to have high effect size and statistical significance.

In the funnel plot, 2 studies diverging from the mean effect size were observed[20,26]. It is noteworthy that the first of the two studies that diverged was conducted on patients who were diagnosed with COVID-19 and died. In the other study, Yang et al.[26] evaluated 52 patients. In this single-center study, the death of 32 patients included in the evaluation explains the distance to the mean effect size. In 7 studies included in the analysis, fatality was not evaluated, but infectivity and clinical course. Due to the time limitation of the research, more and detailed studies are needed in this area.

The first year of the disease was important for the development of diagnosis and treatment protocols. For this reason, many studies were followed, including laboratory, imaging, clinical findings, mutations due to the evolutionary change of the disease, and critical information that should be carefully examined in a new pandemic process. This research has been studied to guide new studies by summarizing the data on infectivity and fatality by including clinical data and comorbidities of cases with a diagnosis of COVID-19 published from the beginning of the pandemic to the first year of the epidemic. A total of 21 studies involving 48,229 patients were included. Since data with high heterogeneity were obtained in the analyses, meta-regression data were also included.

The fact that the mean age of the studies included in the analysis was 52.4 years can be called a limitation of the study. It can be said that the number of studies on individuals in the advanced age group is insufficient and this situation may affect the results of the analysis. It is similar to other studies reporting that men (51.9%) are the most frequently infected gender. In the studies conducted for this condition, it was explained that men lack the protection of the X chromosome and estrogen hormone. This datum was not limited to the studies included in the meta-analysis, but was consistent with other studies[31-33].

The data of the clinical findings of the studies were evaluated and a high level of significance was determined. While common clinical findings and incidence are similar to those in other studies, the most common ones are fever, cough and burnout. It was also found to be significant in the subgroup analyses[34].

When the comorbidity data were evaluated, it was observed that some of the patients had more than one chronic disease. The most common complication was hypertension. This is followed by liver disease and diabetes. We can say that the low mean age of the patients evaluated in our study affected the analysis of comorbidity status. Considering the basic limitations of our research, comorbidity was among the findings obtained in the study. In order to evaluate this situation in more detail, studies with high evidence value examining the relationship between COVID-19 and comorbidity should be included by keeping the time limitation of the research wide[35].

Study Limitations

There are some limitations of this research. The number of studies included in the study is small. Since most of the studies were conducted in China, it would be better to include them in studies conducted in a wider geography, given the difference in infectivity and fatality in other countries. Studies published in English or Turkish are included in the search criteria. It is important to expand the language and time limit in order to gain more comprehensive information on the subject.

CONCLUSION

There is a great increase in the infectivity and fatality of COVID-19 disease in the first year of its emergence. This disease has affected the whole world. When infectivity and fatality were evaluated by gender, it was observed that men were exposed to more than women. This disease, which is known to be more deadly in advanced ages, was observed to have affected middle age at the time of the study. All of the studies are retrospective and differ in terms of methodological quality. It has been determined that the most common clinical features in COVID-19 cases are high fever and cough, which are the general symptoms of viral infections. It has become important in chronic diseases in patients infected with COVID-19. The most common chronic diseases encountered in the studies include hypertension, chronic liver diseases and diabetes. It can be said that gender, the clinical picture of the disease and the presence of comorbidity have a significant effect on the infectivity and fatality of the epidemic. Considering that the majority of the studies were conducted in China and the

difference in infectivity and fatality in other countries, there is a need for further studies conducted in a wider geography.

Ethics

Ethics Committee Approval: Since the research is not a study conducted on living things, ethical permission was not obtained since there was no situation that would constitute an ethical violation. Additionally, the studies used in the research It was selected from studies with available full texts and is located in the reference section.

Informed Consent: Meta-analysis study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Design: E.K., T.O., Data Collection or Processing: E.K., Analysis or Interpretation: E.K., T.O., Literature Search: E.K., Writing: E.K., T.O.

Conflict of Interest: No conflict of interest was declared by the authors.

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The Effects of Time of Watching Television and Food Advertisements on Nutritional Status of Preschool Children

Televizyon İzleme Süresi ve Gıda Reklamlarının Okul Öncesi Çocukların Beslenme Durumuna Etkisi

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ABSTRACT

Aim: Watching television for a long time affects the nutrition of children, and the effect of orientation on these foods is quickly seen in children watching food advertisements. The aim of this study was to determine the nutritional status and television viewing time of children aged 3-4 and 5 years, to examine the effect of food advertisements on the nutritional status of children, and to determine how this effect impacted the nutrient intake of children.

Materials and Methods: The study was conducted with 215 adult parents who volunteered to collect information about children in order to determine the nutritional status and television watching time of 3-4 and 5-year-old children studying in a kindergarten in Edirne city center and to evaluate these situations. Children's socio-demographic characteristics, anthropometric measurements, food consumption frequency and food consumption records, nutrition and television watching habits were evaluated.

Results: It was found that 49.8% of children watched television for 2-3 hours a day on average, and 36.7% demanded the food they saw in food advertisements 1-2 times a week. A positive and significant relationship was determined between the fact that the food advertisements on the television attracted the attention of children and the frequency of the children wanting the food they saw in the advertisements, like acidic beverages, fruit juice, chips, chocolate, wafers, sweets, biscuits and crackers (p<0.05). Daily energy intake, the percentage of carbohydrates in the food pattern, and daily sugar consumption were found to be higher in children who paid attention to food advertisements compared to those who did not (p<0.05). It was determined that the consumption of chocolate, wafer, soda and fruit juice increased as the television viewing time increased (p<0.05).

Conclusion: As a result, television viewing time and food advertisements on television can be effective on children's food choices and thus their nutritional status.

Keywords: Child, nutrition, food advertisement

ÖZ

Amaç: Uzun süreli televizyon izlemek çocukların beslenmesini etkiler ve gıda reklamlarını izleyen çocuklarda bu gıdalara yönelim etkisi hızlı bir şekilde görülmektedir. Bu çalışmanın amacı 3-4 yaş ve 5 yaş grubu çocukların beslenme durumları ve televizyon izleme sürelerini belirlemek, gıda reklamlarının çocukların beslenme durumuna etkisini incelemek ve bu etkinin çocukların besin alımını nasıl etkilediğini belirlemektir.

Gereç ve Yöntem: Çalışma, Edirne Şehir Merkezi'nde bir anaokulunda okuyan 3-4 ve 5 yaşındaki çocukların beslenme durumunu ve televizyon izleme süresini belirlemek için gönüllü olan 215 yetişkin ebeveyn ile gerçekleştirildi. Çocukların sosyo-demografik özellikleri, antropometrik ölçümler, gıda tüketimi sıklığı ve gıda tüketimi kayıtları, beslenme ve televizyon izleme alışkanlıkları değerlendirildi.

Bulgular: Çocukların %49,8'inin günde ortalama 2-3 saat televizyon izledikleri ve %36,7'sinin haftada 1-2 kez gıda reklamlarında gördükleri yiyecekleri talep ettikleri saptanmıştır. Televizyonda yer alan gıda reklamlarının çocukların ilgisini çekmesi ile çocukların reklamlarda gördükleri asitli içecekler, meyve suyu, cips, çikolata, gofret, tatlılar, bisküviler ve krakerler gibi yiyecekleri isteme sıklıkları arasında pozitif yönlü ve anlamlı bir

ÖZTÜRKLER ÇAKIR et al. Effect of Television Watching Time on Nutritional Status

Nam Kem Med J 2023;11(4):363-369

ilişki saptanmıştır. Gıda reklamlarına dikkat eden çocuklarda dikkat etmeyenlere göre günlük enerji alımı, besin örüntüsündeki karbonhidrat yüzdesi ve günlük şeker tüketimi daha yüksek bulunmuştur. Televizyon izleme süresi arttıkça çikolata, gofret, soda ve meyve suyu tüketiminin arttığı tespit edildi.

Sonuç: Sonuç olarak, televizyon izleme zamanı ve televizyonda gıda reklamları çocukların gıda seçimleri ve dolayısıyla beslenme durumları üzerinde etkili olabilir.

Anahtar Kelimeler: Çocuk, beslenme, gıda reklamı

INTRODUCTION

The effect of watching television on children depends mainly on two factors. These are content and viewing time. The rate of exposure also increases during the viewing period. For example, it is successful in attracting the attention of even children as young as 1-year-old when advertising messages are accompanied by more colorful, entertaining and unique music. It is a fact created in the group that is most open and sensitive to the influence of television. Considering that children start regularly watching television between the ages of 2 and 2.5 years, this is obviously a pretty big problem[1].

Nutrition is defined as adequate and balanced intake of nutrients containing nutrients necessary for living things and their correct use[2]. The period in which children's physical, mental, emotional and social development accelerates and basic habits are taught to the child, in which their character and personality traits begin to take shape, is the pre-school period, which covers the age range of 0-6 years.[3] Preschool period, in addition to being a time when children grow up rapidly and become more active, it is an important period when children learn about food and determine their food preferences[4]. The foundations of eating habits in later ages are laid in this period. For this reason, preschool children should learn to eat adequate, balanced and healthy nutrition and make it a habit. At this age, the child's family and social environment are very important, as the child begins to imitate those around him. The preschool period is the most appropriate period for the child to focus on nutritious foods and regular physical activities and to acquire a healthy meal order and a healthy life habit[5].

The most effective environment in the development of children's eating habits is the family environment. Children learn first by imitating family members. The content and environment of meals eaten with the family is a very good example in shaping the child's eating habits[6].

The goal of children's balanced and healthy nutrition is that the child can develop at the desired level. The foods that make up the diet should be present in certain proportions from the basic food groups. For example, when there is no food variety, a child's health is adversely affected by a diet based on fat and carbohydrates. Excessive consumption of high-calorie foods also causes overnutrition and, as a result, obesity. The child's consumption of high-calorie foods or overeating does not mean adequate and balanced nutrition. For this reason, it should be present in various and sufficient proportions of food groups in the daily diet of children[7].

Television is the most widely used and most active mass media and it affects children as well as the whole society[8]. According to the Radio and Television Supreme Council (RTÜK)-2018 data, an average of 3 hours and 34 minutes of television is watched per day in Turkey[9]. Preschoolers are the most sensitive target audience of television. In studies conducted, the average daily television watching time of preschool children varies between 2.5 and 3.5 hours[10]. From a pedagogical point of view, preschool children have many needs and television programs specially prepared for them are one of these needs. According to studies, the nutrition of children is affected by the media and the media can cause health problems related to nutrition[11]. The effect of advertisements is not the same on every child. The child's age, gender, social environment, family environment, education level of the family, the conditions in which the advertisement is delivered, the type of the advertised product and many similar factors change the effect of the advertisement[12].

Television significantly affects children's eating habits. As the time spent by children in front of the television increases, the consumption of ready-made food also increases. At the same time, as the time spent watching television increases, children lead a sedentary life. As a result, obesity is more common in children who watch television for a long time. Television can also be used by families as a way of feeding especially young children[8].

Wartella[13] found that television advertisements had a significant impact on the food choices, food purchasing demands and short-term food consumption of children between the ages of 2 and 11 years. In addition, in the same report, it was determined that the media affected the behavior and nutritional routines of 2-5-year-old children towards food and beverages.

Food advertisements with messages for preschool children are widely broadcast on television. Through these messages, it directs children to demand the food products in these advertisements. As a result of these, the healthy eating behaviors of children are replaced by a single type of diet with various additives added, or a similar diet. With the effect of food

advertisements on television, children may start to consume the food they see in the advertisement, although they do not like it before. When television advertisements draw attention to food products that are rich in sugar, poor in protein, and that have added many additives, they lead children to want and consume them[8].

As television viewing time increased, daily energy expenditure decreased, and besides, nutrition in the form of snacking in front of television increased. Television food advertisements make high-calorie foods more attractive and increase the desire to eat these foods. These behaviors, which develop in parallel with the duration of watching television, are among the important factors that increase the incidence of obesity in the society[14]. In this study, it was aimed to determine the nutritional status and television watching time of 3-5-year-old children studying in a kindergarten in Edirne city center and to examine the effects of food advertisements on television on the nutritional status of children.

It was shown that in children aged 2-5 years who watched a special television program for children, exposure to food advertisements within this program was positively associated with more eating behaviors. According to the study, watching and being influenced by food advertisements, usually in early childhood, leads to eating behaviors that promote weight gain[15].

It was observed that children sacrifice their main meals and increase their snack food consumption with the effect of food advertisements. The effect of orientation to these foods is seen rapidly in children who watch food advertisements[16].

Parents and then the child's immediate environment should be adequately informed about the negative effects of television and the media, which have a significant impact on children's eating habits. The effect of the nutrition education given to children in the preschool period continues on the individual in later ages, and this effect can even reach the social level. For this reason, the wrong nutritional behaviors acquired in this period significantly affect the level of social health[11].

Given the seriousness of this public health problem, it is important to inform the public, especially parents of young children, about this issue and to learn more about the underlying factors. The aim of this study was to determine the nutritional status and television viewing time of children aged 3-4 and 5 years, to examine the effect of food advertisements on the nutritional status of children, and to determine how this affected the nutrient intake of children. Although there are similar studies investigating the nutritional status of preschool children affected by food advertisements, our study is more comprehensive than other studies and is thought to contribute to the literature.

MATERIALS AND METHODS

This study was approved by the Trakya University Faculty of Medicine Scientific Research Ethics Committee (protocol no: TÜTF-BAEK 2018/387, date: 19.11.2018). This research was conducted between October 2018 and April 2019. It was conducted cross-sectionally and descriptively with 250 people who were their parents and 215 adults who were volunteers, in order to obtain information about the children aged 3, 4-5 years, who were studying at Ayşekadın Kindergarten in the city center of Edirne. The sample size was determined by the full count method, without the need for any calculations, based on the total number of students in the kindergarten where the research would be conducted. The data of the study were obtained by socio-demographic characteristics of children and their families, anthropometric measurements of children, nutritional habits, television watching habits and a questionnaire including children's interest in advertisements, 48-hour food consumption record, food consumption frequency record. The amounts of all foods consumed by the children included in the study for two days were questioned. The data obtained were entered into the BEBIS 6.1 program. The children included in the study were questioned about which foods they consumed and how often in the last month. The participant was given 6 options for consumption frequency. The participant who chose one of these options was asked how much of that food he consumed at one time. The answers to the questions "Do food advertisements on television attract your child's attention and does your child demand from you the foods he sees in food advertisements?" were used to measure interest in the advertisements.

Statistical Analysis

While evaluating the findings obtained in the study, Statistical Package for the Social Sciences 25 software was used for statistical analysis. While evaluating the study data, in addition to descriptive statistical methods such as numbers, percentages, averages, and standard deviations, the chi-square test was used for differences between the groups of qualitative data, and the Mann-Whitney U tests were used for pairwise comparisons of non-normally distributed variables. The results were evaluated at the 95% confidence interval and the significance level of $p<0.05$.

RESULTS

It was determined that 54% of the children were girls and 46% were boys. It was determined that the mean body mass index (BMI) value was 15.86 ± 2.84 kg/m² in girls and 16.29 ± 2.49 kg/m² in boys (Table 1).

It was revealed that 41.9% of children were fed four meals a day. While 38.8% of girls ate three meals, 47.5% of boys ate

ÖZTÜRKLER ÇAKIR et al. Effect of Television Watching Time on Nutritional Status

Nam Kem Med J 2023;11(4):363-369

four meals. 80.5% of children regularly had breakfast every day. 67.0% of them sometimes skipped their meals. Of those who skipped meals, 40.1% skipped snacks and 37.9% skipped lunch. 54.2% of them cited lack of appetite as the reason for skipping meals. Daily water consumption of 42.8% was determined as 501-1000 mL.

49.3% of children had a television at home and 41.3% had two televisions. 49.8% of them watched television for 2-3 hours a day on average. 48.4% of children whose attention was attracted by television advertisements demanded the foods they saw in food advertisements 1-2 times a week. A statistically significant difference was found between the number of televisions at home and the frequency of children's demand for the foods they saw in food advertisements, and whether television advertisements attracted the child's attention (p=0.001) (p<0.05).

It was determined that the children who attracted the attention of food advertisements on television consumed packaged products more frequently, and there was a significant difference between them (p<0.05). While 50.7% of children never consumed soda, 42.8% consumed acidic beverages 1-2 times a week. A significant difference was found between the frequency of consumption of soda and the way food advertisements on television attracted the attention of the child (p=0.001) (p<0.05). A statistically significant difference was found between the number of televisions in the house and the ability of food advertisements to attract the child's attention (p=0.035). No relationship was found between the number of televisions in the house where the child lived, the frequency of requesting the foods he saw in food advertisements, and the average daily television viewing time and whether food advertisements attracted the child's attention (p=0.099).

87.7% of children whose attention was drawn by television advertisements consumed milk, 45.2% coke, 74.2% fruit juice, 7.7% other carbonated beverages. 62.8% of children did not consume coke and 92.6% did not consume other carbonated drinks. 16.7% of children who did not notice television commercials did not consume milk, 83.3% did not consume coke, 45% did not consume fruit juice, and 93.3% did not consume other carbonated drinks. In the statistical analysis, a significant difference was found between the

consumption of coke and fruit juice and the attention of the child by the food advertisements on the television (p=0.006) (p<0.05). No significant difference was found between the consumption of milk and other carbonated beverages and the attention of the child by the food advertisements on the television (p>0.05). 36.7% of children consumed chips, 72.6% consumed chocolate-wafers, 23.3% consumed sweets, 65.1% consumed biscuits-crackers. Of the children whose attention was attracyed by television advertisements, 43.9% consumed chips, 80.6% chocolate-wafers, 27.1% candies, 69.7% biscuits-crackers. 76.7% of children did not consume sweets and 63.3% did not consume chips. 81.7% of children who did not notice television advertisements did not consume chips, 48.3% did not consume chocolate wafers, 86.7% candies, 46.7% did not consume biscuits-crackers. In the statistical analysis, a significant difference was found between the consumption of chips, chocolate, wafers, sweets, biscuits and crackers and the attention of the child by the food advertisements on the television (p<0.05). There was no significant difference between boys and girls in terms of junk food consumption (p=0.0371). It was found that children who were attracted to television advertisements consumed junk food 3-4 times a week and every day significantly more than those who were not (p=0.001).

In Table 2, the relationship between children's television food commercials attracting the attention of the child and the average daily energy, sucrose and fiber intake, and the protein, carbohydrate and fat percentages of children's daily diets are compared. Daily energy intake and nutrient patterns are associated with the attention of children in food advertisements on television. The daily energy intake of children who were attracted by food advertisements was found to be higher. While the average daily energy intake of the children who were influenced by the food advertisements on the television (1st group) was 1743.50±215.92 calories, the daily energy intake of the children who did not notice the food advertisements on the television (2nd group) was 1648.38±195.59 calories. A statistically significant difference was found between the two groups (p=0.001). The daily protein percentages of children who were attracted to food advertisements were found to be lower. There was a significant difference between the groups (p=0.002). When the groups were compared in terms

Table 1. Anthropometric measurement averages of children by gender and 50th percentile range according to 3–5 years of age						
Anthropometric measurements	Girl			Boy		
	Mean	Standard deviation	50th percentile	Mean	Standard deviation	50th percentile
Height (cm)	112.58	6.90	99-112.2	114.15	7.08	100-112.9
Body weight (kg)	20.06	3.86	15-19.1	21.21	4.17	15.3-19.4
BMI (kg/m²)	15.86	2.84		16.29	2.49	
BMI: Body mass index						

Table 2. The relationship between television food commercials attracting the attention of the child and daily energy and some nutrient intakes

Daily average	Child's attention to television food commercials				
	Attention	No attention	Total	TÜBER reference values	p values
	n (155) $\bar{x}\pm$SD	n (60) $\bar{x}\pm$SD	n (215) $\bar{x}\pm$SD		
Energy intake (kcal)	1743.50±215.92	1648.38±195.59	1716.96±214.31	1200	**0.001***
Protein percentage (%)	15.89±2.06	16.87±1.92	16.16±2.07	5-20	**0.002***
Fat percentage (%)	37.81±4.05	38.53±4.70	38.01±4.24	20-35	0.231
Carbohydrate percentage (%)	46.24±4.88	44.48±5.41	45.75±5.08	45-60	**0.033***
Sucrose intake (gr)	38.07±15.51	30.97±12.44	36.09±15.03	<25	**0.003***
Fiber intake (gr)	15.73±3.54	15.63±3.33	15.70±3.48	14	0.623
SD: Standard deviation, TÜBER: Turkey Nutrition Guide					

of fat intake percentage, the difference was not statistically significant (p=0.231). The daily carbohydrate percentages of children who were attracted to food advertisements were found to be higher. There was a significant difference between the groups (p=0.033). The daily sucrose intake of children who were attracted to food advertisements was found to be higher. There was a significant difference between the groups (p=0.003). The average daily fiber intake of the children in the 1st group was 15.73±3.54 g, the average daily fiber intake of the children in the 2nd group was 15.63±3.33 g. The mean of all children was 15.70±3.48 grams. There was no statistically significant difference between the groups (p=0.623). It was determined that the daily average energy intake, fat percentage, sucrose and fiber intake were above the recommended amounts.

DISCUSSION

Children's nutritional status, feeding frequency, skipping meals, and one-way eating habits are effective on their health status[17]. Tuna et al.[18] found in their study that 79.1% of obese children ate at least 3 main meals, while 20.9% ate 2 main meals.

Tokuç et al.[19] reported that 53.4% of the students watched television for 2 hours or more per day. 49.3% of the children in this study had one television at home, and 37.7% had two televisions at home. In the RTÜK-2018 data, it has been determined that an average of 3 hours and 34 minutes of television is watched daily in Turkey[9]. Similar to this and other studies, 49.8% of the children in this study watched television for an average of 2-3 hours a day.

Media is among the factors affecting the food choice of preschool children. Studies have found that food advertisements on television have strong effects on the food choices and food demands of children aged 2-11 years[13]. In another study, it was found that 52.5% of the children sometimes wanted to have the products they saw in the advertisements, 35% wanted the product they saw in the advertisements, and 12.4% did not[20].

The eating habits of preschool children are influenced by the media, especially television advertisements. In a study, it was found that 33.55% of children asked their parents for the foods they saw in advertisements, while 15.31% did not[21].

36.7% of the children in this study demanded the foods they saw in food advertisements 1-2 times a week, 32.1% 1-2 times a month, 8.4% every day.

Food advertisements affect children's food choices and demands. Günlü and Derin[20] determined that after watching the advertisements, 48.5% of the students bought chocolate, chips, cake and candy, 58.7% of them bought coke drinks and 19% of them bought fruit juice. It was determined that 11.5% of children consumed sugar-containing coke drinks, 3.6% consumed diet coke drinks and 25.4% consumed candy, confectionery, bars, wafers and chocolate every day according to a study conducted throughout Turkey[22]. It was determined that 45.1% of the children in this study consumed packaged products such as chips-chocolate-biscuits 1-2 times a week, 28.8% consumed 3-4 times a week, 25.2% every day.

It is thought that food advertisements targeting children and socioeconomic status of families affect children's consumption of junk food, and parents and themselves should be conscious about reducing their consumption of junk food.

Eating in the form of snacks during the time spent in front of the television and computer causes weight gain in children. Francis et al.[23] found that television had a significant effect on the increase in BMI of television viewers. In this study, daily energy intake and nutrient patterns were associated with children who were attracted to food advertisements on television. The daily energy intake of children who were attracted to food advertisements was found to be higher. Daily energy intake was found to be above the energy reference value that should be taken daily according to Turkey Nutrition Guide (TÜBER). In a study, the amounts of energy, nutrients and fiber intake of children and the adequacy level of these amounts according

ÖZTÜRKLER ÇAKIR et al. Effect of Television Watching Time on Nutritional Status

Nam Kem Med J 2023;11(4):363-369

to the amounts recommended in TÜBER were evaluated. It was found that the average energy intake (1189.75±213.82 kcal) was inadequate, while protein (46.87±8.74 g) and carbohydrate (131.51±28.97 g) intakes were adequate[21].

In studies conducted with children aged 2-5 years in the USA and England, it was determined that approximately one-fourth of the children were overweight or obese, and this situation was associated with the media[24].

Unlike other studies, no significant relationship was found between media and BMI. In this study, age was found to be associated with attention to food advertisements on television. The age of the children who were attracted by television advertisements was found to be higher. It is thought that the reason why the advertisements attract the attention of children more as they get older is related to the increase in the development of the child's mental and perception skills.

Study Limitations

The limitations of the study were that it was conducted in a single center, the number of study groups was small, and the survey data were based on parental statements.

CONCLUSION

According to the results obtained from this study, it was observed that children's television viewing time decreased as the mother's education level increased (p<0.05). It was found that children who were attracted by food advertisements on television consumed more packaged products and unhealthier snacks (p<0.05). As the duration of television viewing increased, children's consumption of some unhealthy foods increased (p<0.05). No relationship was found between the duration of watching television and the attention of children with food advertisements. A positive and significant relationship was found between the frequency of children's demand for the foods they saw in food advertisements and the status of television advertisements attracting the attention of the child. It has been determined that the rate of television advertisements attracting the attention of children increases with the increase in the number of televisions at home. The daily energy intake and sucrose consumption of children who were attracted to food advertisements were found to be higher, and the percentages of fat and carbohydrate in the daily food pattern were found to be higher and protein was found to be lower. A significant correlation was found between energy intake, protein and carbohydrate percentages and food advertisements attracting their attention (p<0.05). Age and weight were found to be associated with the attention of food advertisements on television, and the age and weight

of children who were attracted to television advertisements were found to be higher. There is no difference in junk food consumption according to the gender of the children. We can say that television viewing time and food advertisements can have an impact on children's food choices and thus their nutritional status, and this is important for the healthy development of the child. In terms of preventive health services, it is recommended that this situation be taken into account in the primary care providing health services, and parents should be given education and information about it.

Ethics

Authorship Contributions

Surgical and Medical Practices: U.B.Ö.Ç., S.D., Concept: U.B.Ö.Ç., S.D., S.C., E.M.Ş., Design: U.B.Ö.Ç., S.D., S.C., E.M.Ş., Data Collection or Processing: U.B.Ö.Ç., S.D., Analysis or Interpretation: U.B.Ö.Ç., S.D., Literature Search: U.B.Ö.Ç., S.D., S.C., Writing: U.B.Ö.Ç., S.D., S.C., E.M.Ş.

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Impact of Hiatal Hernia on CT-Severity Scores and Survival of COVID-19 Patients

Hiatal Herninin BT-Şiddet Skorlarına ve COVID-19 Hastalarının Sağkalımına Etkisi

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ABSTRACT

Aim: We aimed to investigate the impact of hiatal hernia (HH) on computed tomography (CT) findings, CT-severity scores (CT-SS), intensive care unit (ICU) admission, and mortality rates of patients with Coronavirus disease-2019 (COVID-19).

Materials and Methods: Our study was a single-center retrospective analysis of COVID-19 patients admitted to our hospital between July and October 2021. One hundred-forty four of these patients had HH. We also randomly selected 144 COVID-19 patients without HH as the control group. So, the total study population included 288 patients aged ≥18 years. Chest CT scans examined by the radiologist for the presence of HH and CT-SSs were calculated using a visual scoring system.

Results: The presence of HH was statistically associated with COVID-19 Reporting and Data System classification of initial CT (p=0.002) and CT-SS (p<0.001). Also, HH was statistically associated with ground-glass opacity, pericardial effusion, crazy paving pattern, pleural, bronchial wall thickening and reversed halo sign in CT imaging. Multivariate analysis showed that HH was associated with a higher rate of need for ICU admission [odds ratio (OR): 2.47, p=0.01] and mortality (OR: 2.04, p=0.02).

Conclusion: The presence of a HH has important implications for the severity of pneumonia and increased ICU admission and mortality in COVID-19 patients.

Keywords: Hiatal hernia, COVID-19, computed tomography, pneumonia

ÖZ

Amaç: Hiatal herninin (HH) Koronavirüs hastalığı-2019 (COVID-19) hastalarının bilgisayarlı tomografi (BT) bulguları, BT-şiddet skorları (BT-ŞS), yoğun bakım ünitesine (YBÜ) yatış ve ölüm oranları üzerindeki etkisini araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmamız Temmuz-Ekim 2021 tarihleri arasında hastanemize başvuran COVID-19 hastalarının tek merkezli retrospektif analiziydi. Bu hastaların 144'ünde HH vardı. Ayrıca kontrol grubu olarak HH'si olmayan 144 COVID-19 hastası rastgele seçildi. Dolayısıyla, toplam çalışma popülasyonu 18 yaş ve üstü 288 hastayı içermekteydi. Toraks BT taramaları, bir radyolog tarafından HH varlığı açısından incelendi ve BT-ŞS'leri, görsel bir puanlama sistemi kullanılarak hesaplandı.

Bulgular: HH varlığı istatistiksel olarak ilk BT'nin COVID-19 Raporlama ve Veri Sistemi sınıflandırması (p=0,002) ve BT-ŞS'si (p<0,001) ile ilişkiliydi. Ayrıca HH, BT görüntülemede buzlu cam opasitesi, perikardiyal efüzyon, kaldırım taşı paterni, plevral, bronşiyal duvar kalınlaşması ve ters halo işareti ile istatistiksel olarak ilişkiliydi. Çok değişkenli analiz, HH'nin daha yüksek oranda YBÜ'ye yatış [odds oranı (OR): 2,47, p=0,01] ve mortalite (OR: 2,04, p=0,02) ile ilişkili olduğunu gösterdi.

Sonuç: HH'nin varlığının, COVID-19 hastalarında pnömoninin ciddiyeti ve YBÜ'ye yatış ve mortalite artışı için önemli etkileri vardır.

Anahtar Kelimeler: Hiatal herni, COVID-19, bilgisayarlı tomografi, pnömoni

INTRODUCTION

The Coronavirus disease-2019 (COVID-19) was first seen in December 2019, in Wuhan and the disease spread rapidly all over the world[1]. The real-time reverse transcriptase-polymerase chain reaction (RT-PCR) is the standard diagnostic method for COVID-19, which is taken from oropharyngeal and nasopharyngeal swab samples[2]. However, the RT-PCR test has high initial false-negative results. Computed tomography (CT) plays a major role in the diagnosis of early-stage disease and follow-up of pneumonia[3]. Bilateral, multiple, generally peripheral and basal localized ground-glass opacities (GGOs) with or without consolidation and bronchovascular thickening are the typical chest CT findings of COVID-19 pneumonia[4].

A hiatal hernia (HH) occurs when the intra-abdominal organs, most commonly the stomach, herniate through the esophageal hiatus into the thorax[5]. Most HHs have no symptoms and are diagnosed incidentally[6]. As a result of increased intra-abdominal pressure, the stomach and other intra-abdominal organs may protrude into the mediastinum[7,8]. Obesity and being elderly are the most important causes of HH[9,10]. Most patients with HH have various nonspecific symptoms resulting from gastroesophageal reflux disease (GERD). Rarely, large HHs can cause atypical symptoms such as shortness of breath and chest pain and uncommon complications such as pulmonary edema and heart failure due to compression of the heart and pulmonary veins by organs protruding into the chest cavity[11]. There are case reports in the literature that show a large HH can cause acute heart failure attacks and acute angina as a result of compression[12-14].

CT is not usually used routinely to diagnose HH, but it can provide useful additional information about the location and type of HH[15,16]. It is often diagnosed incidentally when performing a CT scan for a different indication[17]. The frequency of GER and esophagitis in patients with HH is higher than in the normal population. In clinical studies, HH and concomitant reflux esophagitis have been associated with respiratory symptoms, most commonly asthma[18] and laryngitis[19].

In the literature, there are many studies on comorbidities as risk factors for COVID-19 patients, especially diabetes, hypertension, respiratory and cardiovascular system diseases [20]. However, there is no study investigating the effects of HH as a gastrointestinal comorbidity in COVID-19 patients. Jiang et al.[21] investigated the prevalence of laryngopharyngeal reflux disease (LPRD) in hospitalized COVID-19 patients. They reported that LPRD was common in hospitalized COVID-19 patients and independently increased the risk of serious illness and poor prognosis[21].

In our study, we detected HHs incidentally in non-contrast chest CT of COVID-19 patients and the patients were categorized as the groups with and without HH. The purpose of this study was to look at the association between HH and CT findings, CT-severity scores (CT-SS), ICU admission, and mortality rates in COVID-19 patients.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice and was approved by the Ethical Committee of Amasya University Faculty of Medicine (approval number: 148, date: 4 November 2021). Informed consent was not considered necessary as the study was conducted retrospectively.

Study Population and Data Collection

Our study was a single-center retrospective analysis of a total of 288 patients who applied to the Amasya University Faculty of Medicine Emergency Service and COVID-19 policlinic between July and October 2021. We included only laboratory-confirmed patients who were determined by positive RT-PCR tests. All patients underwent at least one chest CT scan in the radiology department of our hospital. Patients with at least 3 negative RT-PCR tests, pediatric patients, were excluded from the study.

We collected the data for retrospective analysis including demographic characteristics, comorbidities, laboratory findings, length of stay in the hospital, length of stay in ICU and clinical outcomes, and mortality rates from the electronic medical records.

Sample Size

Using the G-power (latest version 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) program, the study sample was determined to be 280 patients, with at least 140 in each group with an effect size of 0.39, α=0.05, and power (1-β)=0.90 using the G-power program. There were two groups: 144 patients with HH and 144 patients without HH (control). We selected randomly 144 COVID-19 patients without HH as the control group.

Laboratory Procedures

RT-PCR for Severe acute respiratory syndrome-Coronavirus-2 was performed on the oropharyngeal and nasopharyngeal swab specimens of all patients according to World Health Organization interim guidelines. RT-PCR tests were repeated in patients with a high clinical and radiologic suspicion of COVID-19 when the initial PCR test was negative. The laboratory tests obtained within 1 day from the performance of thorax CT at the patient's admission, including complete blood count, serum biochemistry parameters, inflammatory

markers such as C-reactive protein (CRP), creatine kinase, and lactate dehydrogenase level, erythrocyte sedimentation rate (ESR) and ferritin, were recorded from the hospital records.

Computed Tomography Protocol

All non-contrast chest CT scans of the study patients were performed in a supine position using the multidetector CT scanners 128-slice GE Healthcare Revolution EVO CT (GE Medical Systems; Milwaukee, WI). The following acquisition and reconstruction parameters were used: Tube potential was 120 kV; tube current ranged from 100 to 450 mA; beam pitch was 1.375; beam collimation was 64 mm×0.625 mm; gantry rotation was 0.4 seconds; acquisition direction was craniocaudal; reconstruction kernel was standard; slice thickness and section overlap was 0.625 mm. The chest CT scans were evaluated at 1500 WW and 450 WL for the lung window and 400 WW and 40 WL for the mediastinal window. The non-contrast chest CT was obtained in one breath-hold. Multiplanar reconstruction images (axial, coronal, and sagittal images) were used for diagnosing HH.

Image Analysis

A radiologist with more than 15 years of experience in chest CT imaging retrospectively analyzed the CT images in a standard clinical picture archiving and diagnostic system Workstation, blinded to the clinical and laboratory data. A semi-quantitative 25 Point CT-SS system, which was previously used in the literature, was used to visually calculate the involvement of each pulmonary lobe[22]. It was calculated as follows: 0 no involvement; 5% involvement; 25% involvement; 26-49% involvement; 50-75% involvement; and >75% involvement. The total CT-SS was then calculated by adding the CT scores of the five lobes, which ranged from 0 (none) to 25 (maximum).

The imaging findings of chest CTs and COVID-19 Reporting and Data System (CO-RADS) classification of the initial CTs were analyzed. With the CO-RADS classification, the evaluation of thorax CT scans of patients with COVID-19 was standardized and classified between 1 (very low) and 5 (very high) for suspected COVID-19 pneumonia[23].

CT scans were also evaluated for HH using axial, coronal images, and sagittal images with mediastinal window settings. The lower parts of the posterior mediastinum were examined for the presence of HH in thorax CTs using 3D post-processing images. We defined the CT findings for HHs as soft tissue fullness adjacent to the esophagus, the definition of rugal folds of the stomach, above the esophageal hiatus, a lobulated or asymmetrical enteral shape, or a mixture of these findings. HH was considered present if the gastroesophageal junction was displaced approximately 2 cm or more above the esophageal hiatus[24,25].

Firstly, we draw a line through the anterior and posterior parts of the esophageal hiatus on the sagittal planes to determine how much the gastroesophageal junction migrated above the diaphragm. Then, a vertical line from the hiatus line to the gastroesophageal junction was drawn and the distance was measured (Figure 1).
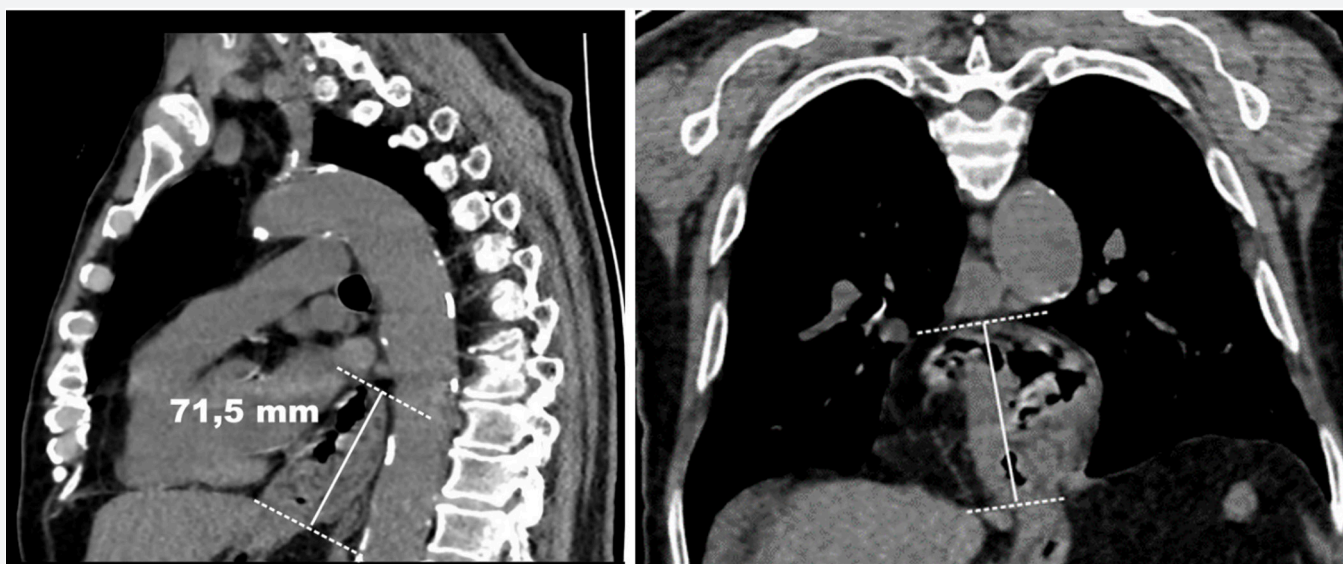

Figure 1. Sagittal (a) and coronal (b) computed tomography scan of a 75-year-old male patient with a hiatal hernia. The length of the hernia was measured (straight line) from the level of the esophageal hiatus (dashed line) to the upper direction of the contour change (dashed line)

Statistical Analysis

Using IBM Statistical Package for the Social Sciences statistics for Windows, version 22.0, statistical analyses were carried out. Descriptive statistics of patients' demographics and laboratory results, total CT scores, and mortality rates were reported as frequency rates, percentages, and mean or median values. Using the Kolmogorov-Smirnov, the variables' conformity to the normal distribution was evaluated. For comparing continuous variables with and without HH, the Mann-Whitney U test was employed for variables with non-normal distribution and Student's t-test for variables with normal distribution. The chi-square test was used instead of the comparison of categorical variables according to the presence of HH. To identify independent risk variables for ICU admission and death of COVID-19 patients, multivariate logistic regression was performed. Statistics were determined significant at $p<0.05$.

RESULTS

Demographic Features

The study population included 288 patients with a mean age of 65 ± 13.9 years; 156 (54.2%) were male. In the group of HH, 79 (54.9%) patients were male with a mean age of 71 ± 11.3 years. Hiatal hernia was statistically associated with older age ($p<0.001$). No significant differences were found between HH and gender. Also, we found no significant difference between gender and mortality ($p=0.896$). However, there was a statistical association between the male gender with high ICU admission ($p=0.02$).

The most common comorbidities of the study population were hypertension (HT) (143/288; 49.7%) and diabetes mellitus (96/288; 33.3%). There was no significant difference between HH and comorbidities.

In addition, when we correlated HH with laboratory findings, statistical associations were found between HH and elevated serum inflammatory markers such as CRP, ESR, increased neutrophil, decreased lymphocyte percentage, and multiple abnormal laboratory findings shown in Table 1.

The patients' median length of stay in the hospital was 13 days, while the median length of stay at the ICU was 10 days. While there was a significant relationship between HH and the length of stay in the ICU ($p=0.015$), there was no significant relationship between the length of stay in the non-ICU ($p=0.221$) (Table 1).

Of all 288 patients, 29 (10.1%) patients were treated as outpatients. Of all inpatients, 66/259 (25.48%) patients were treated in ICU. Of the patients with HH, 48/66 (72.7%) patients were treated in ICU. 84/288 (29.2%) patients died in the total

study population, and 61/84 (72.6%) patients died in the group with HH (Figure 2).

We found a particularly strong association between HH and mortality rates ($p<0.001$) and the need for ICU treatment ($p<0.001$) (Table 2).

The multivariate analysis suggested that HH was related to a higher rate of ICU admission [odds ratio (OR): 2.47, 95% confidence interval (CI): 1.24-4.93, $p=0.01$] and mortality (OR: 2.04, 95% CI: 1.05-3.96, $p=0.02$) (Table 3, 4). There was a significant relationship between mortality with older age (OR: 1.07, 95% CI: 1.03-1.10, $p<0.001$), hiatal hernia (OR: 2.04, 95% CI: 1.05-3.96, $p=0.02$) and chronic heart disease (OR: 3.38, 95% CI: 1.15-9.97, $p=0.02$) shown in Table 3.

The Results of Computed Tomography Images

The median CT-SS of total patients was 10 (0–25). The median CT-SS was 12.5 in the group with HH and the median CT-SS was 8 in the group without HH. There was a statistically significant association between HH with CO-RADS classification of the initial CT imaging ($p=0.002$) and CT-SS ($p<0.001$).

In addition, HH was statistically associated with GGOs ($p=0.003$), pericardial effusion, crazy paving pattern, reticular pattern, linear opacity, intra-interlobular septal thickening, pleural, bronchial wall thickening ($p<0.001$), and reversed halo sign ($p=0.002$) in the CT imaging (Table 2).

DISCUSSION

In this retrospective analysis, we evaluated the association between the presence of hiatal hernia in CT-SS, ICU admission, and mortality rates of patients with COVID-19. According to our study presence of HH was associated with increased mortality and ICU admission rates in COVID-19 patients. Also, our results showed that the COVID-19 patients with HH had higher CT-SS and severe pneumonia CT findings.

The association of many respiratory diseases with HH and GERD has been reported in the literature. In patients with idiopathic pulmonary fibrosis, Tossier et al.[26] found a relationship between the presence of HH and an increased risk of mortality from respiratory causes. They also investigated the presence or absence of HH in coronal and sagittal images of chest CT scans. We also defined HH in the non-contrast chest CT imaging of COVID-19 patients incidentally. The association of hospitalization for reflux esophagitis with hospitalization for numerous upper respiratory and lung diseases was reported. The most common association was seen with sinusitis, laryngitis, pharyngitis, chronic bronchitis, bronchiectasis, asthma, pneumonia, and pulmonary fibrosis[27]. Since HH is associated with reflux esophagitis, it has been reported to be associated with respiratory conditions in various studies[28,29] but it has some limitations in terms of cause and effect.

Table 1. Comparison of age, CT–severity score, laboratory findings and length of stay in hospital or ICU according to the presence of hiatal hernia

	Hiatal hernia	n	Mean	Standard deviation	95% confidence interval for mean		Minimum	Maximum	p value
					Lower bound	Upper bound			
Age[a]	Y	144	58.86	13.60	56.62	61.10	24	93	**<0.001**
	V	144	71.23	11.37	69.36	73.10	39	94	
	T	288	65.05	13.96	63.43	66.66	24	94	
CT–severity score	Y	144	8.38	7.02	7.23	9.54	0	25	**<0.001**
	V	144	12.75	8.17	11.40	14.09	0	25	
	T	288	10.56	7.91	9.65	11.48	0	25	
Neutrophil percentage (40.1–67)[a]	Y	144	69.78	13.58	67.55	72.02	8.13	97.2	**0.025**
	V	144	73.31	12.94	71.18	75.44	34.6	96.6	
	T	288	71.55	13.36	70	73.1	8.13	97.2	
Lymphocyte percentage (23.6–48)[a]	Y	144	20.68	10.16	19	22.35	0.13	68.5	**0.036**
	V	144	18.44	10.60	16.69	20.18	2.30	54.1	
	T	288	19.56	10.42	18.35	20.77	0.13	68.5	
LDH (135–225; U/L)	Y	140	307.65	149.99	282.58	332.71	121	1422	**0.037**
	V	144	343.93	162.94	317.09	370.77	140	1167	
	T	284	326.04	157.46	307.65	344.43	121	1422	
CRP (0–5; mg/L)	Y	144	54.09	64.18	43.51	64.66	0.1	347	**0.05**
	V	144	65.14	61.77	54.97	75.32	0.06	304.19	
	T	288	59.618	63.12	52.29	66.93	0.06	347	
Ferritin (22–322; ug/L)	Y	142	322.95	459.97	246.64	399.26	5.2	3500	0.933
	V	142	353.81	566.26	259.86	447.75	5.5	4892.5	
	T	284	338.38	515.1	278.2	398.556	5.2	4892.5	
ESR (0–30; mm/H)	Y	142	48.09	27.71	43.49	52.68	6	118	**0.003**
	V	142	57.746	28.06	53.091	62.4	11	139	
	T	284	52.91	28.25	49.61	56.2	6	139	
Fibrinogen (200–400; mg/dL)	Y	138	495.17	154.35	469.18	521.15	30.7	975	**0.001**
	V	142	541.67	138.05	518.77	564.57	222	901	
	T	280	518.75	147.9	501.35	536.15	30.7	975	
D-dimer (0–0.5; μg/mL)	Y	142	1.02	1.94	0.7	1.3483	0.02	15.2	**<0.001**
	V	141	2.04	5.28	1.16	2.92	0.15	46.6	
	T	283	1.53	4	1.06	2	0.02	46.6	
BUN (16.6–48.5; mg/dL)	Y	143	39.33	28.37	34.64	44.02	13	267	**<0.001**
	V	143	45.61	24.74	41.52	49.70	17	171	
	T	286	42.47	26.76	39.36	45.59	13	267	
Length of stay in hospital	Y	124	15.37	11.68	13.29	17.45	1	59	0.221
	V	134	15.53	8.921	14.01	17.05	2	42	
	T	258	15.45	10.32	14.19	16.72	1	59	
Length of stay in ICU	Y	18	19.00	12.40	12.83	25.17	0	53	**0.015**
	V	49	11.82	8.31	9.43	14.20	0	33	
		67	13.75	10.01	11,30	16.19	0	53	

[a]Independent samples test was used.

Mann-Whitney U test was used.

A: Absent, P: Present, T: Total, CT: Computed tomography, ICU: Intensive care unit, LDH: Lactate dehydrogenase, CRP: C-reactive protein, BUN: Blood urea nitrogen

Figure 2. An 86-year-old man with confirmed Coronavirus disease-2019. His initial computed tomography (CT)-severity scores was calculated as 12. Axial (a) and coronal (b) lung window of unenhanced chest CT scans show bilateral ground-glass opacities with interlobular and intralobular septal thickening (crazy-paving pattern) with hiatal hernia. The coronal (c) mediastinal window of CT shows a part of the stomach sliding up into the chest through the hiatus. The patient died after being treated in the intensive care unit for 5 days

Table 2. Comparison of demographic parameters and chest CT findings according to the presence of hiatal hernia

		Hiatal hernia		Total	p value*
		A n (%)	P n (%)		
Gender	Male	77 (49.4%)	79 (50.6%)	156	0.813
	Female	67 (50.8%)	65 (49.2%)	132	
Mortality or surviving	Alive	121 (59.3%)	83 (40.7%)	204	**<0.001**
	Death	23 (27.4%)	61 (72.6%)	84	
ICU	ICU	18 (27.3%)	48 (72.7%)	66	**<0.001**
	Non-ICU	126 (56.8%)	96 (43.2%)	222	
GGOs	A	35 (68.6)	16 (31.4)	51	**0.003**
	P	109 (46)	128 (54)	237	
Consolidation	A	107 (53.8)	92 (46.2)	199	0.056
	P	37 (41.6)	52 (58.4)	89	
Crazy paving pattern	A	103 (61.3)	65 (38.7)	168	**<0.001**
	P	41 (34.2)	79 (65.8)	120	
Reticular pattern	A	64 (86.5)	10 (13.5)	74	**<0.001**
	P	80 (37.4)	134 (62.6)	214	
Intralobular septal thickening	A	91 (67.3)	55 (36.7)	146	**<0.001**
	P	53 (37.3)	89 (62.7)	142	
Interlobular septal thickening	A	54 (83.1)	11 (16.9)	65	**<0.001**
	P	90 (40.4)	133 (59.6)	223	
Linear opacities	A	36 (78.3)	10 (21.7)	46	**<0.001**
	P	108 (44.6)	134 (55.4)	242	
Adjacent pleural thickening	A	83 (64.3)	46 (35.7)	129	**<0.001**
	P	61 (38.4)	98 (61.6)	159	
Vascular thickening	A	39 (67.4)	15 (32.6)	54	**<0.001**
	P	105 (44.9)	129 (55.1)	234	
Bronchial wall thickening	A	58 (57.6)	28 (42.4)	86	**<0.001**
	P	86 (42.6)	116 (57.4)	202	

Table 2. Continued					
Bronchiectasis	A	95 (63.8)	54 (36.2)	149	<0.001
	P	49 (35.3)	90 (63.7)	139	
Reversed halo	A	111 (56.3)	86 (43.7)	197	0.002
	P	33 (36.3)	58 (63.7)	91	
Halo present	A	85 (52.8)	76 (47.2)	161	0.285
	P	59 (46.5)	68 (53.5)	127	
Tree- in-bud sign	A	139 (53.3)	119 (46.7)	205	0.002
	P	8 (24.2)	25 (75.8)	33	
Pericardial effusion	A	136 (54.4)	114 (45.6)	250	<0.001
	P	8 (21.1)	30 (78.9)	38	
Pleural effusion	A	130 (52.0)	120 (48.0)	250	0.082
	P	14 (36.8)	24 (63.2)	38	
Mediastinal lymph node enlargement	A	123 56.7)	94 (43.3)	217	<0.001
	P	21 (29.6)	50 (70.4)	71	

*All p-values of reports comparisons were analyzed by the chi-square test.

±p-values of reports were analyzed by logistic regression.

A: Absent, P: Present, ICU: Intensive care unit, GGOs: Ground-glass opacities, CT: Computed tomography

Table 3. Univariate and multivariate analysis of mortality								
Mortality								
	Univariate				Multivariate			
	p value	OR	95% CI		p value	OR	95% CI	
			Lower	Upper			Lower	Upper
Age	<0.001	0.921	0.898	0.945	<0.001	1.07	1.038	1.103
CT–severity score	0.004	0.954	0.923	0.985	0.29	1.022	0.982	1.064
CRP	0.014	0.995	0.991	0.999	0.169	1.003	0.999	1.008
Lymphocyte count	0.036	1.486	1.026	2.154	0.801	0.946	0.615	1.456
Hiatal hernia	<0.001	0.259	0.148	0.451	0.035	2.041	1.051	3.963
Chronic heart disease	0.001	0.223	0.089	0.56	0.027	3.388	1.151	9.971
Hypertension	<0.001	0.372	0.218	0.633	0.406	1.335	0.675	2.639
Cerebrovascular disease	0.02	0.296	0.106	0.824	0.102	2.74	0.818	9.183
Hyperlipidemia	0.017	0.509	0.292	0.888	0.458	1.313	0.639	2.695

Hosmer and Lemeshow test=0.917.

CI: Confidence interval, CRP: C-reactive protein, CT: Computed tomography, OR: Odds ratio

The high risk of hospitalization for respiratory disease after hospitalization for reflux disease supports the causal effect of reflux in respiratory diseases. These respiratory symptoms associated with HH may occur as a result of acid refluxing into the airway due to microaspiration of acid in the esophagus causing vagal-mediated neurogenic reflex and airway contraction[30]. And also, Ruhl et al.[31] reported that hospitalization due to HH and reflux esophagitis increases the risk of hospitalization due to many respiratory diseases. For this reason, patients with respiratory diseases that are resistant to treatment or whose etiology cannot be determined should be evaluated in terms of HH and reflux esophagitis and necessary treatment plans should be applied. Also, in our study, HH was statistically associated with multiple abnormal CT findings including GGOs, pericardial effusion, crazy paving pattern, reticular pattern, linear opacity, intra-interlobular septal thickening, pleural and bronchial wall thickening and reversed halo sign in the CT imaging. In this study, it was observed that in COVID-19 patients with HH, the pulmonary findings and the prognosis may be worse and should be considered in the treatment of these patients.

In patients with hiatal hernia, pulmonary edema or heart failure may occur as a result of compression of the heart and pulmonary vessels by organs protruding into the chest cavity[11]. In our study, consistent with the literature, 78.9% (30/38; p<0.001) of patients with pericardial effusion had HH, which was statistically significant. Also, the effect of acid reflux into the airway can increase respiratory symptoms and

Table 4. Univariate and multivariate analysis of ICU admission

ICU admission								
	Univariate				**Multivariate**			
	p value	**OR**	**95% CI**		**p value**	**OR**	**95% CI**	
			Lower	**Upper**			**Lower**	**Upper**
Age	<0.001	1.045	1.022	1.069	0.124	1.022	0.994	1.051
CT–severity score	0.002	1.057	1.021	1.095	0.253	1.025	0.983	1.068
CRP	0.028	1.005	1	1.009	0.269	1.003	0.998	1.008
Lymphocyte count	0.053	0.668	0.444	1.006	0.719	0.918	0.576	1.464
Gender	0.021	0.509	0.286	0.905	0.073	0.545	0.281	1.059
Hiatal hernia	<0.001	3.5	1.915	6.398	0.01	2.478	1.245	4.931
Chronic heart disease	0.092	2.218	0.877	5.606	0.536	1.392	0.488	3.967
Hypertension	0.005	2.281	1.288	4.038	0.218	1.55	0.772	3.112
Cerebrovascular diseases	0.049	2.808	1.003	7.857	0.275	1.933	0.593	6.309
Hyperlipidemia	0.031	1.916	1.061	3.46	0.332	1.425	0.696	2.918

Hosmer and Lemeshow test=0.233.

CI: Confidence interval, CRP: C-reactive protein, CT: Computed tomography, OR: Odds ratio, ICU: Intensive care unit

complications. In addition, HH reduced total lung capacity and vital capacity as well as gas trapping leading to restrictive lung disease symptoms. Restrictive lung disease may also develop as a result of a HH due to pulmonary fibrosis induced by gastric reflux[32]. In the current study, 75% (21/28; p=0.005) of patients with pulmonary fibrosis had HH, which was statistically significant. These reasons may explain the high rates of ICU admission and mortality of patients with HH in our study.

The most prevalent comorbidities among COVID-19 patients, according to the literature, were hypertension and diabetes, cardiovascular disorders, and respiratory system diseases[20]. However, there are few studies about the effect of digestive system diseases on the prognosis of COVID-19 patients. Jiang et al.[21] investigated the impacts of LPRD on COVID-19 patients who were hospitalized. They reported that LPRD was common in hospitalized COVID-19 patients and was independently related to the risk of serious or critical infection. Since the incidence of LPRD was higher in patients with HH, mortality and ICU admission were also significantly higher in patients with HH in our study.

To our knowledge, this is the first study to investigate the association between hiatal hernia, CT-SS, and death rates in COVID-19 patients. Our results showed a significant relationship between hiatal hernia and high CT-SS, ICU admission and mortality. Our study contributed to the literature with its results and emphasized the importance of the presence of HH in patients with COVID-19. If there is a filling defect in the inferior mediastinum in the X-rays of COVID-19 patients, HH should be considered and thorax CT should be performed. Also, when evaluating thorax CT of COVID-19 patients, coronal and sagittal images should be examined in terms of hiatal hernia. In

addition, the echocardiographic examination is recommended for the evaluation of cardiac and pulmonary venous system compression with large HH.

Study Limitations

The current study has some limitations. The first limitation is that our study was a single-center retrospective analysis. Therefore, a multicenter study with a large sample size is needed for more validation. Second, since our study was retrospective, patients could not be evaluated for GERD. Third, we evaluated the initial chest CT images of the patients. We did not use follow-up CTs, so we cannot review late-period CT finding changes.

CONCLUSION

We found that the presence of a hiatal hernia has important implications for the severity of pneumonia and increased ICU admission and mortality in COVID-19 patients. Therefore, when evaluating chest CT scans of COVID-19 patients, the investigation in terms of hiatal hernia should also be made for the prognosis and treatment planning of the patient.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice and was approved by the Ethical Committee of Amasya University Faculty of Medicine (approval number: 148, date: 4 November 2021).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.T.K., Design: B.A., Data Collection or Processing: A.T.K., B.A., Analysis or Interpretation: A.T.K., B.A., Literature Search: B.A., Writing: B.A.

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Comparison of Absorbable Tuckers and N-Butyl Cyanoacrylate Glue in Mesh Fixation for Laparoscopic Extraperitoneal Inguinal Hernia Repair: A Single General Surgeon's Experience

Laparoskopik Ekstraperitoneal Kasık Fıtığı Onarımı için Meş Fiksasyonunda Emilebilir Zımba ile N-Butil Siyanoakrilat Yapıştırıcıların Karşılaştırılması: Tek Bir Genel Cerrahın Deneyimi

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ABSTRACT

Aim: Laparoscopic inguinal hernia surgery is performed in many centers today. Mesh fixation is an important step in this procedure. In this study, we aim to show our results in total extraperitoneal repair of inguinal hernias using absorbable tuckers and n-butyl cyanoacrylate glue for mesh fixation.

Materials and Methods: All surgeries were performed by a single surgeon. The surgeries were performed in Private Erdem Hospital İstanbul and Al Zahra Hospital Dubai between January 2015 and February 2022. Mesh fixation of the patients was applied in a randomized manner using absorbable tucker and glue. The patients were compared in terms of postoperative visual pain score (VAS), opioid need, length of hospital stay, duration of surgery, presence of recurrence and chronic pain.

Results: A total of 226 patients were operated. Absorbable tucker were used for mesh fixation in 138 patients, and glue was used in 88 patients. The age and gender distribution of the patients were similar in both groups. No patient had any major complications or mortality during or after surgery. There was no conversion to open surgery. Operation time was similar in both groups. In the glue group, VAS was found to be significantly lower at the 8th hour after surgery. There was also a significant reduction in opioid requirement in the same group. The same-day discharge rate in the glue group was statistically significantly higher than in the absorbable tucker group. All patients were followed up for at least six months. Recurrence was observed in two patients (one patient in each group). The number of patients with chronic pain was significantly higher in the absorbable stapler group than in the other group.

Conclusion: The use of glue for mesh fixation in laparoscopic extraperitoneal inguinal hernia repair is a safe method. Fixation with glue is clearly advantageous comparing to absorbable tuckers in terms of postoperative pain, early discharge, and chronic pain.

Keywords: N-butyl cyanoacrylate, laparoscopic hernia repair, mesh fixation, total extraperitoneal hernia repair

ÖZ

Amaç: Laparoskopik fıtık cerrahisi günümüzde birçok merkezde uygulanmaktadır. Meşin fiksasyonu ameliyatın önemli bir aşamasıdır. Biz bu çalışmada total ekstraperitoneal laproskopik fıtık tamirinde emilebilir zımba ile n-butil siyanoakrilat yapıştırıcısı kullanımının kısa ve uzun dönem etkilerini araştırdık.

Gereç ve Yöntem: Bütün ameliyatlar tek bir cerrah tarafından laparospik ekstraperitoneal yöntemle yapıldı. Ameliyatlar İstanbul ve Dubai'de 2015 Ocak ve 2022 Şubat ayları arasında yapıldı. Hastaların meş fiksasyonu randomize bir şekilde emilebilir zımba ve yapıştırıcı kullanılarak uygulandı. Hastalar, ameliyat sonrası vizuel ağrı skoru (VAS), opioid ihtiyacı, hastanede kalış süresi, ameliyat süresi, nüks ve kronik ağrı varlığı açısından karşılaştırıldı.

Bulgular: Toplam 226 hasta ameliyat edildi. Yüz otuz sekiz hastada meş fiksasyonu için emilebilir zımba, 88 hastada yapıştırıcı kullanıldı. Hastaların yaşı, cinsiyet dağılımı her iki grupta da benzerdi. Hiçbir hastada ameliyat sırasında veya ameliyat sonrasında herhangi bir majör komplikasyon veya mortalite görülmedi. Her iki grupta da açık cerrahiye geçiş olmadı. Ameliyat süresi her iki grupta da benzerdi. Yapıştırıcı grubunda ameliyattan

hemen sonra 8. saatte VAS belirgin düşük olarak saptandı. Ayrıca opioid ihtiyacında anlamlı bir şekilde azalma mevcuttu. Yapıştırıcı grubunda aynı gün taburcu oranı emilebilir zımba grubuna göre istatistiksel olarak anlamlı bir şekilde daha fazla idi. Bütün hastaların en az altı aylık takibi yapıldı. Her iki grupta da birer hastada nüks görüldü. Kronik ağrı tespit edilen hasta sayısı emilebilir zımba grubunda diğer gruba göre belirgin olarak fazla idi.

Sonuç: Laparoskopik ekstraperitoneal kasık fıtığı tamirinde meş fiksasyonunda yapıştırıcı kullanımı güvenli bir yöntemdir. Ameliyat sonrası ağrı, erken taburcu, kronik ağrı açısından emilebilir zımba ile tespitte belirgin olarak avantajlıdır.

Anahtar Kelimeler: N-butil siyanoakrilat, laparoskopik fıtık tamiri, meş fiksasyonu, total ekstraperitoneal fıtık tamiri

INTRODUCTION

Dating back to ancient Egypt, hernia surgery is one of the oldest known surgical techniques. Bassini's discovery of the hernia sac for the first time in 1884 and his repair of the defect with stitches were considered as the revolution of hernia surgery. With the better understanding of anatomy over time, rapid advances were made in hernia surgery, and laparoscopic hernia surgery showed rapid progress with the frequent use of laparoscopy in surgery in the early 1990s[1,2]. It is now an accepted fact that laparoscopic hernia surgery has advantages such as less pain, shorter hospital stay, earlier return to work, and better cosmetic results compared to open surgery[3]. Total extraperitoneal and trans abdominal pre-peritoneal repairs are the most commonly used methods. The advantages and disadvantages of the two have been shown in many publications[4-7]. There are differences depending on the surgeon in both types of surgery, such as port locations, which type of mesh is used, and mesh fixation[8,9]. Mesh fixation can be done with sutures, absorbable or non-absorbable staples, and glue. Although suture fixation has a cost advantage, it has technical difficulties[10]. With the emergence of absorbable staples, it has become widely used. Despite the ease of use of staples, they also had disadvantages such as chronic pain and nerve compression, so surgeons began to look for other methods[11]. Alternatively, in extraperitoneal repairs, 3D mesh has not been used for fixation and has been shown to be safe[12,13]. With the introduction of fibrin glues in laparoscopic hernia surgery, a new method for mesh fixation has begun to be adopted[14,15]. Although it has the potential for allergic reactions due to its animal origin, no such case report has been found today; however, its weak binding and slow effect have been reported as its disadvantages[16]. Although there are many publications in the literature on the method of providing mesh fixation in laparoscopic hernia repair, there is still no definitively accepted method. Studies showing that alternative methods are associated with less pain compared to absorbable staples are still up to date[17]. In this study, we compare n-butyl-cyanoacrylate (NBSA) adhesive fixation with absorbable stapler mesh fixation. The main purpose of this study is to compare important criteria such as pain in the early period after surgery, length of hospital stay, chronic pain, need for painkillers, chronic pain and recurrence that affect patient comfort and cost, in both methods, and to investigate the reliability of them.

MATERIALS AND METHODS

To compare mesh fixation methods in laparoscopic inguinal hernia repair, patients who underwent surgery at Private Erdem Hospital and Al Zahra Hospital Dubai between September 2018 and March 2023 were retrospectively examined.

The patients were divided into two groups as A and B. In Group A patients, the mesh was fixed with absorbable staples. In Group B patients, mesh fixation was done with NBSA adhesive. All patients were evaluated according to the visual pain scale (VAS) documented at the eighth hour after surgery. Patients' opioid requirements were recorded during hospitalization. Surgery times and hospital stays were documented, and finally, all patients were recalled at least in the sixth month after surgery and examined for chronic pain and recurrence. All patients were operated by a single surgeon who had already performed more than 1000 laparoscopic hernia repairs. All patients underwent total extrapertoneal laparoscopic hernia repair. Two hours after the surgery, all patients were mobilized and oral liquid nutrition was started. Soft diet was allowed after four hours if the patient tolerated it. The patients were given a dose of nonsteroidal analgesic (injection diclofenac 50 mg) in the recovery room. Then, analgesics (diclofenac 50 mg + paracetamol 500 mg tablet) were administered if needed. Patients were asked to rate their pain on VAS at the sixth hour after surgery. If the patient's pain severity was VAS 7 or above, an ampoule of opioid analgesia (2 mL 100 mg pethidine hydrochloride) was applied. Patients were discharged on the same or the next day. Patients were called for follow-up visits on the third day, first month, sixth month, 12th month, and 24th month. At each follow-up, the groin was evaluated for pain, swelling, and foreign body sensation. If there were suspicious findings for recurrence, the patient underwent groin ultrasound. Chronic groin pain was defined as discomfort in the groin area on the operated side lasting more than six months.

In order to conduct the research, ethical approval was obtained from the Al Zahra Hospital Dubai (date: 11.07.2023, no: 14/301) and informed consent was obtained from all patients.

The procedures followed in conducting the study were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 1975 Helsinki Declaration, as revised in 2000 and 2013.

Statistical Analysis

Statistical analysis was performed using the chi-square test. Differences between independent variables were evaluated using the chi-square test. P value <0.05 was considered statistically significant.

Surgery Technique

The patients were operated under general anesthesia in the supine position with both hands closed. All patients were administered 1 g of ceftriaxone in the perioperative period. A urinary catheter was applied to all patients after anesthesia, and the catheter was removed before the patient woke up. The laparoscopy tower was at the patient's foot level. By making a transverse incision in the subumbilical extra-peritoneal space, a 10 mm trocar of any brand is used in surgeries performed in Turkey and a 10 mm ©Kii Balloon Blunt-Tip System access balloon trocar is used in surgeries performed in Dubai (applied medical resources cooperation, Dubai, UAE). The Surgeon in Charge stood on the opposite side of the hernia and the assistant/nurse stood on the same side (Figure 1). A zero degree telescope was used to examine the preperitoneal space and

Figure 1. Patient position, surgeons' location, port locations

for dissection. The working ports were placed under vision in the midline at a distance of 5 mm. The lower port was placed just above the symphysis pubis and the third port was placed between the other two ports. The dissection area was provided as wide as possible. In all cases, the preperitoneal space was opened from the symphysis pubis medially to the psoas muscle laterally and the hernia sac was exposed. In direct hernias, the entire sac is separated from the fascia transversalis. In all direct hernia cases, the presence of cord lipoma and synchronous indirect hernia was investigated. In indirect hernias, the peritoneal sac was carefully dissected from the cord structures. When the peritoneal cavity had to be opened in large or very adherent indirect sacs, the neck of the opened sac was closed with the help of endoloop and endoclip. In the follow up of these stages, when it was decided that sufficient extraperitoneal space was created and the hernia sacs were completely reduced, 3D Max™ Mesh (Bard Mesh, BD Company, Switzerland) was folded and inserted through the camera port and left in the operating field. All potential hernia areas were tried to be closed by regularly opening the rolled mesh laterally from the pubic symphysis to the psoas muscle. In Group A patients, the mesh was fixed to the ipsilateral Cooper ligament with two absorbable staples. In Group B, the mesh was fixed by spraying 0.5 mL of NBSA adhesive (Aesculap, B. Braun, Brazil) onto the Cooper ligament and various places on the mesh. The mesh was kept pressed on the mesh with the tip of the adhesive apparatus at the spray point for not less than five seconds and not more than ten seconds (Video 1).

RESULTS

A total of 149 patients were included in the study between January 2015 and February 2023. One hundred thirty eight patients were included in Group A (fixation with absorbable staples) and 88 patients were included in Group B (fixation with adhesive). Their ages ranged from 25 to 76. 200 (88.4%) of the patients were male. All patients underwent surgery for unilateral inguinal hernia under general anesthesia. Mean surgery times were similar in both groups (Group A: 44.5 minutes, Group B: 43.1 minutes). All surgeries were completed laparoscopically and no complications were observed during the surgeries. A total of five patients returned to the hospital after discharge. Urinary catheterization was applied to four of them because urinary retention developed (Group A: 2, Group B: 2). One of them applied to the emergency department due to severe pain and was taken into observation for pain treatment. This patient was a Group A patient (Table 1). All patients were mobilized under nurse supervision approximately two hours later and allowed to take clear liquids orally. Patients were allowed to take soft diet after four hours. Forty-six patients could not switch to soft food after four hours because they could not tolerate it. More patients in Group A (32 patients) could not tolerate soft food, but no statistical difference

Table 1. Characteristics of the patients	
Gender	
Male	200 (88.4%)
Female	26 (11.6%)
Age (year)	
Range	25-76
Mean	42.9
Mean surgery time (minute)	
Group A	44.5
Group B	44.1
Complications during surgery	
Group A	Not seen
Group B	Not seen
Returning back to the hospital	
Group A	3 (2 patients with urinary retention, 1 patient with pain)
Group B	2 (urinary retention)
Switching to open surgery	
Group A	Not seen
Group B	Not seen

Table 2. Parameters			
	Group A (%)	**Group B (%)**	**p value**
Switching to soft nutrition at the 4th hour	106 (76.8)	74 (84)	0,18
VAS score measured at the 6th hour <4	51 (36.9)	58 (65,9)	**0,009**
Opioid requirement	61 (44.2)	2 (2.2)	**<0.0001**
Same day discharge	14 (10)	68 (70)	**<0.0001**
Recurrence	1 (0.7)	1 (1.1)	0.747257
Chronic pain	17 (12.3)	2 (2.2)	**0.0079**
VAS: Visual pain score			

was detected between the two groups (p=0.18). The average VAS score measured at the sixth postoperative hour was 5.5 in Group A and 4.5 in Group B. Considering the number of patients with a VAS score of four and below in both groups, the number of patients in Group B was higher (p=0.009). Opioid requirement was found to be 61 in Group A and two in Group B. Opioid requirement was significantly reduced in Group B patients (p<0.00001). All patients were discharged on the same day or the next day. After the patients were checked at the evening visit, they were allowed to be discharged on the same day, depending on the patient's condition or at his/her own request. 77% of the patients in Group B were discharged on the same day. When Group A and B were compared, there was a significant superiority of patients in Group B who were discharged on the same day (Group A 14 patients, p<0.00001). Recurrence was detected in only two patients (1 patient in Group A and B). All patients were called for a check-up at least

six months later and questioned about pain or foreign body sensation in the groin. Findings considered as chronic pain were detected in a total of 19 patients. Seventeen of them were Group A patients and two were Group B patients. Patients with chronic pain in Group A were statistically significantly higher than those in Group B (p=0.0079). These findings are summarized in Table 2.

DISCUSSION

NBSA, a butyl polymer of cyanoacrylate, an acrylic resin, reacts in the presence of water, forming a bond within seconds and providing mesh fixation. Therefore, after application, the mesh must be fixed to the underlying tissue for at least 5-10 seconds. In laparoscopic hernia surgery, mesh fixation is a critical step of the procedure. Pain and doom area triangles are important anatomical regions in the total extraperitoneal hernia repair technique. The doom area triangle is a triangle bounded by the vas deferens, testicular vessels, and peritoneal fold. The importance of this triangle is that it covers major arteries and veins. The pain triangle includes the lateral femoral cutaneous nerve, the femoral branch of the genitofemoral nerve, and the femoral nerve. Avoiding these areas, especially when fixing the mesh with staples, is one of the most important steps of the surgery.

Otherwise, it may lead to complications such as chronic pain, major vascular injuries or nerve damage. Although the cause of chronic pain seen after surgery is not fully known in the literature, genitofemoral nerve, iliohypogastric nerve, and ilioinguinal nerve close to the surgery area, damage to the nerve and lateral cutaneous nerve of the thigh during dissection and accidental stapling are the most likely causes[18,19]. In the literature, postoperative neuralgia is generally reported to be between 0.5-14%[20]. In our study, chronic pain was seen in 8.4%. Most of the patients were in the staple group (Group A) and there was a statistically significant difference between them and the adhesive group (Group B). Chronic pain is an important factor in quality of life after laparoscopic hernia surgery. Many authors, who think that mesh fixation is associated with chronic pain, have tried to complete the surgery without fixing the mesh, especially by using wide meshes in extraperitoneal hernia repair, opening the dissection area widely, removing the air under vision after fully opening the mesh, and they have achieved a decrease in the incidence of chronic pain as well as showing that there was no difference in long-term complications such as recurrence[21-23].

Despite many studies conducted over many years, no conclusion has been reached as to what the mesh will be fixed with or even whether it will be fixed at all. In cases where the mesh is not fixed, some surgeons still have reservations about the possibility of the mesh shifting, folding, and consequently

increasing the likelihood of hernia recurrence in the long term[24]. The negative effect of absorbable staples on chronic pain and the possibility of causing some serious complications have led surgeons to look for alternative methods, and the idea of non-mechanical fixation of the mesh using various adhesive materials has emerged. Many studies have begun to be carried out, such as cost calculations and its effect on chronic pain[25]. Comparing cost-effectiveness is controversial, as is the issue of mesh fixation. Some studies also report that fibrin glues are expensive[26,27]. Considering the fact that disposable absorbable staple guns are re-sterilized and used in underdeveloped and developing countries and reduce the cost, we think that adhesives are less costly in terms of material cost. However, it is another research topic that in common laparoscopic hernia surgeries, the cost is calculated not only on the materials used, but also on the basis of daily discharge, surgery time, anesthesia time, and feasibility of being performed at lower costs in medical centers[28]. In our study, while there was a statistically positive difference in the tendency to be discharged on the same day in the adhesive group (Group B), there was no significant difference in surgery times. Inguinal hernia repair is one of the most frequently performed operations in general surgery. More than 20 million patients undergo inguinal hernia repair every year. There are many different approaches to treatment, with laparoscopy and, in recent years, even robotic surgery contributing to inguinal hernia repair. In 2018, the HerniaSurge community presented international recommendations for inguinal hernia in a study involving many experienced hernia surgeons. According to these recommendations, laparoscopic surgery for inguinal hernia is still performed at a rate of 55% even in developed countries, and there is no consensus on a standard surgical technique. According to the same recommendation, it is recommended not to use mesh in laparoscopic hernia repairs in almost all types of hernia, except for large defects. The HerniaSurge group has a consensus on mesh fixation in large defects and atraumatic fixation techniques (fibrin glue, cyanoacrylate) are recommended[29]. In another study conducted by Novik et al.[30] in 2022, 25190 laparoscopic hernia surgeries were examined and recurrence was found to be high in the groups where mesh was not detected and adhesives were recommended for fixation. In the same study, it was shown that there was no difference in the effect of absorbable staples on relapse in both standard propylene meshes and light weight meshes compared to the adhesive group. As stated in extensive studies conducted in recent years, there is no fully accepted method in hernia surgery, and there is no standard for mesh fixation in laparoscopic hernias. In this study, we wanted to share in the Turkish literature the results of a surgeon with advanced laparoscopy experience, who used mesh absorbable staples and glue in laparoscopic total extraperitoneal hernia repair.

Study Limitations

This study has some strengths and limitations. The strength of our study is that all surgeries were performed by a single surgeon. One limitation is the retrospective design of the study. Prospective studies in which many variables will be examined in the future may provide more valuable results. Second, our sample size was relatively small.

CONCLUSION

In laparoscopic total extraperitoneal hernia repair, NBSA adhesive fixation is associated with lower pain scores in the acute phase compared to absorbable staple fixation. In addition, the ability to discharge patients in the same manner also reduces cost calculations. When chronic pain is examined, there is a significant statistical advantage in the adhesive group. There is no significant difference in both groups in terms of surgery times, complications, transition to oral nutrition and recurrence. Fixation of mesh with NBSA is a feasible and safe method in total extraperitoneal hernias.

Ethics

Ethics Committee Approval: In order to conduct the research, ethical approval was obtained from the Al Zahra Hospital Dubai (date: 11.07.2023, no: 14/301).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

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Video 1. Surgical view of fixation with absorbable staples and glue

http://glns.co/x9ur4

Clinicopathological Characteristics and Real-life Data of Patients Receiving Tyrosine Kinase Inhibitor in Metastatic EGFR Mutant Non-small Cell Lung Carcinoma

Metastatik EGFR Mutant Küçük Hücreli Dışı Akciğer Karsinomunda Tirozin Kinaz İnhibitörü Alan Hastaların Klinikopatoloik Özellikleri ve Gerçek Yaşam Verisi

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ABSTRACT

Aim: The desired survival times could not be achieved with conventional treatments in lung cancer. Tyrosine kinase inhibitors (TKIs) are used in the treatment of non-small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) mutations. The aim of this study was to investigate the clinicopathologic features of EGFR mutant NSCLC patients and the effects of TKIs on progression-free survival (PFS) and overall survival (OS).

Materials and Methods: A total of 61 patients who were admitted to Cerrahpaşa Faculty of Medicine, Department of Internal Medicine, Department of Medical Oncology, between 2012 and 2022, who were EGFR mutants with NSCLC and who used TKI as treatment were included in the study. Demographic and pathological characteristics of the patients, treatments used in the patients, progression and death dates were examined retrospectively.

Results: Age at diagnosis, serum creatinine, last visit ECOG score and progression rate were significantly higher in the deceased group. The rate of T790M mutation and osimertinib use was significantly higher in the surviving group. Last visit ECOG score was significantly higher in the progression group. Survival time was significantly shorter in the group with visceral metastasis than in the group without visceral metastases, in the group with a last visit ECOG score II-III-IV compared to those with 0-I, and in the group without the T790M mutation than in the group with the T790M mutation. The progression-free survival time was significantly shorter in the group with ECOG score II-III-IV at the last visit compared to those with 0-I and in the group with visceral metastasis than in the group without. There was no statistically significant difference between erlotinib, afatinib and gefitinib in terms of PFS and OS. PFS calculated as 27.4 months and OS 49.2 months. The median PFS duration of 13 patients receiving osimertinib was calculated as 18.5 months.

Conclusion: Age, diagnosis and ECOG performance score at the last visit, visceral metastasis at the beginning of TKI, T790M mutation and therefore osimertinib use had a significant effect on the OS, while on PFS, the significant effect of the visceral metastasis at TKI initiation, stage at the time of diagnosis, the ECOG performance score at the last visit was seen.

Keywords: Lung cancer, EGFR, tyrosine kinase inhibitors

ÖZ

Amaç: Akciğer kanserinde konvansiyonel tedaviler ile istenen sağkalım süreleri sağlanamamıştır. Epidermal büyüme faktörü reseptörü (EGFR) mutant olan küçük hücreli dışı akciğer kanseri (KHDAK) hastalarının tedavisinde tirozin kinaz inhibitörleri (TKİ) kullanılmaktadır. Çalışmamızda EGFR mutant KHDAK hastaların klinikopatolojik özelliklerinin ve TKİ'lerin progresyonsuz sağkalım (PS) ve genel sağkalım (GS) üzerindeki etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Cerrahpaşa Tıp Fakültesi İç Hastalıkları Medikal Onkoloji Polikliniği'ne 2012-2022 arasında başvurmuş KHDAK tanılı EGFR mutant ve TKİ kullanmış olan 61 hasta çalışmaya alınmıştır. Hastaların demografik ve patolojik özellikleri, kullanılan tedaviler, progresyon ve ölüm tarihleri incelendi.

Bulgular: Ölen grupta tanı yaşı, serum kreatinini, son vizit ECOG skoru, progresyon oranı anlamlı olarak daha yüksekti. Hayatta olan grupta T790M mutasyonu ve osimertinib kullanım oranı anlamlı olarak daha yüksekti. Progresyon olan grupta son vizit ECOG skoru anlamlı olarak daha yüksekti. TKİ başlandığında visseral metastaz olan grupta olmayana göre, son vizit ECOG skoru II-III-IV olan grupta 0-I olana göre ve T790M mutasyonu olmayan grupta olana göre sağkalım süresi anlamlı olarak daha kısaydı. Son vizit ECOG II-III-IV olan grupta 0-I olana göre ve TKİ başlandığında visseral metastaz olan grupta olmayana göre PS süresi anlamlı olarak daha kısaydı. Erlotinib, afatinib ve gefitinib arasında PS ve GS açısından istatistiksel anlamlı fark saptanmadı, PS 27,4 ay, GS 49,2 ay olarak hesaplandı. Osimertinib kullanan 13 hastanın medyan PS süresi 18,5 ay olarak hesaplandı.

Sonuç: GS üzerinde yaşın, tanıda ve son vizitteki ECOG performans skorunun, TKİ başlandığında visseral metastazın, T790M mutasyonun ve dolayısıyla osimertinib kullanımının anlamlı etkisi görülmüştür. PS üzerinde ise TKİ başlandığında visseral metastazın, tanı anındaki evrenin, son vizitteki ECOG performans skorunun anlamlı etkisi görülmüştür.

Anahtar Kelimeler: Akciğer kanseri, EGFR, tirozin kinaz inhibitörü

INTRODUCTION

Lung cancer is the cancer that causes death most frequently in the world[1]. Lung cancer is divided into two main groups as pathological small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLCs constitute 80% of lung cancers and are divided into 3 groups: large cell lung cancer, squamous cell lung cancer (SCC) and adenocarcinoma. The 5-year survival rate of NSCLCs is considered low (19.1%) despite recent advances in imaging, diagnosis and treatment[1]. Although tobacco and tobacco product use are etiologically leading in the development of lung cancer with the epidemiological increase in non-smoking-related lung cancers, specific molecular and genetic tumor characteristics have been identified. Somatic genomic alterations called driver mutations include epidermal growth factor receptor (EGFR), ALK, ROS-1, BRAF, HER2, MET, RET, KRAS, NTRK mutations[2]. Among the mutations defined as driver mutations in this group, mutations in the Epithelial Growth Factor receptor (EGFR) gene are the most common.[3] The side effect profile of conventional chemotherapy (CT) and radiotherapy treatments in the treatment of lung cancer is wide and that the desired survival times could not be achieved has led to significant advances in treatments. Treatments called tyrosine kinase inhibitors (TKI) are primarily used in the treatment of patients with NSCLC who have a driver mutation in the *EGFR* gene. In addition, PD-1 and PDL-1 expression levels are measured and immunotherapies are used in treatment.

Our study mainly aimed to examine the clinicopathological features of patients with EGFR exon 19 and exon 21 L858R mutations and to investigate to what extent the clinicopathological features and TKI used for treatment affect the progression-free survival (PFS) and overall survival (OS).

MATERIALS AND METHODS

A total of 61 patients who were diagnosed with NSCLC, EGFR mutant, and who used TKI as treatment, who applied to the outpatient clinic of Cerrahpaşa Faculty of Medicine, Department of Internal Medicine. Medical oncology department between 2012 and 202, were included in the study. Patients with non-

EGFR driver mutations or EGFR mutant patients who did not use TKIs were excluded from the study.

Patients' age, gender, body mass index (BMI), smoking history, NSCLC diagnosis date, ECOG performance score at diagnosis, NSCLC histological subtype, type of EGFR mutation, stage at diagnosis, brain and visceral metastasis status at diagnosis, serum creatinine and bilirubin values at diagnosis, whether or not they received CT before TKI, the TKI they received, TKI start date, side effect status under TKI, the reason if TKI was discontinued, T790M mutation status, whether they used Osimertinib, last visit dates, ECOG performance scores at the last visit and, if death occurred, death dates were examined retrospectively.

This thesis study was found to be in compliance with medical ethics and the Helsinki Declaration of Human Rights with the decision of the Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee dated 15.02.2023 and numbered E-83045809-604.01.01-620248.

Statistical Analysis

Mean, standard deviation, minimum and maximum median, frequency and ratio values were used in the descriptive statistics of the data. The distribution of variables was measured with the Kolmogorov-Smirnov test. Mann-Whitney U test was used in the analysis of quantitative independent data. Chi-square test was used in the analysis of qualitative independent data, and Fisher's test was used when chi-square test conditions were not met. Cox-regression (univariate-multivariate) and Kaplan-Meier were used in survival analysis. Statistical Package for the Social Sciences 28.0 program was used in the analyses.

RESULTS

Sixty-one patients diagnosed with metastatic NSCLC and EGFR mutant who used TKI were included in the study.

The median age at diagnosis of the patients was 63 years. Of the 61 patients, 36 were female (59%) and 25 were male (41%). The median BMI was calculated as 26.12. It was observed that 31 of 61 patients had never smoked (50.8%), 14 of them had

smoked before (23%), 8 of them were still active smokers (13.1%), and 8 patients (13.1%) were smokers. No information was available about whether 8 patients (13.1%) smoked or not. The median serum creatinine values of the patients were calculated as 0.76, and the median total bilirubin values were calculated as 0.46 (Table 1).

It was observed that 60 of 61 patients were histologically in the Adenocarcinoma subtype (98.4%), and 1 patient was in the SCC subtype (1.6%). When the EGFR mutation distribution was examined, it was seen that 40 patients had Exon 19 mutations (65.6%), 16 patients had Exon 21 mutations (26.2%), and 5 patients had non-classical mutations (8.2%) (Table 2).

When we look at the ECOG performance score of the patients at the time of diagnosis, the score was "0" in 13 patients (21.3%), "1" in 41 patients (67.2%), "2" in 4 patients (6.6%), and "3" in 1 patient (1.6%), and "4" (3.3%) in 2 patients.

Table 1. Findings

		Minimum–maximum	Median	Mean±SD / n	(%)
Age at diagnosis		26.00-86.00	63.00	62.25±13.56	
Gender	Female			36	59.0%
	Male			25	410%
BMI		20.23-41.62	26.12	26.45±3.98	
Smoking status					
Non-smoker				31	50.8%
Ex smoker				14	23.0%
Smoker				8	13.1%
Unknown				8	13.1%
Serum creatinine		0.43-1.82	0.76	0.82±0.28	
Total bilirubin		0.17-1.33	0.46	0.48±0.23	
Histological subtype	Adenocarcinoma			60	98.4%
	SCC			1	1.6%
ECOG at diagnosis	0			13	21.3%
	I			41	67.2%
	II			4	6.6%
	III			1	1.6%
	IV			2	3.3%
EGFR mutation					
Exon 19				40	65.6%
Exon 21				16	26.2%
Non-classical				5	8.2%
Stage at diagnosis					
Suitable for surgery				9	14.8%
Local advanced				1	1.6%
Metastatic				51	83.6%
Brain metastasis at diagnosis	(-)			42	68.9%
	(+)			19	31.1%
Visceral metastasis when TKI is started	(-)			44	72.1%
	(+)			17	27.9%
Location of visceral metastasis					
Lung				8	47.1%
Adrenal				8	47.1%
Kidney				1	5.9%
1st line CT	(-)			46	75.4%
	(+)			15	24.6%

CT: Chemotherapy, SD: Standard deviation, BMI: Body mass index, SCC: Squamous cell lung cancer, TKI: Tyrosine kinase inhibitor, EGFR: Epidermal growth factor receptor

At diagnosis, 51 patients were metastatic (83.6%), 1 patient was at a locally advanced stage (1.6%), and 9 patients were suitable for surgery (14.8%). Nineteen of 61 patients had brain metastases at the time of diagnosis (31.1%). All patients were in the metastatic stage when TKI was started.

At the time TKI was started, 17 of 61 patients had visceral metastases (27.9%). It was observed that 47.1% of visceral metastases were in the liver (8 patients), 47.1% in the adrenal gland (8 patients), and 5.9% in the kidney (1 patient).

46 of the patients had not received CT before TKI (75.4%), and 15 had received first-line CT (24.6%). Of the 15 patients who received first-line CT, 14 received TKI treatment as a second-line treatment, while 1 received it as a third-line treatment. The patient who received third-line treatment was found to have SCC as histological subtype.

While 53 patients received erlotinib as TKI (86.9%), 6 patients received afatinib (9.8%) and 2 patients received gefitinib (3.3%). While side effects were observed in 32 patients receiving TKI (52.5%), no side effects were observed in 29 patients (47.5%). While skin rash was seen as a side effect in 28 of 32 patients with side effects (87.5%), liver enzyme elevation was observed in 4 patients (12.5%). Side effects were graded according to the "Common Terminology Criteria of Adverse Effects" classification. When examined, it was observed that there were grade 1 side effects in 5 patients (16.1%), grade 2 side effects in 15 patients (48.4%), and grade 3 side effects in 11 patients (35.5%). It was observed that no patient discontinued TKI due to side effects (Table 3).

The last check date was determined as September 2022. Thus, the median follow-up period was calculated as 39.4 months. It was observed that 52 of 61 patients progressed during the follow-up period (85.2%), and 9 did not progress (14.8%). It was observed that T790M mutation was detected as positive in a total of 13 patients (21.3%), and since it could not be started in the first step in these patients due to SUT rules, osimertinib treatment was started after progression under TKI.

The ECOG performance score at the last visit date was "0" in 11 patients (18%), "1" in 20 patients (32.8%), "2" in 4 patients (6.6%), and "3" in 12 patients (19.7%), it was found to be "4" in 4 patients (6.6%). During the follow-up period, 40 of 61 patients died (65.6%) and 21 were alive (34.4%).

The relationship between the demographic, clinicopathological characteristics and laboratory findings of the patients and mortality was examined.

The age at diagnosis in the deceased group was significantly higher than the surviving group (p<0.05). Serum creatinine value in the deceased group was significantly higher than the surviving group (p<0.05). The follow-up period in the deceased group was significantly lower than in the surviving group (p<0.05).

The relationship between gender, BMI, smoking history, serum total bilirubin level and mortality did not show any significant difference.

Histological subtype distribution between deceased and surviving groups, ECOG score distribution at diagnosis, EGFR mutation type distribution, stage distribution at diagnosis, brain metastasis rate at diagnosis, visceral metastasis rate when

Table 2. Findings			
TKI received			
Erlotinib		53	86.9%
Afatinib		6	9.8%
Gefitinib		2	3.3%
The stage of TKI as treatment	I	46	75.4%
	II	14	23.0%
	III	1	1.6%
TKI side effect			
(-)		29	47.5%
(+)		32	52.5%
Elevated liver enzyme		4	12.5%
Rash		28	87.5%
TKI side effect CTCAE grade			
Grade I		5	16.1%
Grade II		15	48.4%
Grade III		11	35.5%
T790M mutation	(-)	48	78.7%
	(+)	13	21.3%
Osimertinib	(-)	48	78.7%
	(+)	13	21.3%
Last visit ECOG	0	11	18.0%
	I	20	32.8%
	II	4	6.6%
	III	12	19.7%
	IV	4	6.6%
Progression	(-)	9	14.8%
	(+)	52	85.2%
Exitus	(-)	21	34.4%
	(+)	40	65.6%
TKI: Tyrosine kinase inhibitor, CTCAE: Common Terminology Criteria of Adverse Effects			

Table 3. Follow-up duration			
	Minimum–maximum	Median	Mean±SD / n (%)
Follow-up duration (month)	3.30–108.07	39.47	38.65±25.39
SD: Standard deviation			

TKI is initiated, 1st line CT rate, distribution of TKI received, which step treatment is TKI and the side effect rate of TKI did not show any significant difference (p>0.05).

The T790M mutation rate and osimertinib rate in the surviving group were significantly higher than the deceased group (p<0.05).

Last visit ECOG performance score in the deceased group was significantly higher than the surviving group (p<0.05).

Progression rate in the deceased group was significantly higher than the surviving group (p<0.05).

The relationship between the patients' demographic, clinicopathological characteristics and laboratory findings and progression was examined.

Age at diagnosis, gender distribution, BMI value, smoking rate, serum creatinine value, total bilirubin value, histological subtype distribution, ECOG score distribution at diagnosis, EGFR mutation distribution, stage distribution at diagnosis, brain metastasis rate at diagnosis between groups with and without progression. Visceral metastasis rate when TKI was started, 1st line CT, TKI received, which TKI was the first-line treatment, TKI side effect rate, T790M mutation rate, osimertinib rate did not show any significant difference (p>0.05).

Last visit ECOG rate in the progression group was significantly higher than the non-progression group (p<0.05).

Cox regression analysis was used to predict OS, and significant variables were examined by the Kaplan-Meier method.

In the univariate model, no significant effect of gender, BMI, smoking, EGFR mutation type, stage at diagnosis, serum creatinine value, total bilirubin value, TKI side effect, first-line CT status, and TKI type received was observed in predicting survival time (p>0.05).

In the univariate model, a significant effect of age, ECOG score at diagnosis, visceral metastasis at TKI initiation, T790M mutation, osimertinib use, ECOG score at last visit and progression was observed in predicting survival time (p<0.05).

In the multivariate reduced model, a significant and independent effect of visceral metastasis at TKI initiation, T790M mutation, and last visit ECOG score was observed in predicting survival time (Table 4).

When the survival times of TKI were examined, the predicted survival time with erlotinib was 47 months, while it was 36.4 months with afatinib. It could not be calculated because there were a total of 2 patients using gefitinib. No significant difference was detected between TKI's in terms of OS (Table 5).

The predicted survival time in the group with visceral metastasis at TKI start (26.2 months) was significantly shorter than the

group without visceral metastasis at TKI start (56.0 months) (p<0.05) (Figure 1, Table 6).

The predicted survival time in the group without T790M mutation (40.5 months) was significantly shorter than in the group with T790M mutation (86.8 months) (p<0.05) (Figure 2, Table 7).

Cox regression analysis was used to predict PFS, and significant variables were examined by the Kaplan-Meier method.

In the univariate model no significant effect of age, gender, BMI, smoking, ECOG score at diagnosis, EGFR mutation type, T790M mutation, Osimertinib receipt, serum creatinine value, total bilirubin value, first-line CT status and type of TKi received was observed in predicting PFS time (p>0.05) (Table 8).

In the univariate model, a significant effect of visceral metastasis at TKI initiation, diagnosis stage, TKI side effect, and last visit ECOG score was observed in predicting PFS time (p<0.05).

In the multivariate reduced model, a significant-independent effect of visceral metastasis at TKI initiation and last visit ECOG score was observed in predicting PFS time.

The predicted PFS time in the group with visceral metastasis at TKI initiation (15.1 months) was significantly shorter than in the group without visceral metastasis at TKI initiation (31.2 months) (p<0.05) (Tables 9, 10).

When the PFS durations of TKI were calculated separately, the predicted PFS was calculated as 26 months with erlotinib, 22.9 months with afatinib, and 48.1 months with gefitinib. No statistically significant difference was found between the 3 TKI in terms of PFS.

Progression was observed in 4 of 13 patients using osimertinib during the follow-up period, and the median PFS duration was calculated as 18.5 months. Nine patients continue to use osimertinib.

DISCUSSION

With the development of targeted therapies for driver mutations in NSCLCs, the clinical importance of these mutations has increased. Currently, the most common targetable mutations are mutations in the EGFR gene. In our study, we examined the demographic and clinicopathologic characteristics of NSCLC patients with EGFR exon 19 and exon 21 L858R mutations and the effects of these characteristics on PFS and OS.

Inoue et al.[4] published a study in 2016 in which EGFR mutant NSCLC patients treated with TKI were evaluated and 1660 Japanese patients were evaluated. In the study by Inoue et al.[4], the median OS duration was 30.8 months and the median PFS duration was 11.4 months, while in our study the predicted

Table 4. Overall survival relationship

Overall survival	Univariate model			Multivariate model		
	HR	95% CI	p	HR	95% CI	p
Age at diagnosis	1.03	1.00–1.06	**0.027**			
Gender	1.03	0.54–1.94	0.936			
BMI	0.98	0.88–1.08	0.674			
Smoking	0.82	0.42–1.60	0.553			
ECOG in diagnosis	1.59	1.11–2.29	**0.011**			
EGFR mutant Exon 19	0.87	0.46–1.65	0.665			
EGFR mutant Exon 21	1.24	0.63–2.43	0.541			
EGFR mutant non-classic	0.87	0.27–2.85	0.822			
Stage at diagnosis suitable for surgery	0.38	0.15–1.00	0.050			
Stage metastatic in diagnosis	0.45	0.18–1.09	0.077			
Brain metastasis in diagnosis	1.25	0.62–2.53	0.539			
Serum creatinine in diagnosis	2.00	0.82–4.88	0.130			
Total bilirubin in diagnosis	0.74	0.16–3.45	0.700			
TKI initiation visceral metastasis	2.70	1.35–5.38	**0.005**	2.95	1.33–6.55	**0.008**
TKI side effect	0.93	0.50–1.74	0.813			
1st line CT	0.99	0.49–1.99	0.979			
The TKI received was erlotinib	1.89	0.58–6.15	0.290			
The TKI received was afatinib	0.98	0.30–3.22	0.972			
The TKI received was gefitinib	0.96	0.50–1.85	0.907			
TKi side effect CTCAE grade	0.56	0.22–1.41	0.219			
T790M mutation	0.23	0.07–0.76	**0.015**	0.15	0.03–0.68	**0.013**
Osimertinib	0.23	0.07–0.76	**0.015**			
Last visit ECOG	1.59	1.23–2.05	**0.000**	1.54	1.17–2.03	**0.002**
Progression	29.2	1.04–823.7	**0.048**			

Cox regresyon (forward LR).

CI: Confidence interval, CT: Chemotherapy, BMI: Body mass index, TKI: Tyrosine kinase inhibitor, EGFR: Epidermal growth factor receptor, CTCAE: Common Terminology Criteria of Adverse Effects

Table 5. TKIs–OS periods

	Predicted survival time (month)	95% CI	p
Erlotinib	47.0	36.9–57.0	
Afatinib	36.4	24.0–48.9	0.295
Gefitinib			
Total	49.2	39.4–59.0	

Kaplan-Meier (log-rank).

CI: Confidence interval, TKI: Tyrosine kinase inhibitor

OS duration was 49.2 months and the predicted PFS duration was 27.4 months. Again, the median age at diagnosis was 67 years in the study by Inoue et al.[4]. In our study, the median age at diagnosis was 63 years, the median age at diagnosis of the patients who died was 66 years, while the median age at diagnosis of the survivors was 59 years, and the association of age with mortality was found to be statistically significant (p<0.05), and a significant effect of age at diagnosis on the

prediction of OS was observed in the Cox regression analysis in accordance with the literature. In the study of Inoue et al.[4] 66.7% of the patients were clinically at stage 4 and there was a statistically significant association between clinical stage and OS and PFS in both univariate and multivariate regression analyses. In our study, 83.6% of the patients were in stage 4 at the time of diagnosis, and the rest progressed to metastatic stage during follow-up. In univariate Cox regression analyses, a significant association of clinical stage with PFS was found in accordance with the literature, but not in multivariate analyses. Also, unlike the literature, the association with GS was not statistically significant. In our study, the fact that TKIs were given only in the metastatic stage was thought to be effective in this situation. In our study, in the Cox regression analysis of the ECOG performance score at the time of diagnosis, the association with GS was statistically significant in the univariate model but not in the multivariate model in accordance with the study of Inoue et al.[4]. However, the association of ECOG performance score at the last visit with both OS and PFS was statistically significant in univariate and

Figure 1. Visceral metastasis-OS relationship
OS: Overall survival, TKI: Tyrosine kinase inhibitor

Figure 2. T790M mutation-OS relationship
OS: Overall survival
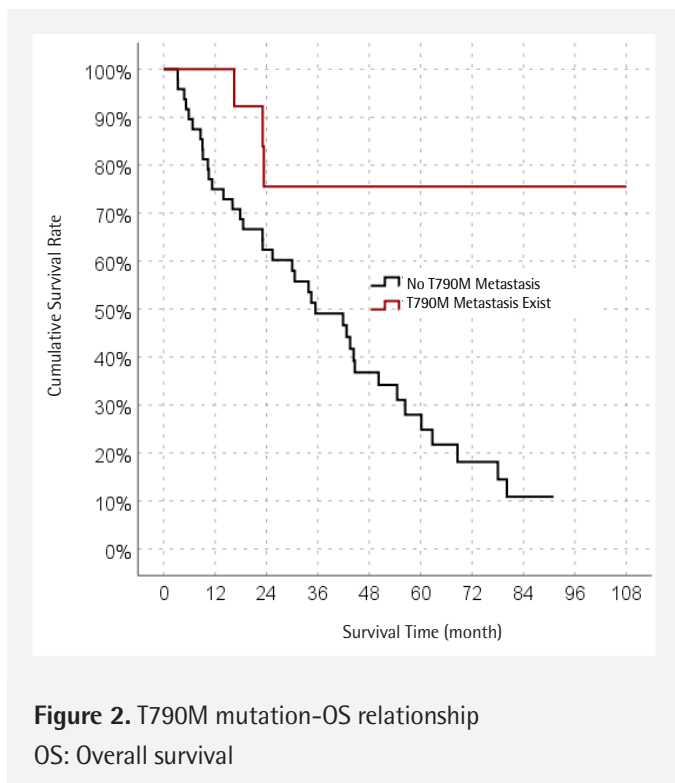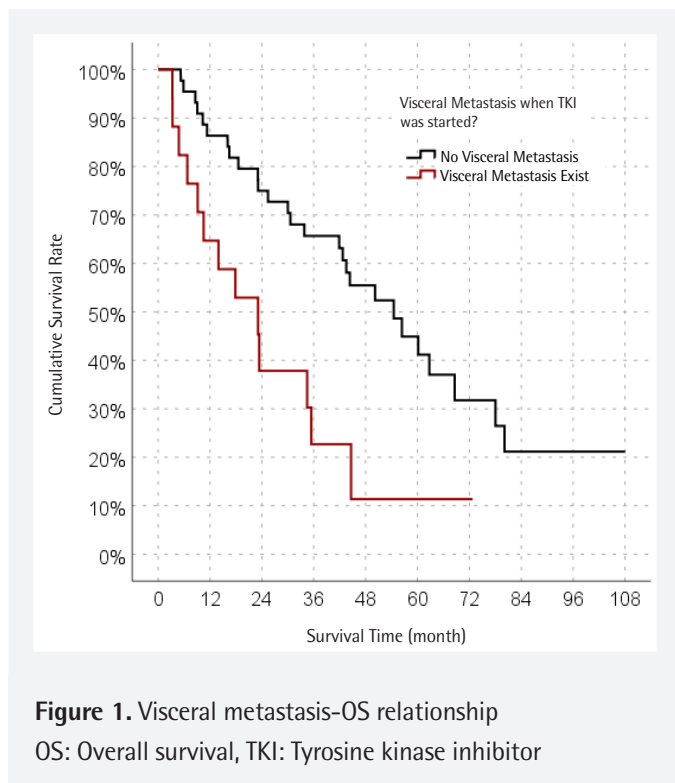
Table 6. Visceral metastasis–OS relationship

		Predicted survival time (month)	95% CI	p
Was there visceral metastasis when TKI was started?	None	56.0	44.7-67.2	0.003
	Yes	26.2	15.3-37.2	
	Total	49.2	39.4-59.0	

Kaplan-Meier (log-rank).
CI: Confidence interval, TKI: Tyrosine kinase inhibitor, OS: Overall survival

Table 7. T790M mutation–OS relationship

		Predicted survival time (month)	95% CI	p
T790M mutation	None	40.5	32.1-48.8	0.008
	Yes	86.8	65.8-107.8	
	Total	49.2	39.4-59.0	

Kaplan-Meier (log-rank).
OS: Overall survival, CI: Confidence interval

multivariate analyses. This supports the idea that deterioration in performance score during clinical follow-up can be used as a guide for prognosis.

The gender distribution in the study by Inoue et al.[4] was 64.8% female and 35.2% male. Similarly, the gender distribution of the patients included in our study was 59% female and 41% male. Although lung cancer is more common in men, the fact that patients with driver mutation are mostly from the non-smoking group and the smoking rate of women is lower compared to men and as a result, EGFR mutant NSCLC is more common in women is consistent with the data[5-7]. In the meta-analysis published by Lee et al.[8] in 2015, it was reported that treatment with TKI was more beneficial in women than men and provided 27% longer PFS (10 months vs. 11.8 months). However, in the EURTAC study published in 2012, no significant difference was observed between male and female genders[9]. In our study, the association of female gender with mortality and OS and PSK was not statistically significant.

In a meta-analysis of 3.688 patients published by Ren et al.[10] in 2012, it was reported that EGFR mutation was more common in patients with no smoking history. In a study published by Lee et al.[11] in 2010 with 324 Korean patients, it was reported that EGFR mutation was more common in patients with less than 25 pack-years of smoking and was more common in women. Again, in the study published by Lee et al.[12] in 2010, it was reported that the incidence of EGFR mutation decreased even in passive smokers. In the meta-analysis published by Lee et al.[8] in 2015, it was reported that treatment with TKI provided 36% more benefit in never smokers than in former smokers and current smokers. In our study, 50.8% of the patients were never smokers, 23% had quit smoking, 13.1% were active smokers, and 13.1% had no smoking-related data. The association of smoking with mortality, OS and PFS was not statistically significant. This situation, which differs from the literature, was thought to be due to the patients in our study whose smoking data could not be accessed.

Table 8. Progression-free survival relationship

Progression-free survival	Univariate model			Multivariate model		
	HR	95% CI	p	HR	95% CI	p
Age at diagnosis	1.01	0.99-1.04	0.206			
Gender	1.06	0.61-1.86	0.827			
BMI	0.96	0.87-1.05	0.346			
Smoking	0.97	0.54-1.75	0.929			
ECOG in diagnosis	1.17	0.84-1.63	0.368			
EGFR mutant Exon 19	1.36	0.76-2.44	0.297			
EGFR mutant Exon 21	0.68	0.36-1.28	0.234			
EGFR mutant non-classic	1.11	0.39-3.12	0.843			
Stage operable in diagnosis	0.39	0.17-0.89	**0.024**			
Stage metastatic in diagnosis	0.42	0.19-0.90	**0.026**			
Brain metastasis in diagnosis	1.31	0.71-2.41	0.381			
Serum creatinine in diagnosis	1.46	0.61-3.51	0.398			
Total bilirubin in diagnosis	0.93	0.25-3.44	0.917			
TKI initiation visceral metastasis	2.22	1.19-4.14	**0.012**	2.19	1.12-4.31	**0.022**
TKI side effect	1.11	0.64-1.92	0.705			
1st line CT	0.68	0.35-1.30	0.241			
TKI erlotinib received	1.50	0.60-3.77	0.391			
TKI afatinib received	0.84	0.30-2.34	0.736			
TKI gefitinib received	0.90	0.52-1.55	0.697			
TKI side effect CTCAE grade	0.40	0.16-0.95	**0.038**			
T790M mutation	1.63	0.86-3.07	0.135			
Osimertinib	1.63	0.86-3.07	0.135			
Last visit ECOG	1.38	1.11-1.71	**0.004**	1.37	1.10-1.71	**0.006**

Cox regression (forward LR).

CI: Confidence interval, CT: Chemotherapy, BMI: Body mass index, TKI: Tyrosine kinase inhibitor, EGFR: Epidermal growth factor receptor, CTCAE: Common Terminology Criteria of Adverse Effects

Table 9. TKIs-PFS times

	Predicted survival time (month)	95% CI	p
Erlotinib	26.0	19.6-32.3	
Afatinib	22.9	12.9-32.8	0.581
Gefitinib	48.1	0.0-103.4	
Total	27.4	20.9-33.9	

Kaplan-Meier (log-rank).

CI: Confidence interval, TKI: Tyrosine kinase inhibitor, PFS: Progression-free survival

Table 10. Osimertinib PFS

Discontinuation of osimertinib	Minimum-maximum	Median	Mean±SD / n (%)	
(-)			9	69.2%
(+) Progression			4	30.8%
PFS	3.27-91.03	18.57	24.00±19.21	

PFS: Progression-free survival, SD: Standard deviation

In a study published by Jiang et al.[13] in 2017, it was reported that in KHDAK patients with EGFR-mutant TKI, liver metastases were associated with shorter PFS duration (7.5 months vs. 11.8 months). In a study published by Luo et al.[14] in 2022, it was reported that bone, liver, and surrenal gland metastases increased mortality in EGFR mutant NSCLC patients. In our study, 17 patients had visceral metastases when TKI was initiated (27.9%). Univariate and multivariate Cox regression analyses revealed statistically significant associations between visceral metastases and OS and PFS. The predicted survival time was significantly shorter in the group with visceral metastasis at TKI initiation than in the group without visceral metastasis at TKI initiation (26.2 months vs 56 months). The predicted PFS time was significantly shorter in the group with visceral metastases on TKI than in the group without visceral metastases on TKI (15.1 months vs 31.2 months).

In our study, 31.1% of patients had brain metastases at the time of diagnosis. There was no significant correlation between the presence of brain metastasis at the time of diagnosis and OS and PFS. In a study published by Ouyang et al.[15] in 2020, it

was reported that metachronous brain metastases (occurring >6 months after the diagnosis of the primary tumor) had shorter OS and PFS times than synchronous brain metastases (22.1 months vs. 30.3 months) and brain metastasis was the most important prognostic factor and metachronous brain metastases should be treated more aggressively. In our study, metachronous brain metastases were not analyzed separately; a separate study on this subject is warranted.

In the study published by Maemondo et al.[16] in 2010, it was reported that the most common side effect after TKI use was skin rash with 71.1%. In the LUX-Lung 3 and LUX-Lung 6 studies, skin rash was reported to be the most common side effect in patients (16-15%)[17]. In our study, 52.5% of the patients had side effects related to TKI use, the most common side effects were skin-related side effects (87.5%) and 48.4% of these were grade 2 side effects, consistent with the literature. TKI was not discontinued in any patient due to side effects.

In the meta-analysis published by Lee et al.[8] in 2015, it was reported that treatment with TKI was more beneficial in patients with exon 19 mutation than those with exon 21 mutation, longer OS was detected in patients with exon 19 mutation compared to those with exon 21 mutation after treatment with TKI, and patients with exon 19 mutation who were not treated with TKI had a worse prognosis compared to those with exon 21 mutation. It was reported that patients with exon 21 mutation treated with TKI had shorter PFS than patients with exon 19 mutation treated with TKI. Similarly, many other studies have also reported longer OS in patients with exon 19 mutations compared to those with exon 21 mutations. In the LUX-Lung 3 and 6 studies published in 2015, it was reported that when afatinib and CT use were compared in exon 19 mutant patients, significantly longer OS was obtained in patients receiving afatinib, but no significant difference was found in patients with exon 21 mutants[17-22]. However, in the LUX-Lung 7 study, no significant difference was found between exon 19 and exon 21 in terms of PSK[23]. In our study, 65.6% of patients had exon 19 mutation, 26.2% had exon 21 mutation, 8.2% had other non-classical mutations and no significant difference was found between exon 19 and exon 21 mutations in terms of OS and PFS.

In the OPTIMAL study published in 2011, erlotinib and CT were compared as first-line treatment in EGFR mutant patients and a significantly longer OS was found in the erlotinib group (13.1 months vs. 4.6 months)[18]. In the LUX-Lung 3 and LUX-Lung 6 trials published in 2015, afatinib and pemetrexed-cisplatin treatments were compared and no significant difference was found in terms of OS, but when the exon 19 mutant subgroup was considered separately, it was reported that longer OS was achieved in the group receiving afatinib in both studies[17]. In our study, 75.4% of the patients received TKI as first-line

treatment, while 24.6% had received CT previously. There was no significant difference in OS and PFS between the group who received 1st-line CT and the group who did not. This was thought to be due to the fact that most of the patients in the group received CT while waiting for EGFR mutation results and after the mutation was detected, the patients were switched to TKI treatment before the completion of CT.

In a meta-analysis of 1821 patients published by Liang et al.[24] in 2014 comparing the use of erlotinib, gefitinib, afatinib and icotinib in EGFR mutant patients, it was reported that no significant difference was found between these 4 TKIs in terms of OS and PFS. Similarly, no significant difference was found between erlotinib, gefitinib and afatinib in terms of OS and PFS in our study.

In the FLAURA study published in 2021 comparing the use of osimertinib, a 3rd generation EGFR TKI, with 1st generation EGFR TKIs as 1st line treatment in EGFR mutant patients, it was reported that significantly longer PFS (18.9 months-10.2 months) and longer OS (38.6 months-31.8 months) were achieved in the osimertinib group[22]. In the study published by Lee et al.[3] in 2021, it was reported that patients receiving 1st-line osimertinib had a longer OS compared to the other TKI group, no difference was observed in terms of OS, but patients who started treatment with afatinib and switched to osimertinib after progression had similar OS times to those receiving 1st-line osimertinib. In a study published by Luo et al.[14] in 2022, it was reported that the use of osimertinib reduced mortality in EGFR mutant NSCLC patients receiving 1st generation TKI as 1st line treatment with or without T790M mutation. Since osimertinib cannot be used in patients with progression under TKI and not positive for T790M mutation according to SUT rules in our country, there were no patients receiving 1st line osimertinib in our study. In our study, a significant difference was found between patients who received osimertinib and those who did not in terms of mortality and OS, similar to the literature, but no significant difference was found in terms of PFS. Since 1st generation and 2nd generation TKIs do not adversely affect the PFS duration of systemic therapies given after them, and since CT is also used as interim treatment in the use of osimertinib after 1st and 2nd generation TKIs, we found that the PFS duration of osimertinib was shorter than expected.

Study Limitations

The small number of patients receiving osimertinib is one of the limiting parts of our study, and the lack of a significant difference in terms of PS between patients receiving and not receiving osimertinib is at a scientifically low level of evidence and has low reliability when evaluated statistically. In our study, the relatively small number of patients, not using all of the TKIs classically used in the world in primary care and the retrospective nature of our study are the prominent limitations of our study.

CONCLUSION

In conclusion, our study showed that age, ECOG performance score at diagnosis and at the last visit, visceral metastasis at TKI start, T790M mutation and thus osimertinib use had a significant effect on OS, whereas visceral metastasis at TKI start, stage at diagnosis, ECOG performance score at the last visit had a significant effect on PFS.

Ethics

Ethics Committee Approval: This thesis study was found to be in compliance with medical ethics and the Helsinki Declaration of Human Rights with the decision of the Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee dated 15.02.2023 and numbered E-83045809-604.01.01-620248.

Informed Consent: Retrospective study.

Peer–review: Externally peer-reviewed.

Authorship Contributions

Concept: N.S.D., Design: N.S.D., Data Collection or Processing: B.E., Analysis or Interpretation: B.E., N.S.D., Literature Search: B.E., Writing: B.E.

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Falsely Elevated Thyroid Stimulating Hormone in Two Cases Requiring Special Follow-up

Özel Takip Gerektiren İki Olguda Yalancı Tiroid Stimulan Hormon Yüksekliği

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ABSTRACT

"Inappropriate thyroid stimulating hormone (TSH)" refers to an elevation in TSH levels that does not match the clinical findings and free T3 and free T4 levels. Several conditions can cause this, such as pituitary tumors that produce TSH, resistance to thyroid hormones, macro-TSH, and antibody interference. Macro-TSH is a condition that causes TSH to be measured high in the blood for a long time by forming a complex with immunoglobulins, mostly IgG. However, patients are clinically euthyroid because macro-TSH is not a bioactive complex. It is essential to exclude the diagnosis of falsely elevated TSH to protect patients from unnecessary or high-dose levothyroxine therapy. In our first case, we presented a patient in whom subclinical hypothyroidism was detected during in vitro fertilization treatment, and levothyroxine was started. The other case was an operated papillary thyroid cancer patient. In both cases, although the dose of levothyroxine was increased, insufficient TSH response to increased fT4/fT3 levels suggested inappropriate TSH elevation. The polyethylene glycol (PEG) precipitation method was used to detect the assay variability. TSH recovery after PEG was 0.96% and 21%, respectively, supporting the diagnosis of macro-TSH. In both cases, detecting Macro-TSH was crucial in preventing thyrotoxicosis caused by excessive levothyroxine dosage. In addition, delay in treatment for infertility was prevented in the first case.

Keywords: Macro-TSH, subclinical hypothyroidism, polyethylene glycol precipitation method, interference, heterophilic antibody

ÖZ

"Uygunsuz tiroid stimüle edici hormon (TSH)" terimi, klinik bulgularla ve serbest T3/T4 düzeyleriyle uyumlu olmayan TSH yüksekliğini ifade eder. TSH üreten hipofiz tümörleri, tiroid hormon direnci, makro-TSH ve antikor interferansı gibi çeşitli durumlar buna neden olabilir. Makro-TSH, başta IgG olmak üzere immünoglobulinler ile TSH'ın kompleks oluşturarak kanda uzun süre yüksek olarak ölçülmesi durumudur. Ancak hastalar klinik olarak ötiroiddir; çünkü makro-TSH biyoaktif bir kompleks değildir. Hastaları gereksiz veya yüksek doz levotiroksin tedavisinden korumak için hatalı TSH yüksekliğini dışlamak önemlidir. İlk olgumuzda yardımcı üreme teknikleri tedavisi sırasında subklinik hipotiroidi saptanan ve levotiroksin başlanan bir olguyu sunduk. Diğer olgumuz, opere papiller tiroid kanseri hastasıydı. Her iki olguda da, levotiroksin dozu artırılmış olmasına rağmen, artan serbest T3/T4 seviyelerine yetersiz TSH yanıtı, uygunsuz TSH yüksekliğini akla getirmiştir. Laboratuvar interferansını değerlendirmek için polietilen glikol (PEG) ile çöktürme metodu kullanıldı. PEG sonrası TSH recovery oranları sırasıyla %0,96 ve %21 olup, makro-TSH tanısını desteklemekteydi. Her iki olgumuzda da, yüksek levotiroksin dozunun neden olabileceği tirotoksikozun önlenmesinde makro-TSH'nin saptanması önem arz etmekteydi. Ayrıca, ilk olguda infertiliteye yönelik tedavide gecikmenin önüne geçilmiş oldu.

Anahtar Kelimeler: Makro-TSH, subklinik hipotiroidizm, polietilen glikol çöktürme yöntemi, interferans, heterofil antikor

INTRODUCTION

The term inappropriate thyroid stimulating hormone (TSH) describes a TSH elevation inconsistent with clinical findings and free T3 (fT3) and free T4 (fT4) levels[1-3]. TSH-producing pituitary adenomas (TSHoma), thyroid hormone receptor resistance (RTH), macro-TSH, and interference by endogenous antibodies may cause this situation[4]. The first condition to be evaluated in the differential diagnosis is the presence of clinical findings.

YANIK ÇOLAK et al. Falsely Elevated Thyroid Stimulating Hormone

Nam Kem Med J 2023;11(4):395-407

In the presence of thyrotoxicosis symptoms and signs, TSHoma and RTH should be considered first. However, depending on the mutation subtype in RTH, it may also present with hypothyroidism. Sometimes the symptoms of both conditions can be seen together in patients with RTH. In addition, in the differential diagnosis of TSHoma and RTH, pituitary magnetic resonance imaging (MRI), TR-β mutation analysis, TSH alpha subunit level, thyrotropin releasing hormone (TRH) stimulation test, and t3 suppression test are performed as further tests[5,6].

In the absence of hypothyroid symptoms, the situations causing falsely elevated TSH levels, such as macro-TSH and interference by endogenous antibodies, should be considered as rare causes. The concept of interference by endogenous antibodies is used whenever one suspects a patient's sample contains antibodies that cause false results by binding to the assay antibodies[7]. There are three types of endogenous antibodies that cause interferences in immunoassays: autoantibodies, heterophilic antibody, and antianimal antibody[8]. Macro-TSH is caused by a large amount of monomeric TSH complexed with anti-TSH antibodies-mostly immunoglobulin G (IgG)[9,10]. Macro-TSH is at least 150 kDa that likely accumulates in the circulation, resulting in measurements indicating falsely increased TSH levels[9,11,12]. This molecule is not bioactive but immunoreactive. It is crucial to identify laboratory interferences to ensure the accurate diagnosis of thyroid diseases, proper treatment, and prevention of the adverse effects of levothyroxine overtreatment.

In this article, we discuss two cases where precise TSH level measurement is crucial for treatment planning and monitoring. The first case involves a female patient who is undergoing in vitro fertilization (IVF) treatment, while the second case is a patient with papillary thyroid cancer, who requires TSH suppression therapy after surgery.

CASE REPORTS

Case 1

A 32-year-old female patient was referred to our clinic after detecting TSH: 25 µIU/mL (reference interval: 0.27-4.20), fT4: 1.3 ng/dL (reference interval: 0.93-1.7) fT3: 2.6 pg/mL (reference interval: 2.0-4.4) in the tests performed during IVF treatment. There were no signs or symptoms of thyroid dysfunction and no personal or family history of thyroid or autoimmune disorders. In the patient's control examination, TSH: 14.8 µIU/mL, fT4: 1.41 ng/dL and fT3: 2.8 pg/mL were detected. Anti-thyroglobulin and anti-thyroid peroxidase were found to be negative. The thyroid ultrasound pattern was normal. The patient had a pregnancy plan. Levothyroxine was started at a dose of 1.25 mcg/kg. Six weeks later, TSH: 11.1 µIU/mL, fT4: 1.56 ng/dL and fT3: 3.21 pg/mL were detected. The levothyroxine dose was adjusted to 1.6 mcg/kg in the

patient. In the follow-up examination one month later, TSH: 10.2 µIU/mL, fT4: 1.68 ng/dL and fT3: 3.70 pg/mL were detected. Although the levothyroxine dose was increased, sufficient TSH response to the increase in fT3 and fT4 levels could not be obtained. The patient had drug compliance. There was no drug use to interact with levothyroxine. There were no laboratory or clinical findings suggestive of malabsorption. Rheumatoid factor was negative. In the differential diagnosis of the patient who was clinically euthyroid since diagnosis, polyethylene glycol (PEG) precipitation test was applied to exclude laboratory interference and to screen for monomeric TSH. The post-PEG TSH recovery was 0.96% (Table 1).

Case 2

A 31-year-old male patient was referred to our clinic for follow-up after a total thyroidectomy operation. The pathology result was reported as encapsulated follicular variant papillary thyroid carcinoma (pT1bNx Mx). The postoperative laboratory evaluation results were as follows: TSH: 30.8 µIU/mL, fT4: 0.05 ng/dL and fT3: 1.32 pg/mL. Levothyroxine treatment was started at a dose of 1.6 mcg/kg/day. At the follow-up evaluation after six weeks, TSH: 27.5 µIU/mL, fT4: 1.32 ng/dL and fT3: 2.1 pg/mL were detected. Therefore, the levothyroxine dose increased to 1.9 mcg/kg/day. One month later, TSH: 15.0 µIU/mL fT4: 1.34 ng/dL, fT3: 2.2 pg/mL were detected. Macro-TSH was considered in the differential diagnosis of the patient since the suppression in the TSH level did not show sufficient correlation with the increase in the fT4/T3 levels. The patient was previously evaluated for other possible causes. The patient had drug compliance. There was no clinical or laboratory finding to suggest malabsorption. Rheumatoid factor was negative. PEG precipitation test was performed on the patient (Table 1). The post-PEG TSH recovery rate was 21%.

The recovery rates following PEG indicated that both patients had macro-TSH. Since the levels of monomeric TSH were normal after PEG precipitation, conditions such as TSHoma and RTH were ruled out as potential diagnoses. As a result, there was no need to conduct further tests such as pituitary MRI, TR-β mutation analysis, TSH alpha subunit level assessment, TRH stimulation test, and t3 suppression tests. Also, endogenous antibodies might have caused falsely elevated TSH results as an assay interference in our patients, and PEG precipitation

Table 1. PEG precipitation			
	TSH concentration (µIU/mL) Before PEG	TSH concentration (µIU/mL) After PEG	Recovery rate (%)
Case 1	12.5	0.12	0.96
Case 2	15.24	3.16	21
PEG: Polyethylene glycol, TSH: Thyroid stimulating hormone			

Nam Kem Med J 2023;11(4):395-407

YANIK ÇOLAK et al. Falsely Elevated Thyroid Stimulating Hormone

might have precipitated these antibodies as well. In any case, monomeric TSH detected after PEG determines our clinical approach.

In the first case, our patient's monomeric TSH level was at the target value (TSH <2.5) before undergoing IVF, so we continued their current levothyroxine treatment dose. For the second case, we adjusted the levothyroxine suppression dose to maintain fT4 at the upper limit of 1/3, and no recurrences were observed during the patient's follow-up.

PEG Precipitation

The patients' serum samples were mixed with equal volumes of a 25% solution of PEG 6000 (Sigma, dissolved in distilled water) and equal volumes of distilled water (as a control). The mixtures were centrifuged at 10000 g for 5 minutes and the supernatants were collected for TSH assay. TSH levels were measured by Roche E801 analyzer using its original kits with electrochemiluminescence immunoassay (ECLIA) method and results were given by multiplying by two (dilution factor). The recovery (%) rates were calculated by using following formula: Recovery %=(the TSH value measured after the addition of PEG/the TSH value measured after the addition of distilled water)×100. A recovery rate that was lower than 40% suggests the high molecular weight proteins, such as immunoglobulins[4]. In the case reports in the literature, the TSH recovery after PEG precipitation was less than 25%[10]. In only two cases, the recoveries were about 50%[13,14]. According to Sakai et al.[4], a recovery rate lower than 40% suggests high molecular weight proteins, such as immunoglobulin. We based this study's method and cut-off values to evaluate our cases.

Other Laboratory Assays

Serum TSH, fT4, fT3, anti-thyroid peroxidase assays were also measured by Roche E801 analyzer using its original kits with ECLIA method. Anti-thyroglobulin was measured through Siemens Immulite 2000 Xpi by using its original kit with chemiluminescent immunometric method. Rheumatoid factor levels were measured by Roche C702 chemistry analyzer using its original kits.

DISCUSSION

Measurements of serum TSH levels are the first step test in the thyroid function evaluation algorithm. If there is an increase in TSH levels, the next step should be to check the fT4/fT3 levels. Subclinical hypothyroidism is characterized by normal levels of serum fT4/fT3 and high levels of serum TSH. Moreover, there are various situations where a high serum TSH concentration does not accurately fit the subclinical hypothyroidism definition. These include TSHoma, RTH and occasional mutations of the TSH receptor, variability in assays, the phase of recuperation from nonthyroidal illness[5].

Elevated TSH in patients with TSHoma or RTH is generally associated with high serum fT4 and/or fT3 concentrations. Diagnosing this case is relatively straightforward, but sometimes fT4/T3 levels can be normal. Thyrotoxicosis clinic is a common symptom of TSHoma. In addition, symptoms and signs of hyperthyroidism or hypothyroidism may be observed depending on the subtype of the mutation in RTH. To confirm our diagnoses, laboratory tests were conducted to prevent assay variability that might lead to inappropriate TSH elevation. In both cases, normal monomeric TSH levels were found after precipitation with PEG, which ruled out the diagnoses of TSHoma and RTH. As a result, further tests such as pituitary imaging, genetic mutation analysis, TSH alpha subunit level, TRH stimulation test, and t3 suppression test were not required.

Interference by endogenous antibodies (autoantibodies, heterophilic antibody, and antianimal / human anti-mouse antibodies), rheumatoid factor and macro-TSH should be kept in mind at differential diagnosis[15]. TSH is a small bioactive hormone of 28 kDa easily filtered by the kidney, macro-TSH is a large molecule of at least 150 kDa that likely accumulates in the circulation, resulting in measurements indicating falsely increased TSH levels[9,11,12]. The binding of IgG to TSH has been reported as the leading cause of macro-TSH[12,16]. Macro-TSH is not bioactive but immunoreactive. The etiology of macro-TSH is unknown.

The PEG precipitation method to screen for macroprolactinemia has also been transposed to macro-TSH detection[9]. Multiple PEG precipitation procedures are available, with percent recovery typically performed[16]. Information about monomeric TSH levels can be obtained by precipitating macro-TSH and interfering endogenous antibodies with PEG. If TSH recovery is low, macro-TSH and interferences by endogenous antibodies should be kept in mind.

The prevalence of macro-TSH has yet to be well known but is considered a rare condition. Hattori et al.[9] evaluated 681 patients with elevated TSH concentrations; macro-TSH was detected in 11 patients from 681 serum samples (1.61%). Ismail et al.[17], in their study, found that 6 of 5310 patients had high TSH levels due to interference by endogenous antibodies. In some of these 6 cases, falsely elevated TSH might occur due to macro-TSH.

One of our cases is the first macro-TSH case diagnosed during IVF treatment in the literature. For women with laboratory values for subclinical hypothyroidism and scheduled for IVF, achieving a TSH concentration of <2.5 mU/L with levothyroxine replacement is recommended.[18] However, in the presence of macro-TSH, patients may receive levothyroxine treatment unnecessarily. Therefore, especially in clinical and laboratory incompatibility cases, the diagnosis of macro-TSH

YANIK ÇOLAK et al. Falsely Elevated Thyroid Stimulating Hormone

Nam Kem Med J 2023;11(4):395-407

will be important in terms of not delaying IVF and protecting the mother and fetus from exogenous thyrotoxicosis caused by the overtreatment of levothyroxine during pregnancy. Hattori et al.[19] proposed that Macro-TSH should be excluded before giving levothyroxine replacement therapy in patients with subclinical hypothyroidism to avoid unnecessary treatment. However, it is challenging to confirm every case routinely. It is also not cost-effective. Therefore, it seems reasonable to keep it in mind in the differential diagnosis and to perform a further examination for macro-TSH in selected cases. In our case, the clues that initiated further investigation were the consistently high TSH levels despite appropriate levothyroxine treatment and the fT4 level close to the upper limit of the reference range. Moreover, malabsorption, drug incompatibility, and interactions with other drugs were all ruled out, and there were no clinical signs of hypothyroidism since diagnosis. Although there is no guideline for treatment monitoring in the presence of Macro-TSH during IVF treatment in the literature, since TSH levels of individuals planning pregnancy cannot be trusted, it may be a reasonable approach to set fT4 levels at 1/3 upper limit as the treatment goal before IVF.

No data are available on thyroidectomized patients with macro-TSH except for two case reports with thyroid cancer and nodular goiter. Macro-TSH was present in one female patient with a history of low-risk papillary thyroid carcinoma[20]. Our second case is the second macro-TSH case reported in the literature, diagnosed due to insufficient TSH suppression after a papillary thyroid cancer operation.

In cases of operated papillary thyroid cancer followed up with TSH suppression, macro-TSH should be considered if a higher than expected dose of levothyroxine is needed to achieve euthyroidism. There are no definitive data on levothyroxine dose adjustment in these patients. Levothyroxine dose adjusted for body weight is the main factor in TSH suppression therapy. In such cases, levothyroxine replacement dose can be determined so that fT4 is kept at the upper limit of 1/3. Thus, patients are protected from exogenous hyperthyroxinemia. In the adults, while macro-TSH disappears and serum TSH level returns to normal in some patients, macro-TSH may persist for up to 4 years in some patients[10]. Considering that the renal clearance of macro-TSH is slower than monomeric TSH, long-term follow-up of patients is required. More studies are needed to assess the balance between TSH suppression and avoiding unnecessary exogenous hyperthyroxinemia.

CONCLUSION

In conclusion, falsely elevated TSH should be kept in mind in clinically euthyroid[18] patients who are planning pregnancy and having subclinical hypothyroidism, and the patients with operated thyroid cancer who require a higher-than-expected dose of levothyroxine to achieve targeted TSH suppression.

Ethics

Authorship Contributions

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2023 Referee Index

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Ayhan AKÖZ

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Ayhan SARITAŞ

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Banu SARIFAKIOĞLU

Barış YILBAŞ

Başak BÜYÜK

Batuhan ÜSTÜN

Beril KADIOĞLU

Beyhan ÖZYURT

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Cem BAŞATAÇ

Cem ÇELİK

Cem Zeki ESENYEL

Cengiz GAZELOĞLU

Cihan KAYA

Cihan ÖZGÜR

Çağrı DOĞAN

Çoşkun BAKAR

Damla BİNNETOĞLU

Derya GÜNER

Duygu KESKİN GÖKÇEELİ

Duygu KÖSE

Elmas BEYAZYÜZ

Emel ALTUNCU

Emre GÜNDOĞDU

Ercan AKŞİT

Erdem BAGATUR

Erkut ÖZTÜRK

Erman ÇİLSAL

Evrim ŞENKAL

Eyyüp ÇAVDAR

Fahri BAYIROĞLU

Fethiye KILIÇASLAN

Fettah EREN

Fevzi CELAYİR

Gamze VAROL

Gökay TAYLAN

Gökhan CANSABUNCU

Gökmen Umut ERDEM

Gülcan GÜCER ŞAHİN

Gülşah ELBÜKEN

Gülsüm YİTİK TONKAZ

Hakan ÇAKIR

Hamdi ER

Hamit BAŞARAN

Hasan Cem AYKUTLU

Hasan KARANLIK

Hilal ERMİŞ

Hüray KÖK

Hüseyin ŞAHİN

İlhan OCAKCIOĞLU

İlkay ER

İlke Evrim SEÇİNTİ

İlke ÖZER ASLAN

İlknur UCUZ

İrem ATEŞ

İrfan ÇINAR

İsmail ACAR

İsmail ÖZKAYA

İstemi SERİN

Kemal KEF

Kerem TEKE

Kubilay KARABOYUN

Kübra USTAÖMER

Kürşat Rahmi SERİN

Leman ŞENTURAN

Levent CANSEVER

Leyla BOZATLI

Lora KOENHEMSI

Mahmut BODUR

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Nusret ERDOĞAN

Onur BARAN

2023 Referee Index

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Özkan ALAN

Özlem KARAARSLAN CENGİZ

Pınar MUTLU

Pınar TARKUN

Pınar TOSUN TAŞAR

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Sercan ERGÜN

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Şevket Barış MORKAVUK

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2023 Author Index

2023 Author Index

2023 Author Index

2023 Subject Index

2023 Subject Index