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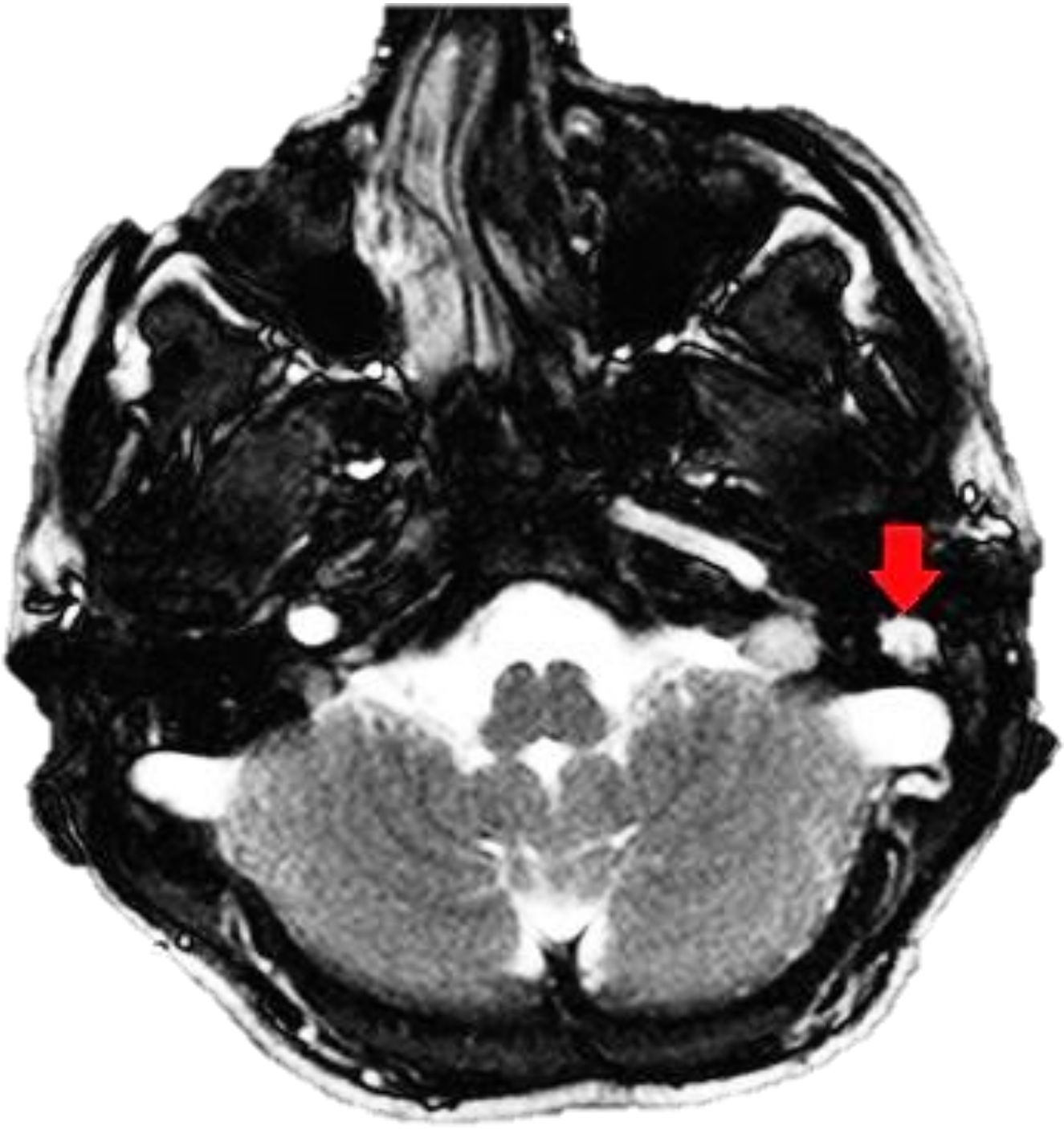

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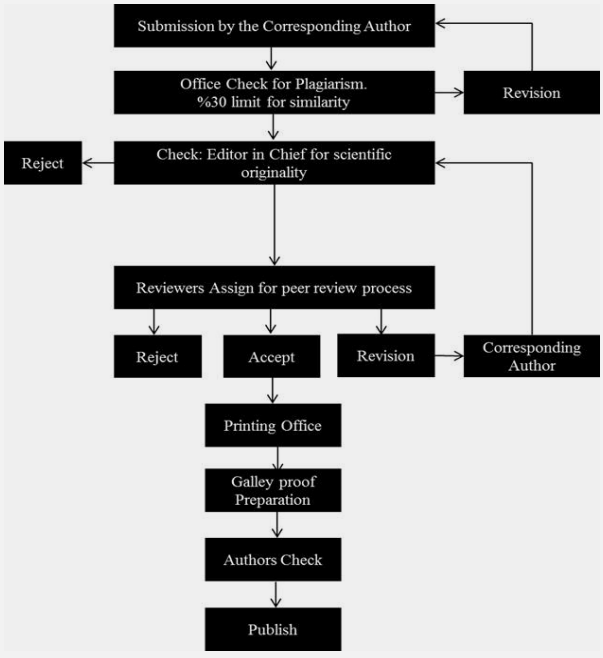

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Serum paraoxonase enzyme activity after balneotherapy in patients with fibromyalgia

Ahmet Karadağ[1*], Halef Okan Doğan[2]

Abstract

Objective: The aim of this study was to compare the serum paraoxonase (PON 1) levels between patients with fibromyalgia syndrome (FMS) and healthy control subjects, and to investigate the possible effect of balneotherapy (BT) on PON 1 enzyme activity in FMS patients.

Methods: The study included 45 female patients with FMS, and 35 healthy female volunteers. To measure PON 1 enzyme activity, venous blood samples were taken twice from the FMS group, before and after BT, and once from the control group. The Visual Analogue Scale (VAS) and Fibromyalgia Impact Questionnaire (FIQ) scales were applied to the FMS patients before and after BT.

Results: There was no difference between the FMS group and healthy control group in terms of serum PON1 activity ($p>0.05$). The comparison of the serum PON 1 activity of the FMS group before and after BT revealed a statistically significant increase after BT ($p=0.001$). A statistically significant decrease was determined in the VAS and FIQ scores of the FMS group after BT compared to the pre-treatment values ($p=0.002$, $p=0.001$, respectively).

Conclusion: BT is an effective non-pharmacologic method in the treatment of FMS. There was an increase in serum PON 1 activity in patients with FMS after BT. BT may have a regulatory effect on the antioxidant system of patients with FMS.

Keywords: Fibromyalgia syndrome, balneotherapy, paraoxonase

Introduction

Fibromyalgia syndrome is a clinical condition that is characterized by chronic widespread pain, sleep disturbance fatigue, and cognitive dysfunction (1). Genetic, environmental and immunological factors, as well as central and peripheral mechanisms are known to play a role in the etiopathogenesis (2, 3). Despite the many clinical trials carried out to date related to the issue, the etiology of FMS is still not clearly understood, although recent clinical studies have indicated that oxidative stress may play a role in the pathogenesis of FMS (4,5).

Paraoxonase-1 (PON 1) is a protein that hydrolyzes lipid peroxides, and has the ability to protect low density lipoproteins (LDL) from oxidation. It also has an antioxidant function, being able to neutralize other radicals, including hydrogen peroxide (6,7). Previous studies have suggested that degradation of the antioxidant system in FMS may lead to oxidative damage in the muscles, and therefore, the antioxidant role of PON 1 may be important in FMS (8).

The management of FMS is based on symptomatic multidisciplinary treatment through pharmacological and non-pharmacological strategies. Balneotherapy (BT) is a non-pharmacologic treatment method that has seen success in the treatment of FMS, in which thermal and/or mineral waters, peloids and gases are applied repeatedly as a cure at various intervals (9). There is a regulatory effect of BT on the antioxidant system that removes free radicals from the body, and clinical trials have shown that after BT sessions, significant reductions have been noted in superoxide dismutase, catalase and glutathione peroxidase enzymes (9,10).

There have been reports in literature of clinical trials evaluating PON 1 enzyme activity levelsin patients with FMS. However, to the best of our knowledge there has been no clinical study examining the effects of BT on PON 1 enzyme activity levels in FMS treatment. Therefore, the aim of this study was to compare the serum PON1 levels between patients with FMS and healthy control subjects, and to investigate the possible effect of BT on PON 1 enzyme activity in FMS patients.

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Materials and methods

This study was conducted between June 2017 and June 2018, and included female patients aged 18–65 years who agreed to participate in the study. A total of 45 FMS female patients who were diagnosed with FMS according to the American College of Rheumatology (2010) criteria (11), were enrolled and a control group was formed of 35 female healthy subjects.

FMS patients with malignancy, rheumatic disease (osteoarthritis, Behçet's disease, rheumatoid arthritis, etc.) and those with a known history of systemic disease (hypertension, diabetes mellitus, neurological or psychiatric disease, etc.) were excluded. The control group was formed of subjects with no known disease and no medication use, recruited from hospital personnel and the relatives of patients. The age, height, weight and tobacco use of the patients in each group were recorded.

This study was approved by the Ethics Committee of our University (2015-12/07), and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each of the study participants.

The FMS group engaged in 20-minute BT sessions every day for 21 days. During BT, the patients reclined and relaxed in a therapeutic pool containing thermo-mineralized water that was rich in bicarbonate (HCO3) and Calcium (Ca) at a temperature of 40°C. The mineral content of the thermal water included chloride, 257 mg/L; sodium, 337 mg/L; Ca, 655 mg/L; magnesium, 104 mg/L; sulfate, 65 mg/L; HCO3: 2003 mg/L; fluoride, 2.24 mg/L and silicate: 32 mg/L. Venous blood samples were taken from the patients both before and after the treatment, and the scales were applied.

Fibromyalgia Impact Questionnaire (FIQ): An FIQ comprising 20 questions was used to assess the patients' physical function, occupational status, pain, sleep, anxiety, depression, stiffness, fatigue and general health as a means of evaluating the functional status of the patients and the progression and results of the disease (12). The validity and reliability of the study was demonstrated in Turkey by Sarmer et al (13).

Visual Analogue Scale (VAS): The VAS was used to examine pain levels in the patients. On a 10 cm scale where 0 indicates no pain and 10 indicates the most severe pain, the patients were requested to mark the point corresponding to the level of pain they experienced.

Biochemical analyses: Blood samples were collected between 8:00 and 10:00 after 10–12 hours of fasting, before and after BT from the FMS group and once from the control group. The blood samples were centrifuged at 1500 rpm for 15 minutes and then separated into sera. The obtained serum samples were stored at -20 ° C until assay.

Paraoxonase activity analysis: The rate of paraoxone hydrolysis was assessed in accordance with the method described by Eckerson et al (14), with measurements made using an autoanalyzer (Beckman Coulter AU 5800, USA).

Statistical analysis

Data obtained in the study were analysed statistically using IBM SPSS Statistics vn. 22 software (IBM Corp., Armonk, NY, USA). Conformity of the data to normal distribution was analyzed using the Kolmogorov-Smirnov test. The Student's t-test and Pearson's correlation test were applied when the parametric test assumptions were met. When the parametric test assumptions could not be met, the Mann Whitney U-test was used, and the Chi-Square test was used to evaluate categorical data. The Wilcoxon sign test was applied in the comparison of pre-treatment and post-treatment variables. Continuous baseline variables were presented as mean ± standard deviation values, and categorical data as number and percentage (%). A value of $p<0.05$ was considered statistically significant. In this study, using $\alpha=0.05$ $\beta=0.10$ $(1-\beta)=0.90$, it was decided to include 45 FMS patients and 35 control subjects in the groups, and the power of the test was p=0.90120.

Results

The study was conducted on the data of 35 healthy female subjects and 45 female patients with FMS. Both groups were similar in terms of age, body mass index (BMI) and tobacco use (Table 1). Serum PON1 activity was found to be lower in the FMS group than in the healthy control group, but not at a statistically significant level ($p>0.05$). The mean serum PON1 activity of the patients with FMS was 95.66±70.84 before BT and 126.51±81.94 U/L after BT.

The serum PON1 activity after BT was significantly higher compared to the pre-treatment PON1 activity (p=0.01) (Table 1). There was no statistically significant correlation between PON1 activity and disease duration, VAS scores and FIQ scores of patients with FMS ($p>0.05$; r=0.207, r=0.202, and r=-0.051, respectively).

Table 1. Disease duration in patients with FMS and the sociodemographic data of the groups

	FMS (n=45) Mean ± SD	Control (n=35) Mean ± SD	p value
Age (years)	52.1 ± 10.1	48.6 ± 13.3	0.213
BMI (kg/m^2)	29.7 ± 5.6	28 ± 5.4	0.404
Disease duration (month)	57.4 ± 52.6	-	
Smoke, Negative n (%)	37 (82.2)	26 (74.3)	0.278
Smoke, Positive n (%)	8 (17.8)	9 (25.7)	

BMI: Body Mass Index; FMS: Fibromyalgia Syndrome; SD: Standard deviation; n: number of patients

Table 2. The VAS and FIQ score of FMS patients, and serum PON 1 levels of the controls and FMS patients

	FMS (n=45)		Controls (n=35)	
	(Before BT)	(After BT)		
VAS (Mean±SD)	7.6 ± 1.3	4.1 ± 1.7		0.002
FIQ (Mean±SD)	72.6 ± 12.1	49.6 ± 19.2		0.001
PON 1 (U/L) (Mean±SD)	95.6 ± 70.8	126.5 ± 81.9	120.1 ± 102.4	0.232[a] 0.001[b]

*p<0.05 VAS: Visual Analogue Scale; FIQ: Fibromyalgia Impact Questionnaire; FMS: Fibromyalgia Syndrome; PON 1: Paraoxanase 1; SD: standard deviation; n: number of patients [a] Comparison between controls and FMS (Before BT); [b] Comparison between FMS patients before BT and after BT

Discussion

The results of the present study showed that PON 1 enzyme activity in FMS patients was no different to that of the healthy individuals, which is a finding that differs from previous studies in literature (15,16). In addition, a decrease was found the VAS and FIQ scores after BT in the current study patients with FMS. However, no correlation was determined between the VAS and FIQ scores and the PON 1 activity of the FMS patients, and a similar finding has been previously reported in literature (17). The current study results showed an increase in PON1 enzyme activity after BT in the FMS patients. Furthermore, no statistically significant correlation was identified between the PON 1 activity of patients with FMS and disease duration. The present study is the first in literature to report such results.

PON 1 has been shown to act as an antioxidant in many clinical studies (18), and it has been suggested that decreases in PON 1 activity may play a role in the pathogenesis of cardiovascular disease, diabetes mellitus and metabolic syndrome (19). PON 1 activity levels have been found to be lower in rheumatoid arthritis (RA) and systemic lupus erythematosus, when compared to healthy individuals (20, 21). Only two clinical studies were identified in literature evaluating PON 1 enzyme activity levels in FMS. A clinical study by Altındağ et al (16) showed that PON 1 activity was lower in FMS patients than in healthy individuals, while Bozkurt et al. (15), reported no decrease in PON 1 activity in FMS patients, and no correlation between PON 1 activity and the VAS and FIQ scores. Similar to that study, no significant differences were noted in the present study between the PON 1 activity of FMS patients and healthy individuals, and no correlation was observed between PON 1 activity and the VAS and FIQ scores.

There is still no definitive treatment for FMS, and so the use of non-pharmacological and pharmacological methods together remains the optimum approach. BT, a non-pharmacological treatment, is known to have a positive effect on clinical parameters (17,22). In line with previous studies, the findings of the present study demonstrated a significant improvement in post-BT VAS and FIQ scores in patients with FMS. The regulatory role of the antioxidant system is believed to be one of the mechanisms of action of BT. Yamaoka et al. (23) determined the effect of BT on superoxide dismutase and catalase activities, while a further study found decreased superoxide dismutase activity as a result of BT in patients with rheumatoid arthritis (24), and Bender et al. (9) demonstrated the antioxidant effect of BT. The findings of the present study mirror those of previous studies as the serum level of PON1, an antioxidant enzyme, was seen to be elevated after BT in patients with FMS.

The limitations of this study include the narrow patient population studied, the absence of male patients with FMS, the lack of repeated measurements of PON 1 levels after 21 days in the healthy control group, and that no evaluation was made of other factors which can affect the FIQ score, and other antioxidant markers

Conclusion

BT is an effective non-pharmacological method in the treatment of FMS. The results of this study showed an increase in serum PON1 activity in patients with FMS after BT.Previous studies have also shown that the oxidant/antioxidant status could have a role in the pathogenesis of FMS. Therefore, these results suggest BT may have a regulatory effect on the antioxidant system in patients with FMS. The results of the present study may contribute to a better understanding of the pathogenesis of FMS and guide future clinical studies investigating the pathogenesis and treatment of FMS. Nevertheless, there is a need for further clinical studies examining antioxidant enzyme metabolism in FMS.

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Author's Contributions: AK, HOD: Research concept and design, data collection, literature search, preparation of the article, statistical analysis, **AK:** Manuscript revision

Ethical issues: Author declare, originality and ethical approval of research. The study was conducted under defined rules by the Local Ethics Commission guidelines and audits.

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Seasonal clustering in epilepsy

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Abstract

Objective: There are few studies in the literature suggesting that epileptic attacks can cluster especially in winter. We aimed to confirm the most frequent month and season in which our patients with epilepsy visited the emergency room because we had similar observations in our clinical experience.

Material and Methods: Patients admitted to the emergency room due to convulsive epileptic seizures between January 2017 and December 2019 were included in the study. The month of seizures was recorded.

Results: In our study, epileptic seizures clustered significantly in winter.

Conclusion: Although more detailed data should be collected on this subject, we think this is an indirect result of the change in vitamin D metabolism, as suggested in other studies.

Keywords: epileptic cluster; seasonality; human seasonality

Introduction

Seasonal clustering can be seen in many systemic diseases. Although in infectious diseases, the reason of seasonality is easily understood, various reasons are speculated when it comes to psychiatric diseases, coronary artery diseases, ischemic and hemorrhagic stroke, and even for some oncologic diseases (1-5). There are few studies in the literature suggesting that epileptic attacks can cluster, especially in winter (6,7). Studies on seasons and epilepsy are not just about seasonal clustering: there are several studies emphasizing etiologic implications of epilepsy in terms of season of birth, and circadian and seasonal variation of the first febrile seizure and infantile spasm (8-12).

In our own clinical experience, we observed that the number of epileptic attacks was higher in the winter season. We aimed to achieve significant results by converting our observation to quantitative values.

Material and methods

Demographic and medical information of patients

This is a descriptive single-center study conducted in our adult neurology and emergency departments. Patients who were admitted to the emergency room for convulsive epileptic seizures between January 2017 and December 2019 were included in the study. In the retrospective design of the study, the medical history of all patients was investigated, and whether they used antiepileptic drugs and vitamin D supplements, epilepsy follow-up years, and demographic information were recorded.

All patients were using antiepileptic drugs. None of the patients were receiving vitamin D supplements.

Patients aged under 18 years, and patients with suspected pseudo-seizures and incomplete medical data were not included in the study. The month in which the patients had a seizure was noted. The annual data were divided into four seasonal periods (December to February, March to May, June to August, and September to November).

Statistical analysis

The data are expressed in the form of mean, standard deviation, minimum and maximum values and percentages. One-way analysis of variance (ANOVA) was used to show the difference of the number of epileptic seizures according to the seasons statistically.

Results

In the 36-month period between January 2017 and December 2019, a total of 194 seizures of 82 patients who were admitted to the emergency department with convulsive epileptic seizures were distributed by months.

Forty-five female and 37 male patients were included in the study. The ages of the patients were between 18-90 years and the mean age was 47.52 years. The mean follow-up for epilepsy was 9.7 (range, 1-45) years. The most visited month due to epileptic seizures was December (n=25), and the least visited month was July (n=10) (Graph 1).

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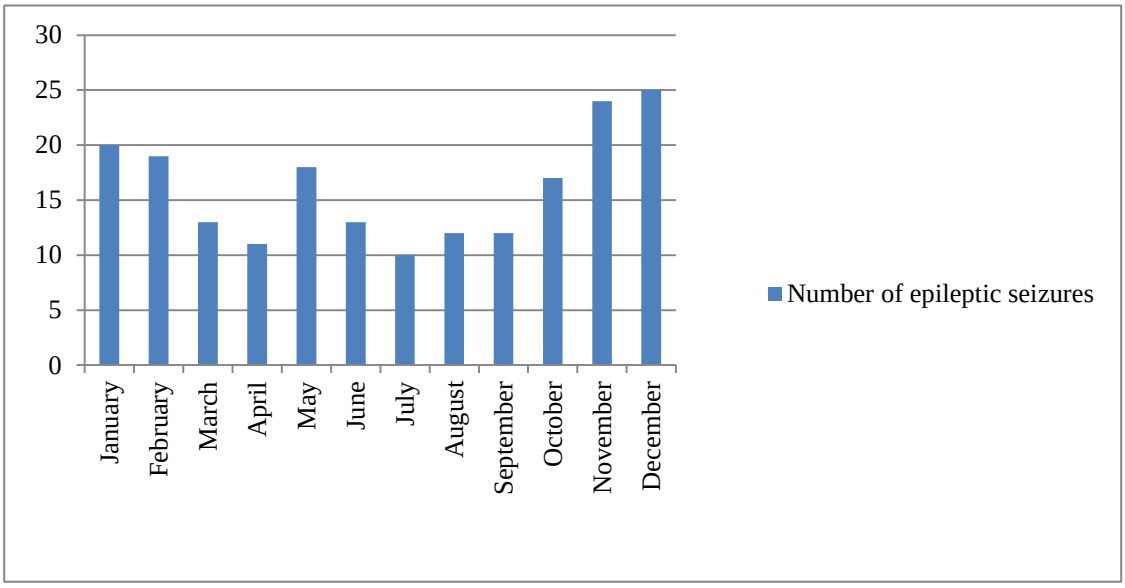
When seizures were analyzed according to seasonal distribution, the least frequent season in which epileptic seizures were seen was summer (n=35), followed by spring (n=42) and autumn (n=53). The most frequent season in which epileptic seizures were seen was winter (n=64). The comparison of the number of seizures experienced by patients with epilepsy during the 36-month period by season was statistically interpreted. Accordingly, the average number of seizures of the patients according to the seasons in this period was as following; 12.67 ± 1.5 in the spring, 10.33 ± 2.1 in the summer, 17.67 ± 6 in the autumn, and 21.67 ± 3.8 in the winter (Table 1).

The number of epileptic seizures experienced by the patients showed a statistically significant difference according to the seasons (one-way ANOVA; $p<0.05$). This distinctness arises from the difference in number of epileptic seizures between summer and winter ($p=0.026$; Tukey arrangement). As can be clearly assessed in Graph 2, the number of epileptic seizures experienced in the winter season was considerably higher than in the summer season, both numerically and statistically.

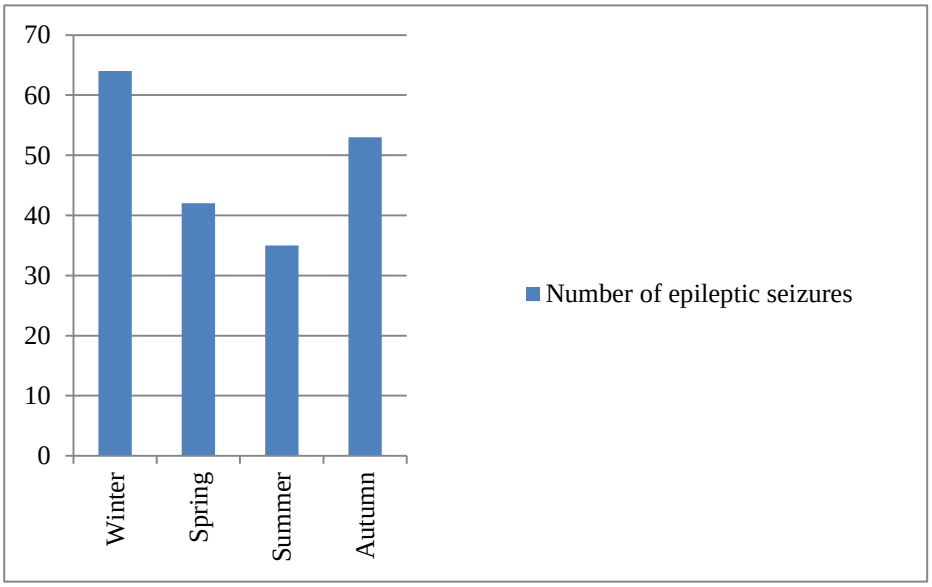
Table 1: Average number of seizures of the patients according to the seasons.

	Spring	Summer	Autumn	Winter	P Value
Seizure frequency Mean±SD	12.67 ± 1.5	10.33 ± 2.1	17.67 ± 6	21.67 ± 3.8	Winter-Summer p = 0.026

One-way ANOVA; p <0.05



Graph 1. Distribution of epileptic seizures by months



Graph 2. Distribution of epileptic seizures by seasons (winter: December to February, spring: March to May, summer: June to August, autumn: September to November).

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There are quite a few studies examining the relationship of epilepsy with seasons. In our study, similar to previous studies, it was found that epileptic seizures were more common in winter season.

While the seizure numbers showed a significant peak in December and a nadir in June in our study, similar results were obtained in January and August in the study of Clemens et al. (6). Procapia et al. presented a number of studies suggesting differences in the seasonality of birth between patients with epilepsy and the general population, which confirmed that births in summer and winter months were related with high incidence of epilepsy. All three studies seemed to confirm the existence of an etiological factor for epilepsy with a seasonal presence in the environment causing disruption of neurodevelopment in the perinatal period (8-10). In 1988, Danesi showed a higher incidence of photo paroxysmal discharges in winter compared to other seasons, suggesting higher cerebral neuronal excitability (13). In the studies of Scorza and Bell, sudden unexpected death due to epilepsy was more common in winter (14-16).

While many studies confirm the relationship between low vitamin D and epilepsy patients, there are possible theories regarding the role of vitamin D deficiency in the etiopathogenesis of epilepsy (17). Vitamin D directly reduces neuronal hyperexcitability, interacts with GABA-A receptors in the brain and maintains the expression of the neuromediator genes involved in neurotransmission (18,19)

In our study, the difference in the number of epileptic seizures between July and December (10 vs 25), and the difference between summer and winter (35 vs 64, $p = 0.026$) are very significant. According to our results, the accumulation of epileptic seizures in the winter season compatible with the literature.

As our study is a retrospective study and there is no definite record of other factors that may affect the frequency of seizures in our study. Fever, bacterial and viral infections, multiple antiviral and antibiotic drug use are other factors that may increase the frequency of epileptic seizures in winter. This is the most obvious limitation of our study.

Conclusion

In conclusion, epileptic seizures are significantly cumulating in winter in our study. Although more detailed data should be collected on this subject, we think that this is an indirect result of the change in vitamin D metabolism, as suggested in other studies.

Conflict of Interest: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. The authors have no independent disclosures or conflicts of interest.

Author's Contributions: MAU: concept, design, literature search, data analysis, manuscript preparation, manuscript editing and manuscript review; **MMA:** manuscript review, statistical analysis, **YO:** data acquisition, data analysis

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The neutrophil lymphocyte ratio may predict the discharge status in patients admitted to the emergency department

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Abstract

Objective: The neutrophil to lymphocyte ratio (NLR) has been investigated as an indicator of mortality and/or morbidity in many clinical pathologies. However, these studies have mostly been conducted for specific diseases. We investigated whether there is a relationship between the NLR and hospitalization or discharge decisions in the emergency department (ED).

Methods: We retrospectively reviewed the medical records of patients admitted to the ED. The NLR values of the patients were calculated, and their demographic characteristics (age and gender) and clinical outcomes were recorded.

Results: Of the 1970 patients, 1400 (71.1%) were discharged from the ED, and 570 (28.9%) were hospitalized. The patients who were discharged were younger and had lower NLR values, and this group had a lower female gender ratio ($p<0.001$, $p<0.001$, and $p<0.001$, respectively). The NLR threshold for discharge was 4.8, with a sensitivity of 70% and a specificity of 60%. Of the 570 hospitalized patients, 478 (83.9%) were discharged from the hospital and 92 (16.1%) died. Deceased patients were statistically significantly older with higher NLR values ($p<0.001$ and $p=0.001$, respectively). The threshold value of NLR for predicting mortality was calculated as 11.5, with a sensitivity of 45% and a specificity of 77%.

Conclusions: Our study reveals that the NLR is an important predictor of hospitalization and mortality in patients admitted to the ED regardless of diagnosis. ED physicians may consider to discharge patients with NLR values below 4.8 instead of spending additional time with advanced tests. In addition, clinicians should review the risk of mortality in patients with NLR values greater than 11.5 more thoroughly concerning mortality and should consider early aggressive treatment options.

Keywords: Complete Blood Cell Count, Emergency Department, Neutrophil Lymphocyte Ratio

Introduction

Determining the clinical course and the severity of patients in the emergency department (ED), where there are frequent and diverse patient admissions, is one of the most important topics in emergency medicine. The neutrophil to lymphocyte ratio (NLR) has been investigated as an indicator of mortality and/or morbidity in many clinical pathologies, especially sepsis, in recent years due to its easy and rapid applicability, low cost, and widespread use (1-5).

The increase in the number of white blood cells (WBCs) in the peripheral blood is a marker of systemic inflammation. The NLR is the ratio of neutrophils to lymphocytes, which comprise the majority of WBCs in the blood. During acute inflammation, the number of WBCs increases due to the number of neutrophils. There are publications showing that physiological stress suppresses the lymphocyte count in the blood (6). Therefore, the NLR has been accepted as a parameter to show the negative effects of both an increased neutrophil count, which reflects acute inflammation, and decreased lymphocyte count, which reflects physiological stress. Numerous published studies have been conducted to determine whether the NLR has a prognostic or diagnostic value in clinical pathologies, such as acute abdominal events, cardiovascular system pathologies, ischemic events (e.g., acute ischemic stroke), pulmonary embolism, vascular events (e.g., aortic dissection), sepsis, and chronic obstructive pulmonary disease exacerbation. However, these studies have mostly been conducted for specific diseases (2,3). There is a lack of data in the literature on the relationship between the NLR and clinical outcomes regardless of the diagnosis from the ED evaluation.

In this study, we investigated whether there is a relationship between the NLR and the decision to admit or discharge patients in patients who presented to the ED.

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Also, the predictive value of NLR for mortality in patients admitted to hospital was investigated in our study.

Material and Methods

This study was performed in accordance with the Declaration of Helsinki, and ethical approval was obtained from the Ethics Committee of Süleyman Demirel University Medical Faculty (2019/263). We retrospectively reviewed the medical records of patients admitted to the ED of Suleyman Demirel University Training and Research Hospital between October 1, 2017 to January 1, 2018. The data of patients who underwent a complete blood cell count (CBC) examination were obtained from the hospital data processing center. Patients with pregnancy, trauma, malignancy, immune deficiency, or drug use that could affect CBC parameters as well as patients who were under the age of 18 and whose medical records could not be reached were excluded. The NLR values of the patients included in the study were calculated, and the demographic characteristics (age and gender) and clinical outcomes were recorded.

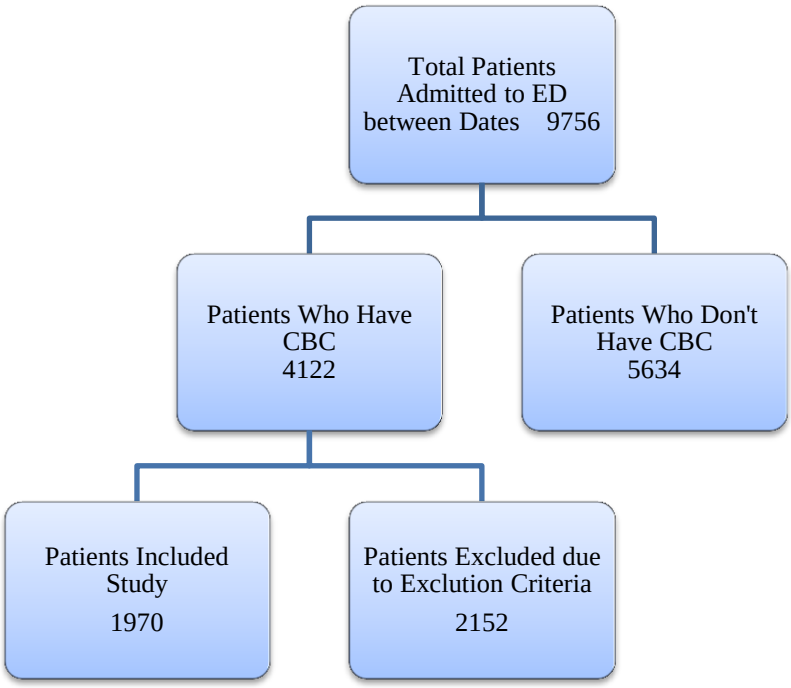
The SPSS 25 program was used for statistical analysis. Since the quantitative data were not normally distributed, the Mann–Whitney U test was used in statistical comparisons, and the descriptive statistics are shown as the median (minimum-maximum). For categorical variables, a chi-square analysis was used in statistical comparisons, and descriptive statistics are shown as the frequency (%). The success of the NLR in predicting in-hospital mortality and patients' outcomes in the ED was evaluated by receiver operating characteristic (ROC) curve analysis. The sensitivity, specificity, positive predictive, and negative predictive values were calculated based on the threshold values obtained. When $p<0.05$, the related result was considered statistically significant.

Results

A total of 9756 patients were admitted to the ED in the study period, and 4122 of these patients underwent a CBC examination. After investigation of the medical records, 2152 patients were excluded from the study according to the exclusion criteria, and a total of 1970 patients were included in the study (Figure 1). Of the patients included in the study, 1011 (51.3%) were male, and 959 (48.7%) were female. The median age was 50 years (18-97) in men, 58 years (18-96) in women, and 55 years (18-97) in total.

A total of 1400 (71.1%) patients were discharged from the ED, and 570 (28.9%) patients were hospitalized. When the patients were compared according to their ED outcomes, the patients who were discharged were younger, had lower NLR values, and had a higher female gender ratio ($p<0.001$, $p<0.001$, and $p<0.001$, respectively) (Table 1). In the structured ROC curve analysis, the threshold value of NLR for discharge was 4.8, with a sensitivity of 70% and a specificity of 60% (Table 2) (Figure 2).

In our study, 478 (83.9%) of the 570 hospitalized patients were discharged from the hospital, and 92 (16.1%) of them died. When these patients were compared, the patients who died were statistically significantly older with higher NLR values ($p<0.001$ and $p=0.001$, respectively). No significant difference was found between the two groups in terms of the gender distribution ($p=0.141$) (Table 1). In the ROC curve analysis of these patients, the threshold for predicting mortality was calculated as 11.5, with a sensitivity of 45% and a specificity of 77% (Table 2) (Figure 3).



Graphic 1. Flow Chart of Study. ED: Emergency Department, CBC: Complete Blood Cell Count

Table 1. Comparison of Patients According to Emergency Room Outcome and Inhospital Mortality

	Gender (m/f)	Mean Age (year)	NLR
Discharged Paitents (n:1400)	666/734	49.2 ±20.2	5.2 ±6.6
Hospitalized Patients (n:570)	345/225	62.7 ±18.7	10.3 ±12.6
P value	**<0.001**	**<0.001**	**<0.001**
Patients Discharged From Hospital (n: 478)	283/195	60.8 ±18.7	9.3 ±11.5
Patients Died in Hospital (n: 92)	62/30	72.7 ±15.2	15.4 ±16.7
P value	0.141	**<0.001**	**0.001**

Table 2. Performance parameters of NLR as a Predictor of Discharge from ED and Inhospital Mortality

	Discharged from ED NLR<4.8) (95% CI)	Inhospital Mortality (NLR>11.5) (95% CI)
Sensitivity	70 (67.52-72.39)	45.65 (35.22-56.37)
Specificity	60.88 (56.74-64.91)	77.41 (73.39-81.08)
Positive predictive value	81.46 (79.15-83.62)	28 (20.98-35.91)
Negative predictive value	45.24 (41.68-48.84)	88.1 (84.61-91.03)
Positive likelihood ratio	1.79 (1.61-1.99)	2 (1.53-2.60)
Negative likelihood ratio	0.49 (0.44-0.55)	0.7 (0.58-0.85)
Receiver operating characteristic curve area	0.68 (0.65-0.71)	0.61 (0.54-0.68)
P value	**<0.001**	**0.001**

CI: Confidence Interval, NLR: Neutrophil to Lymphocyte Ratio, ED: Emergency Department

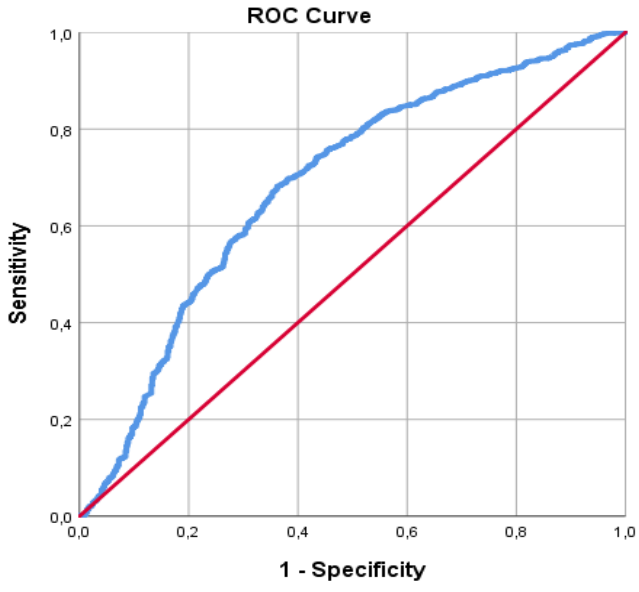


Figure 2. Receiver Operating Characteristic Curve of NLR to Predict Discharge from ED.

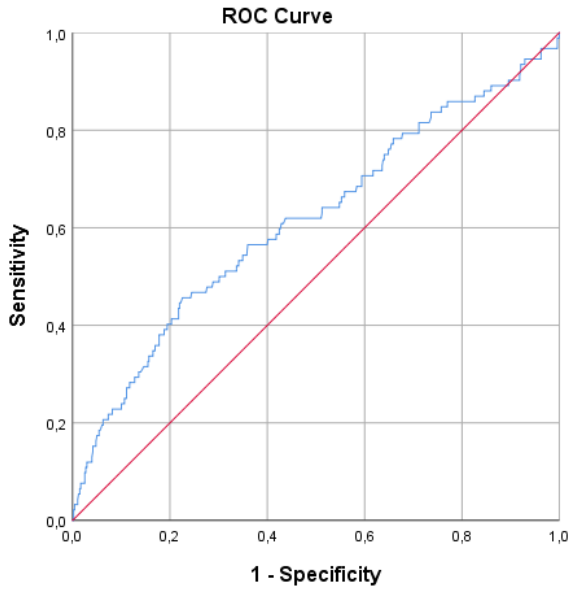


Figure 3. Receiver Operating Characteristic Curve of NLR to Predict Inhospital Mortality.

Discussion

Our study reveals that the NLR is an important predictor of hospitalization and mortality in patients admitted to the ED regardless of diagnosis. As a result of technological developments, the overall lifespan has increased, and chronic diseases have become more common. In parallel, the number of patients being admitted to EDs worldwide is reportedly increasing daily (7). With this increase, patients' lengths of stay in the ED is prolonged, and treatment processes are negatively affected. In this case, emergency physicians must evaluate patients and make the decision to admit or discharge patients quickly. Several risk scoring scales have been developed to enable the rapid assessment of patients (8).

However, remarkably few of these scoring systems use laboratory data. In addition, the effects of laboratory data on clinical evaluation have been questioned in many studies (9,10). There are no published studies considering the ability of NLR to predict patient discharge in the ED. Our study reveals that patients with NLR values less than 4.8 can be safely discharged from the ED. This value, in addition to other scoring methods or vital signs, will be much more sensitive than alternative measures in predicting discharge and will shorten the patient evaluation process in the ED, thus contributing to the overall functioning of the ED. In our study, an NLR value greater than 11.5 significantly predicted mortality in hospitalized patients. Many published studies support the use of the NLR in predicting mortality. In a wide-scale cohort by Proctor et al., an increase in the neutrophil count and a decrease in the lymphocyte count, along with many laboratory values, significantly predicted all-cause mortality (11). Although the sensitivity and specificity of the NLR was not evaluated in their study, the results showed that NLR significantly predicted mortality. Akıllı et al. stated that an NLR greater than 11.9 was an independent risk factor for mortality in patients admitted to the ED and was an indicator of critical illness according to the systemic inflammatory response syndrome criteria (12). Although there was a methodological difference between our study and this study, the findings support each other.

Age is generally used as a parameter to predict mortality rather than patient severity in the ED (8). In our study, the mean age of patients who died was significantly higher than the mean age of patients who were discharged, supporting these mortality predicting scores (Table 1). In addition, the mean age of patients who were hospitalized was over 60 years, and the mean age of patients who were discharged was below 60 years. Significant difference was observed between the two groups (Table 1). Considering these findings, it would be appropriate to add age as a parameter to the scoring systems to estimate the severity of the status of patients in the ED. The most important limiting factors of our study were its retrospective single-center design. A risk score based on the vital signs and physical examination findings of the patients included in the study could not be performed because of the retrospective nature of the study. More comprehensive prospective studies on this topic are required to provide further findings.

Conclusion

The NLR promises hope to clinicians as an easily accessible, fast, and inexpensive parameter that can provide information in the distinction and risk classification of critical patients. Our study showed that the NLR can be a critical guide for emergency physicians in patient evaluation. Emergency physicians may consider to discharge of patients with NLR values less than 4.8 instead of spending additional time with advanced tests. Thus, patients' lengths of stay in the ED can be significantly reduced. In addition, clinicians should review the risk of mortality in patients with NLR values greater than 11.5 more thoroughly and should consider early aggressive treatment options.

Conflict of Interest: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. The authors have no independent disclosures or conflicts of interest.

Author's Contributions: KK, EÇ, EF AO: concept, design, literature search, data analysis, manuscript preparation, manuscript editing and manuscript revision; **KK:** manuscript revision, statistical analysis, data acquisition, data analysis

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Investigation of angiogenic factors in obese rats exposed to low oxygen pressure

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Abstract

Objective: Obesity, which is one of the most important health problems of today's people, remains current due to the risks of illness it brings due to the increase rate in the world.

Material and Methods: Male Sprague Dawley rats were used in our study of obesity. Rats were divided into four groups as standard diet/ normal oxygen, standard diet/low oxygen, high-fat diet/normal oxygen and high-fat diet / low oxygen. For the study, a special cage with a low oxygen level of 17-18% was made in a closed system. After achieving the desired 25% weight increase in obese group rats, blood, liver, lung, white adipose tissue and brown adipose tissue were obtained from the rats. In these tissues, adrenomedullin, hypoxic inducible factor 1-α (HIF1-α) and matrix metalloproteinase-II (MMP-II) levels were measured by ELISA.

Results: According to our results, there was a significant increase in adrenomedullin, HIF1-α and MMP-II in white adipose tissue, and adrenomedullin and MMP-II in brown adipose tissue. It was found that the amount of HIF1-α increased significantly in liver and lung tissues.

Conclusion: According to the metabolic status of adipose tissue, it is thought that the effect of adrenomedullin, HIF1-α and MMP-II can increase vascularization of brown adipose tissue and provide energy consumption.

Keywords: Hypoxia, Obesity, HIF1-α, MMP-II, Adrenomedullin

Introduction

Hypoxia indicates that the arterial oxygen concentration is lower than normal. Hypoxia is a life-threatening risk factor for organisms, and reducing oxygen prevents biochemical reactions (1). The World Health Organization (WHO) has defined obesity as "fat accumulation that adversely affects the health of other organs" (2). Obesity, which has become one of the most serious health problems of people today, continues to be updated due to increased disease risks. In all countries of the world, obesity has negative health effects, social and economic effects on both individuals and living communities (3). Obesity is the cause of many diseases and decreases the quality of life and the life span. Obesity appears as an important increasing health problem affecting more than 20% of the western population. It significantly increases the risks of obesity, type-2 diabetes, hypertension, coronary heart disease, stroke, liver failure, dementia, obstructive sleep apnea, and various cancers. Therefore, diseases associated with obesity and / or diseases such as atherosclerosis, diabetes and cancer significantly affect quality of life (4). Obesity is an energy metabolism disorder that is characterized by physical and mental problems due to the accumulation of too much fat in the body.

Obesity is a multi-factor phenomenon caused by inequality between calorie intake and use (5). The amount of fat in the body is usually indicated by the body mass index (BMI) and is calculated by dividing body weight by height [(kg) / (m2)]. Since the adult height will remain constant, the increase in body weight shows an increase in fat mass. BMI is defined as 25 to 29.9 overweight, 30 to 39.9 obese, more than 40 deadly obese. In short, obesity is the balance in favor of calorie intake between calories intake and calories consumed (6). The development of obesity is associated with a significant change in adipose tissue structure. The plasticity of the adipose tissue reflects its extraordinary ability to expand or its size throughout the life of the adult, and the expansion of adipose tissue is closely related to vascular development (7). In recent years, as a result of obesity research, adipose tissue has been reported to be an active endocrine organ that secretes many factors (8). These factors include adipokines, appetite and satiety control, fat distribution, insulin sensitivity, insulin release, energy consumption, inflammation, blood pressure, and factors that regulate endothelial functions (9,10). Functional disorder of adipose tissue is one of the important causes of obesity defects. It is characterized by impaired adipose

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tissue function, atherogenic adipokine structure and proinflammatory secretion. At the same time, impaired adipose tissue function is affected by genetic, behavioral and environmental factors. Degradation processes in adipose tissue lead to mitochondrial dysfunction, depressive adipose tissue, hypoxia, ectopic fat accumulation and adipose hypertrophy (4). Angiogenesis is the formation of new vessels from existing vessels. Physiologically, new vascular formation, wound healing, embryo development, menstrual cycle and pregnancy occur as well as diseases such as new vessel formation, abnormal vascularity, collagen tissue disorders, retinopathies and psoriasis (11). Also known as a vascular factor, adrenomedullin (ADM) is a multifunctional regulatory peptide that is secreted by various cell types. Inflammatory cytokines such as tumor necrosis factor-a (TNF-a) and lipopolysaccharide are potent stimulants of ADM synthesis in adipocytes. In addition, ADM synthesis and ADM concentration have been found to increase in obese fat tissue. The possible physiological role of ADM secreted from adipose tissue may be related to the prevention of metabolic syndrome, type 2 diabetes and hypertension, characterized by obesity through its antioxidant and potent vasodilatory effects. It has been reported that ADM synthesis in adipose tissue plays a role in the pathogenesis of obesity-related diseases in obese individuals (12). ADM is synthesized in human adipose tissue such as epididymal (13) and adherent omental tissue (14). Hypoxia-inducible transcription factors (HIF) activate various pathways that regulate cellular metabolism, angiogenesis, proliferation and migration in a low oxygen or hypoxic environment. Disruption of mitochondrial function can be caused by reactive oxygen species, stress and viral infection and are regulated by HIFs (15). HIF transcription factors in the form of heterodimers containing the A and p subunits are HIF-1, HIF-2 and HIF-3. Although the subunit kontrol is controlled by oxygen-induced proteolytic degradation, the subunit β is preserved structurally (16, 17). HIFs have also been reported to play a role in the pathogenesis of various liver diseases (15). Matrix metalloproteinases (MMPs, Matrixins) are zinc-containing endopeptidases involved in extracellular matrix (ESM) metabolism and are responsible for the degradation of other proteins such as collagen, gelatin, fibronectin and laminin. The MMP family has 28 enzymes with different functions. These enzymes are called collagenases, gelatinases, stromelysins, matrilizins, and shingles-linked MMPs and are partially classified as groups or substrates (18, 19). The activity of these enzymes is regulated by tissue inhibitors. Most of the studies have been reported in obesity model studies with MMPs (19). MMPs represent the most known members of proteinases associated with cancer formation. In addition to extra cellular matrix expansion and cancer cell migration functions, MMPs have been reported to regulate signaling pathways that can function in cell growth, inflammation or angiogenesis, and even non-proteolytic destruction (20).

Material and Methods

Five months old Sprague Dawley male rats produced by Inonu University Experimental Animal Production and Research Center were used in the study. The rats were housed in special cages, 12 hours light/dark, ventilated, room temperature 24 °C. Rats other than the obesity group were fed with standard rat died and water. Obesity is desired to create a group of high-fat dietary diet and water was given. The feed and water needs of the rats were monitored daily until the study was finished. Once a week and the same day of the week, rats were weighed. Obesity group was provided with 20-25% weight increase in rats. Low oxygen pressure (Low PO2) was provided in an environment containing 17-18 % oxygen. Normal oxygen pressure (NPO2) group rats were allowed to breathe with normal air for 21% oxygen. Rats were kept in low oxygen pressure environment for 24 hours. The carbon dioxide formed due to respiration was removed by the soda-lime, which was put in the cage.

Table 1. Rat groups used in the study

Group-I	Standard diet/Normal P_{O2} (SD/N_{O2})
Group-II	Standard diet/low P_{O2} (SD/l_{O2})
Group-III	High-fat diet/Normal P_{O2} (HFD/N_{O2})
Group-IV	High-fat diet/low P_{O2} (HFD/l_{O2})

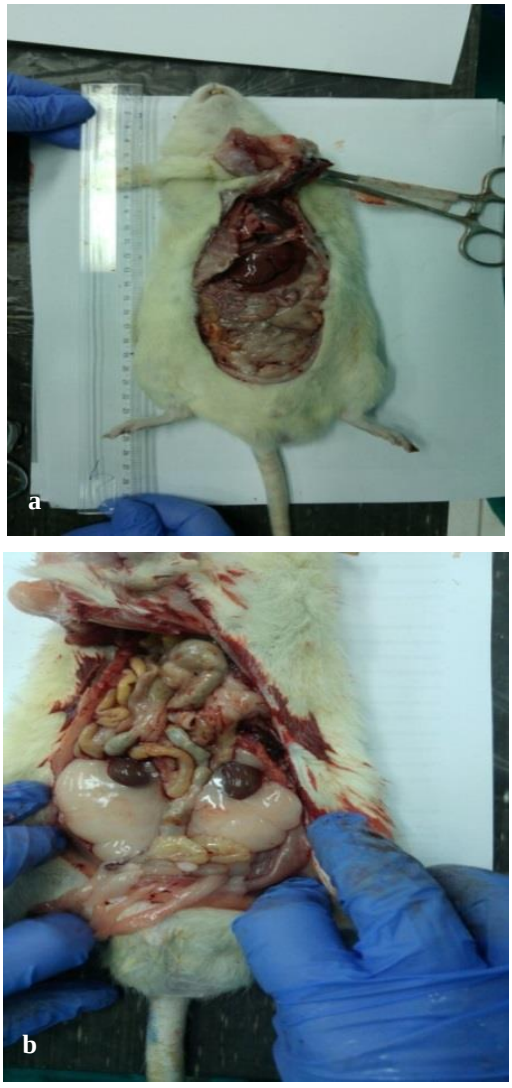

Figure 1. Changes observed during tissue removal from rats. a) Standard diet, b) High-Fat Diet

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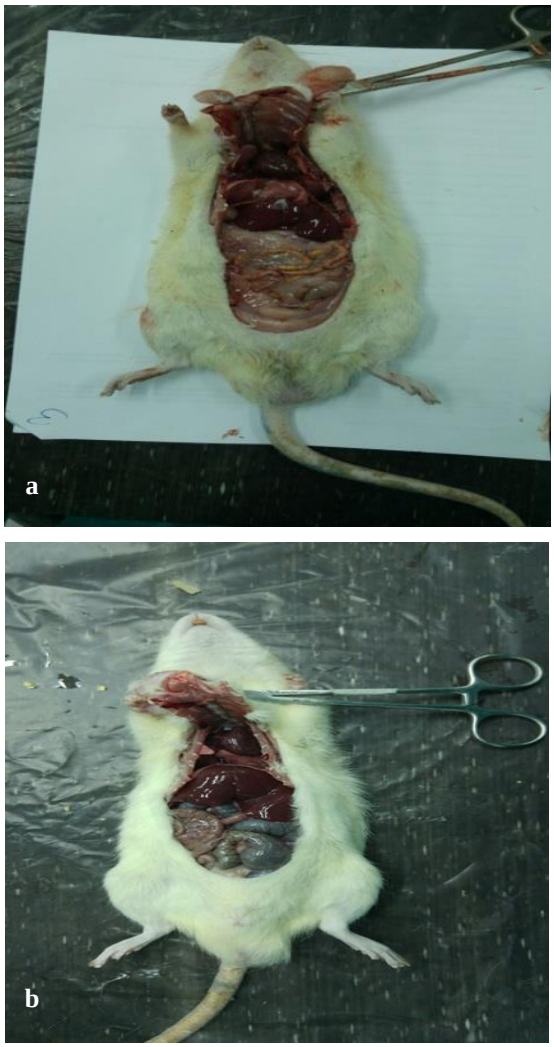

Figure 2. Comparison of normal (a) and hypoxia-exposed rat images (b).

Results

In all working groups; ADM, HIF1-α and MMP-II quantities were measured by ELISA method in liver, lung, WAT, BAT and plasma. Amounts of ADM measured in tissues are shown in Table 2, HIF1-α amounts in Tables 3 and MMP-II in Tables 4.

The greatest increase in ADM levels observed in white adipose tissue, high-fat diet/low oxygen treated group (Table 2). There was no significant increase ($p>0.05$) between SD/lPO2 and HFD/NPO2 groups compared to the ADM levels of liver tissue. Also, there was no significant increase in the amount of ADM in lung tissue and plasma ($p> 0.05$).

According to the results obtained from rat tissues (Table 3), the increase in HIF1-α levels studied in all groups (SD/NPO2, SD/lPO2, HFD/NPO2 and HFD/lPO2) and tissues (liver, lung, WAT, BAT and plasma) ($p <0.05$). When we looked at Table 3, there was a significant increase between SD/NPO2 and HFD/lPO2 groups ($p <0.05$). There was no significant increase between SD/lPO2 and HFD/NPO2 groups ($p> 0.05$).

There was a significant difference between SD/lPO2, HFD/NPO2 groups and SD/NPO2 and HFD/lPO2 groups ($p> 0.05$). There was no significant increase in plasma HIF1-α levels among the groups ($p> 0.05$).

According to the data in Table 4, MMP-II levels studied in all groups (SD/NPO2, SD/lPO2, HFD/NPO2 and HFD/lPO2) and tissues (liver, lung, WAT, BAT and plasma). The significant increase in the amount of MMP-II was found in the WAT and BAT ($p <0.05$).

There was no significant increase in the amount of MMP-II in the liver ($p> 0.05$). There was no significant difference between the SD/lPO2 and HFD/NPO2 groups in the lung tissue ($p <0.05$), whereas the SD/NPO2 group in the lung tissue showed a significant increase in the amount of MMP-II compared to the HFD ($p< 0.05$).

There was a significant increase in the amount of MMP-II between the HFD/lPO2 group and the other three groups (SD/NPO2, SD/lPO2, HFD/NPO2) ($p <0.05$). There was also a significant difference between SD / NPO2 and SD/lPO2 groups ($p <0.05$).

Plasma HFD/lPO2 group showed a significant increase when compared with the other groups ($p <0.05$). There was no significant difference in the increase of MMP-II levels between the other three groups (SD/NPO2, SD/lPO2, HFD/NPO2) ($p> 0.05$).

Table 2. Adrenomedullin (ADM) levels in rat tissues (ng/l). The differences between the different words in the columns are statistically significant. The results are given as mean ± SE.

	Liver	Lung	WAT	BAT	Plasma
SD/NP_{O2}	130.08±3,04^{a}	79.99±2,76^{a}	182.12±13,08^{a}	104.53±5,38^{a}	53.44±1,68^{a}
SD/LP_{O2}	142.41±2,78^{b}	83.21±4,83^{a}	203.56±7,69^{b}	130.36±2,38^{b}	56.34±1,93^{a}
HFD/ NP_{O2}	143.77±6,79^{b}	81.35±4,79^{a}	229.33±12,11^{c}	140.13±4,13^{c}	56.80±2,57^{a}
HFD/LP_{O2}	167.8±6,73^{c}	83.88±8,21^{a}	274.45±10,75^{d}	186.71±0,91^{d}	58.35±6,04^{a}

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Table 3. Hypoxia-inducible factor 1-α (HIF1-α) levels in rat tissues (pg/L). The differences between the different words in the columns are statistically different. The results are given as mean ± SE.

	Liver	Lung	WAT	BAT	Plasma
SD/NP$_{O2}$	66.65±1.99^{a}	32.95±0.70^{a}	43.46±3.49^{a}	50.29±4.84^{a}	30.13±1.51^{a}
SD/LP$_{O2}$	102.35±3.36^{b}	45.20±1.87^{b}	57.15±4.96^{b}	66.08±3.15^{b}	32.71±0.86^{a}
HFD/NP$_{O2}$	95.69±4.38^{c}	52.79±1.33^{c}	74.99±2.99^{c}	59.23±2.44^{b}	30.41±1.33^{a}
HFD/LP$_{O2}$	117.14±3.72^{d}	64.19±3.51^{d}	95.24±3.01^{d}	90.12±4.45^{c}	37.79±2.89^{a}

Table 4. Matrix metalloproteinase-II (MMP-II) level in rat tissues (ng/L). The differences between the different words in the columns are statistically different. The results are given as mean ± SE.

	Liver	Lung	WAT	BAT	Plasma
SD/NP$_{O2}$	23.60±0,45^{a}	20.25±0,36^{a}	22.47±2,21^{a}	25.92±0,97^{a}	18.22±0,32^{a}
SD/LP$_{O2}$	24.31±0,94^{a}	21.13±0,23^{b}	32.05±2,43^{b}	27.01±0,73^{b}	18.48±0,38^{a}
HFD/ NP$_{O2}$	22.94±1,21^{a}	20.47±0,21^{ab}	38.27±1,88^{c}	30.02±0,51^{c}	18.62±0,74^{ab}
HFD/LP$_{O2}$	24.53±0,92^{a}	23.23±0,46^{c}	48.94±2,78^{d}	33.29±0,97^{d}	20.32±0,30^{b}

Discussion

In our study, it was determined that the ADM amount was more increased in obese (HFD/NPO2 and HFD/lPO2) groups compared to the other groups (SD/NPO2 and SD/lPO2) in WAT and BAT. Shibasaki et al. (2010) found that epicardial adipose tissue ADM mRNA levels were elevated in patients with coronary disease in their studies, suggesting that this was a protective effect (21). We also found ADM levels increased in our study. It has been reported that specific binding sites of ADM were mostly in the lung (22). The increase in the amount of ADM in the WAT and HFD / LPO2 group, the onset of angiogenesis in obesity and the increased amount of ADM in the liver also suggest that it may be related to some obesity-related diseases. HIFs activate various pathways that regulate cellular metabolism, angiogenesis, proliferation, and migration in order to be able to respond to a cell in a low-oxygen or hypoxic environment (22). Low oxygen exposure has been reported to be sufficient to induce a hypoxic response that compensates for HIFs (17- 9). Several etiologies of HIFs have been reported to play a role in the pathogenesis of liver disease (16). In our study, an increase in HIF1-α quantities was observed in all tissues except plasma. The increased amount of HIF1-α indicates that HIF has a protective role against obesity in obesity and hypoxia conditions and plays a role in maintaining body homeostasis in hypoxic conditions. At the same time, an increase in the amount of HIF1-α in WAT and HFD, an occurrence of angiogenesis in obesity, and an increase in the amount of HIF1-α in the liver also correlate with some diseases associated with obesity. MMPs are enzymes involved in other proteins such as collagen, gelatin, fibronectin and laminin (23). MMPs represent the most prominent family of proteinases associated with tumor formation. Recent developments have made it clear that MMPs have a prominent role as micro-modulators of tumor. In our study, three angiogenic factors (ADM, HIF1-α and MMP-II) were investigated in five different rat tissues. According to the data we obtained from our study; It was determined that ADM, HIF1-a and MMP-II amounts increased. It was determined that the most significant increase was in WAT.

This indicates that angiogenic factors in obesity, diagnosis and / or treatment pathways will also play an important role. In our study, it was found that MMP-2 activity in WAT and BAT increased significantly compared to rats in the standard diet and normal oxygen group. Based on these evidence, we think that this increase is important in counteracting the adverse conditions arising in the hypoxic state and will positively affect obesity. WAT development can be one of the right approaches to prevent obesity. Therefore, it is unclear which negative or positive angiogenesis regulator can be used in the treatment of obesity.

Conclusion

If the angiogenic vascularization of metabolically active adipose tissue, BAT, increases, it consumes more energy. On the contrary, it has been suggested that inhibition of angiogenesis may be more beneficial in obese individuals with large amounts of metabolically inactive WAT.

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Conflict of Interest: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. The authors have no independent disclosures or conflicts of interest.

Author's Contributions: MD, MY: concept, design, literature search, data analysis, manuscript preparation, **MY:** manuscript revision, statistical analysis, data acquisition, data analysis

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Diagnosis of cholesteatoma by the b1000 value DWI MRI according to the signal intensity

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Abstract

Objective: The cholesteatoma (CL) can be evaluated visually or numerically on an apparent diffusion coefficient (ADC) map, which obtained from at least two different b-valued diffusion-weighted imaging (DWI). In this study, we aimed to evaluate the signal intensity (SI) of the lesion both visually and numerically only on the DWI image without ADC. In case of positive results a second 'b' value is not required, so this method could be shorten the duration of the MRI examination.

Material and Methods: Between January 2017 and May 2018, we included patients with chronic otitis media (COM) with a clinical suspicion of primary CL who underwent DWI. Two radiologists and one ear, nose, throat specialist evaluated the radiological images and the pathology results.

Results: The mean SI measurement was significantly higher in the CL group by both observers (observer LR; CL: 107.94 ± 53.36, COM: 37.34 ± 14.70, observer FC; CL: 108.56 ± 50.00, COM: 37.06 ± 15.44; p<0001). ROC analysis showed that a mean SI value of 48.6 was the cut-off value in predicting the diagnosis of CL. The mean SI was significantly higher in the CL group (p<0.001).

Conclusion: We demonstrated a significant difference between CL and COM concerning the diagnosis by visual and numerical signal evaluation only via b1000 valuable images. In false-positive cases, ADC is still confirmatory for high diagnostic accuracy.

Keywords: Cholesteatoma, diffusion, signal intensity, quantitative assessment, false positive

Introduction

Cholesteatoma (CL) is a keratin collection filling the squamous epithelium sac. This benign but destructive lesion causes bone erosions in the middle ear and mastoid bone and subsequently causes complications such as loss of conductive hearing, facial paralysis, and labyrinthitis (1). The treatment includes an intact canal wall or canal wall down mastoidectomy based on the extent of the disease and the surgeon's preference (2). Both technics carry the risk of residual CL in a wide frequency range (3, 4). Second look surgery is often required 6 - 18 months after surgery. However, advances in radiology over the past decade have given reliable non-invasive hope for the diagnosis of primary and residual cholesteatoma (5).

The high resolution computed tomography (CT) is an excellent imaging tool with its high spatial resolution to demonstrate the localization concerning bony neighbors and extension of the lesion (6). However, its specificity is as low as 48%, and the soft tissue cannot differentiate between CL and other middle ear pathologies (7, 8).

The postcontrast T1-weighted magnetic resonance imaging (MRI) is one of the effective techniques for distinguishing soft tissue pathologies from CL. The CL is avascular and does not enhance with contrast material, whereas others such as granulation tissue are vascularized and contrasted (7). Although the sensitivity and specificity of this technique in the diagnosis of postoperative residual or recurring CL were 90% and 100%, respectively (9), 30-45 minutes of post-contrast imaging decreases practice efficiency and irritate the patients that limit the availability of the technique (1).

Today, the highest sensitivity (100%) and specificity (90%) of the radiological modality is diffusion-weighted imaging (DWI) (10). During the past decade, data have been published advocating DWI for primary diagnosis and evaluation of the residual CL following mastoidectomy (11). Many centers have used non-echoplanar imaging (EPI) for the follow-up of patients for a residual lesion in the place of second-look surgery (12, 13).

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The lesion can be evaluated both visually and numerically on the ADC map obtained from at least two different b-valued DWI images. The limited molecular diffusion with decreased signal intensity (SI) on the ADC map is caused by the excellent keratin content of CL (1). To date, this technique is considered as state-of-the-art cholesteatoma imaging.

In this study, we aimed to evaluate the signal intensity of the lesion both visually and numerically only on the DWI image without creating an ADC map in diffusion MRI where we shortened the examination time by taking a single b value (b 1000) instead of taking multiple b values.

Material and Methods

Study population: The approval for the retrospective study without patient informed consent was taken from the local institutional review board (12/04/2018-18/287). The patients who had been referred to our radiology unit with a clinical suspicion of primary CL and examined with HASTE DWI MRI between January 2017 and May 2018 were included in the study. The radiological reports, physical examination findings, conductive hearing test, and the pathology results, if operated, were evaluated by two radiologists and one ear, nose, throat specialist. The follow-ups of patients who did not undergo surgery were investigated on the hospital system for up to 18 months after the first imaging. As a result, the CL group of 31 patients was formed based on the pathology report (three of 31 patients with no signal in the DWI were excluded from the statistical evaluation). Thirty patients of chronic otitis media (COM) without CL formed COM group. Out of 30, 12 patients were confirmed by surgery, and 18 patients with chronic otitis media had negative otoscopy and hearing test with a negative DWI MRI during 18 months of the follow-up period.

Imagıng technique: MRI was performed on a 1.5-T superconductive unit (MagnetomAvanto; Siemens Medical Solutions, Erlangen, Germany) with the use of the standard Head Matrix coil. Axial 5-mm thick accurate FISP T2-weighted images (TR 4640 ms; TE 103 ms; matrix 245x 384; the field of view 150 x200 mm) were performed. In all patients, a 2-mm thick HASTE DWI sequence was acquired in the coronal plane (TR 2000 ms; TE 147 ms; matrix 134 x 192; the field of view 220x 220 mm; b factors 0 and 1000 mm2/s; acquisition time 3 minutes 38 seconds).

MR imaging analysis

The images were evaluated by one head and neck radiologist who has at least five years of experience in temporal bone imaging (F.C.), one radiology resident (L.R.) and one ear, nose, and throat specialist (H.G.) who were blinded to the radiological and clinical data.

In the first step, the b1000 images were evaluated visually and numerically on DWI. Two of the three observers (F.C. and L.R.) were trained for standardization of the SI measurement, and all of the three observers (F.C., L.R., and H.G.) were trained for visual assessment of signal compared to brain parenchyma in five cases who were not included in the study. The qualitative and quantitative evaluation of the lesion signal was analyzed for each test lesion. In case of any discordance in the assessment of the lesion, a more detailed investigation was performed retrospectively for possible reasons to make a standard evaluation. After the training period was completed, all three observers separately evaluated all of the cases randomly.

Secondly, the ADC maps using b0 and b1000 values were evaluated by both of the observers (F.C. and L.R.).

Determination of the lesions for evaluation

On a standard PACS monitor, axial T2, coronal DWI, and CT images were evaluated for the determination of the middle ear and mastoid lesions. Due to the insufficient spatial resolution of HASTE DWI, CT images were examined to confirm whether the SI was in the mastoid bone and the middle ear or outside both locations. There were no more than two weeks between DWI MRI and CT examinations.

Qualitative analysis by visual inspection

The criteria for the diagnosis of CL at DWI was based on the evidence of a hyperintense middle ear or mastoid lesion, compared with the SI of the brain parenchyma. If the SI of the pathology were hyperintense compared to brain parenchyma on b1000 DWI, it would be diagnosed as CL. During the visual assessment, the SI of the part of the lesion with the highest intensity was taken into account.

To prevent the false-positive results due to some possible hyperintense non-CL lesions, we graded SI in three levels as isointense, mildly hyperintense, and hyperintense compared to the brain parenchyma.

The pathologies were evaluated in two separate groups with different visual signal intensities. In Group 1, the hyperintense lesions were considered as CL, mildly hyperintense, and isointense pathologies as COM. In Group 2, the hyperintense and mildly hyperintense lesions were thought of as CL and isointense lesions as COM.

Quantitative analysis

The SI of the lesions was determined by using a region of interests (ROIs) ranging in size from 3 to 6 mm2 on b1000 DWI images. In the same image, the SI of the adjacent temporal lobe parenchyma was also measured by using a similar-sized ROI.

The central SI of the lesion was usually higher than peripheral, and particular attention was paid to achieve a standardized evaluation by localizing the ROI on the highest signal-containing portion of the pathology and in the adjacent temporal lobe parenchyma. To prevent the partial volume effect that might lead to a decrease in the measured SI of the lesion on DWI, the measurement of the SI was attempted on the image seen by the broadest diameter of pathology and the axial T2 and multiplanar CT images were also used to determine the slice passing through the central part of the pathology.

Quantitative characterization of a lesion with the severity of SI may not be accurate due to scanner related and magnetic susceptibility artifacts in MRI. Thus, we rated the SI of

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lesion and brain parenchyma and performed lesion signal intensity ratio (SIR) to normalize signal differences.

Statistical analysis: The data were analyzed using SPSS version 22 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics related to discrete data were expressed as numbers (n) and percentages (%). Continuous variables were expressed as a mean±standard deviation. A Kolmogorov-Smirnov test was used to test for the normal distribution of continuous variables, and parametric tests were used to compare the normally distributed data between the groups, while nonparametric tests were used for the comparison of data without normal distribution. A parametric paired sample t-test was used to compare two independent groups, and a nonparametric independent sample t-test was used for the comparison of matched groups. Pearson's correlation coefficient was used to evaluate the correlation between variables. The discriminative power of the numerical signal intensity data was predicted using the area under the curve in the ROC curve analysis, along with sensitivity-specificity parameters. A p-value of <0.001 was considered statistically significant in the comparisons.

Results

The study included 28 patients with primary CL (48.3%) and 30 patients with chronic otitis media (COM) without CL (51.7%). The mean age of the patients was 36.53±15.20 (min-max: 12–72 years). The mean age was 32.68±14.07 years (min-max: 12–58 years) in the CL group and 40.13±15.56 years (min-max: 20–72 years) in the COM group. A comparison of age and gender between the groups showed no statistically significant difference (p=0.061 and p=0.754, respectively).

The mean lesion size was 12.71±7.34 mm (min-max: 5.0–42.0) in the CL group (Figure 1) and 12.64±4.30 mm (min-max: 5.4-24.0) in the COM group. There was no statistically significant difference between the groups (p=0.964).

There was a statistically significant difference in the visual SI evaluation between CL and COM groups (p<0.001) (Table 1). The analysis of the correlation between the numerical and visual signal intensities assessed by physicians showed a significantly positive and robust correlation (Table 2).

When a hyperintense lesion was considered diagnostic for CL, and mildly hyperintense and isointense pathologies for COM (Group 1) according to the analysis of visual signal intensity, the sensitivity was 82.14%, specificity was 90%, PPV was 88.46%, and NPV was 84.37%. When hyperintense and mildly hyperintense lesions were considered diagnostic for CL and an iso-intense pathology for COM (Group 2), the sensitivity was 96.43%, specificity was 73.33%, positive predictive value (PPV) was 77.14%, and negative predictive value (NPV) was 95.65%.

In Group 1, there were five false-negative results in the CL group and three false-positive results in the COM group (Figure 2). In Group 2, there was one false-negative result in the CL group and eight false-positive results in the COM group (Table 3).

The mean SI measurement of the observer (LR) was 107.94 ± 53.36 (min-max: 47.75 ± 263.75) in the CL and 37.34 ± 14.70 (min-max: 21.00 ± 94.67) in the COM. The mean SI was significantly higher in the CL group (p<0.001). The SIR measurement value of observer (LR) was 3.270 ± 1.77 (min-max: 1.32 ± 8.09) in the CL group, while it was 1.15 ± 0.46 (min-max: 0.61 ± 3.04) in the COM group and SIR rates were significantly higher in the CL group (p < 0001). The mean SI of the brain parenchyma did not differ significantly between the CL and COM groups (p=0.159 and p=0.823, respectively). Therefore, we were able to evaluate the SIR between the two groups.

In the measurements of other observer (FC), both SI means (CL: 108.56 ± 50.00, min-max; 40.25 ± 220.96 and COM: 37.06 ± 15.44, min-max; 19.75 ± 100.50) and SIR values (3.34 ± 1.71, min-max; 1.10) ± 7.56 and COM: 1.14 ± 0.50, min-max; 0.59 ± 3.24) were higher in the CL group (p <0.001, p <0.001, respectively).

Based on the results of the ROC curve analysis, a mean SI value of 48.625 was considered as the cut-off value in predicting a diagnosis of CL. The reference value was 1.322 when the SIR was taken into account for diagnosis.

The rate of patients diagnosed with CL according to the maximum and mean SI values was 56.9% (n=33). Furthermore, five patients were falsely diagnosed with CL according to the numerical SI value. The sensitivity was 96.43%, and the specificity was 80% based on the mean SI values. The PPV was 81.82%, and the NPV was 96%. The diagnose of CL was also predicted in 34 patients according to the reference value of SIR. Accordingly, sensitivity was 96.43%, specificity was 76.67%, PPV was 79.41%, and NPV was 95.83%.

The mean numerical SI value was 48.58 ± 6.89 (n=5, min-max: 38.67–54.00) in five patients in the COM group who had a mildly hyperintense lesion upon a visual SI evaluation, and 65.56 ± 25.26 (n=3, min-max: 49.50–94.67) in three patients who had a hyperintense pathology (Table 4).

Only one patient was found to have an isointense lesion in a visual SI evaluation in the CL group. The mean numerical SI value of this CL was 47.75.

The mean ADC values were 1.1 ± 0.1 ×10-3 mm2/s for the CL group and 1.9 ± 0.6 ×10-3 mm2/s for the COM group. If the ADC value of the lesion is ⩽1.1 ×10-3 mm2/s, CL could be diagnosed with 97 % sensitivity, 89% specificity, 91% PPV, and 96% NPV.

Table 1: Comparison of visual signal intensity evaluation among three observers (LR, FC ve HG).

Observers	Groups	Isointense n (%)	Mildly hyperintense n (%)	Hyperintense n (%)	Total n (%)	P*
LR	CL	1 (3.6)	4 (14.3)	23 (82.1)	28 (100.0)	**<0.001**
	COM	22 (73.3)	5 (16.7)	3 (10.0)	30 (100.0)	
	Total	23 (39.7)	9 (15.5)	26 (44.8)	58 (100.0)	
FC	CL	-	5 (17.9)	23 (82.1)	28 (100.0)	**<0.001**
	COM	24 (80.0)	5 (16.7)	1 (3.3)	30 (100.0)	
	Total	24 (41.4)	10 (17.2)	24 (41.4)	58 (100.0)	
HG	CL	-	5 (17.9)	23 (82.1)	28 (100.0)	**<0.001**
	COM	20 (66.7)	9 (30.0)	1 (3.3)	30 (100.0)	
	Total	20 (34.5)	14 (24.1)	24 (41.4)	58 (100.0)	

*Pearson chi-square test, CL: Cholesteatoma, COM: Chronic otitis media without cholesteatoma

Table 2: Correlations of observers (LR, FC ve HG) in evaluating the numerical and visual signal intensity

SI evaluation _ Observer		r	p*
Numerical SI_LR	SIR_LR	0.987	<0.001
	Numerical SI_FC	0.981	<0.001
	SIR_FC	0.971	<0.001
	Visual SI_LR	0.711	<0.001
	Visual SI_FC	0.740	<0.001
	Visual SI_HG	0.737	<0.001
SIR_LR	Numerical SI_FC	0.960	<0.001
	SIR_FC	0.976	<0.001
	Visual SI_LR	0.687	<0.001
	Visual SI_FC	0.713	<0.001
	Visual SI_HG	0.709	<0.001
Numerical SI_FC	SIR_FC	0.982	<0.001
	Visual SI_LR	0.755	<0.001
	Visual SI_FC	0.783	<0.001
	Visual SI_HG	0.768	<0.001
SIR_FC	Visual SI_LR	0.732	<0.001
	Visual SI_FC	0.758	<0.001
	Visual SI_HG	0.743	<0.001
Visual SI_LR	Visual SI_FC	0.950	<0.001
	Visual SI_HG	0.904	<0.001
Visual SI_FC	Visual SI_HG	0.917	<0.001

*Pearson Correlation Test (Correlation is significant at the 0,005 level. (2-tailed)), SI: Signal intensity, SIR: Signal intensity ratio

Table 3: Comparison of pathology results with visual signal evaluation. In group 1, a hyperintense lesion was considered diagnostic for cholesteatoma. In group 2, hyperintense and mildly hyperintense lesions were deemed to be symptomatic for cholesteatoma.

Group 1 (Hyperintense=CL) Pathology	Visual SI	n	%
COM (n=30)	COM	27	90.0
	CL	3*	10.0
CL (n=28)	COM	5*	17.9
	CL	23	82.1
	Total	28	100.0
Group 2 (Hyperintense ve mildly hyperintense=CL) Pathology	**Visual SI**	**n**	**%**
COM (n=30)	COM	22	73.3
	CL	8**	26.7
CL (n=28)	COM	1**	3.6
	CL	27	96.4
	Total	28	100.0

SI: Signal intensity, CL: Cholesteatoma, COM: Chronic otitis media without cholesteatoma. *(If mildly hyperintense and isointense lesions were considered diagnostic for COM (group 1) in visual SI evaluation, there were five false-negative results in the CL group and three false-positive results in the COM group.) **(If hyperintense and mildly hyperintense lesions were considered diagnostic for CL (group 2) in visual SI evaluation, there was one false-negative result in the CL group and eight false-positive results in the COM group. Of the eight false-positive results, three were hyperintense, and five were mildly hyperintense.)

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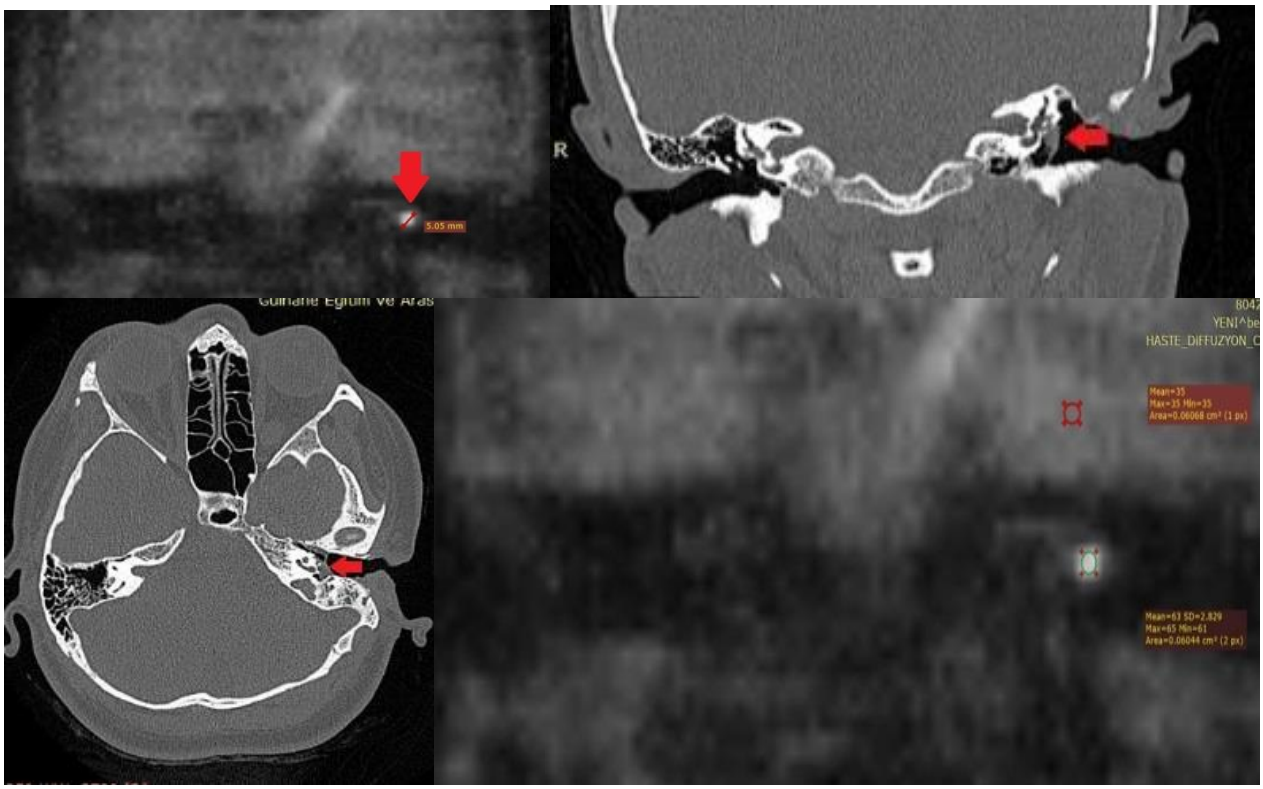

Figure 1. Cholesteatoma (red arrow) in the left middle ear cavity. A. Round shape lesion with 5 mm diameter was hyperintense compared to the brain parenchyma on HASTE DWI at b=1000 mm^2/s. B-C. On the temporal bone CT, the cholesteatoma as a nodular soft tissue density localized at mesotympanum. D. The SI ratio (SIR) of cholesteatoma to the brain parenchyma(cholesteatoma SI/Brain parenchyma SI) was 1.8.

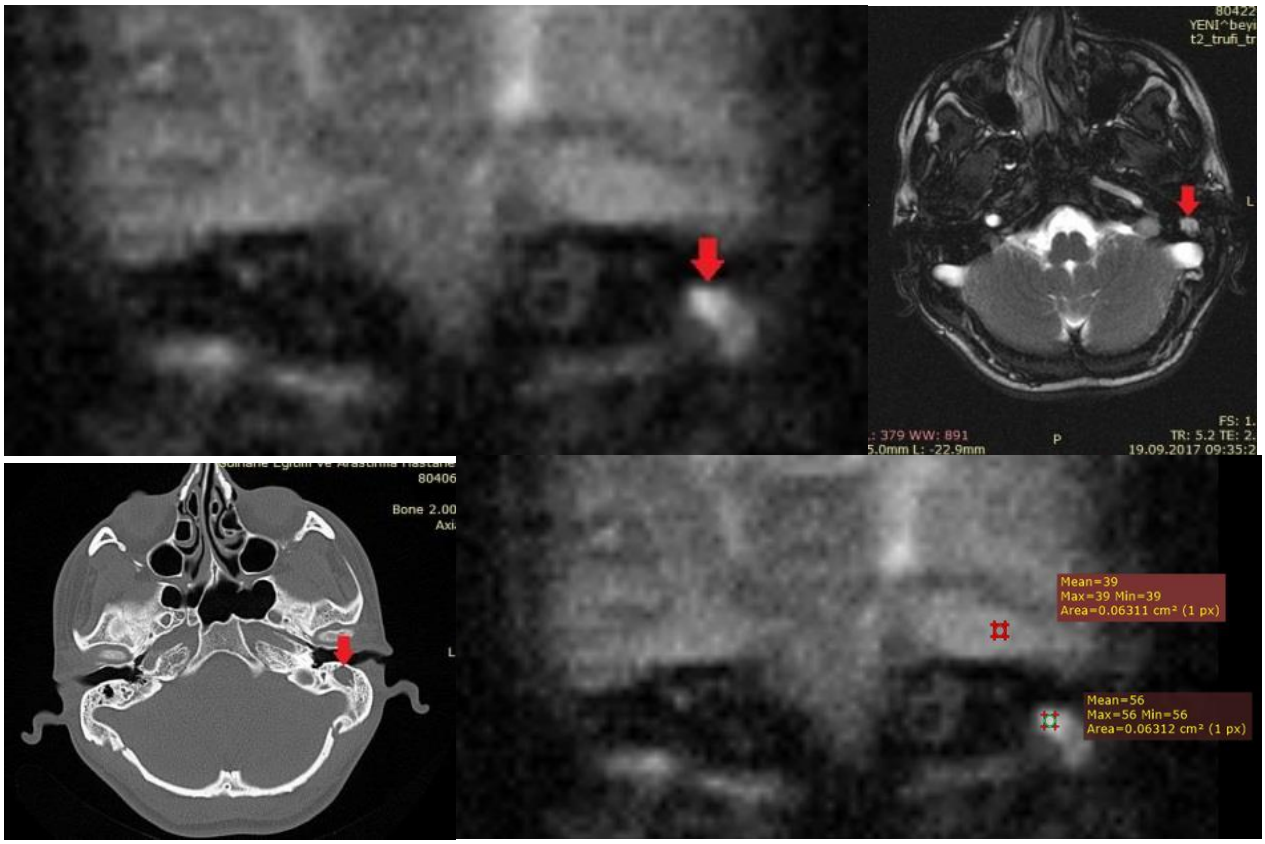

Figure 2. False positive non-cholesteatoma lesion (red arrow) with the histopathologic diagnosis of chronic inflammation. A. Nodular mildly hyperintensity compared to the brain parenchyma on HASTE DWI. B. Axial T2-weighted image showed hyperintense lesion at the mastoid cavity. C. The lesion observed at the mastoid tip within no bone trabeculation on axial CT image. D. The SIR of non-cholesteatoma lesion to brain parenchyma was 1.4.

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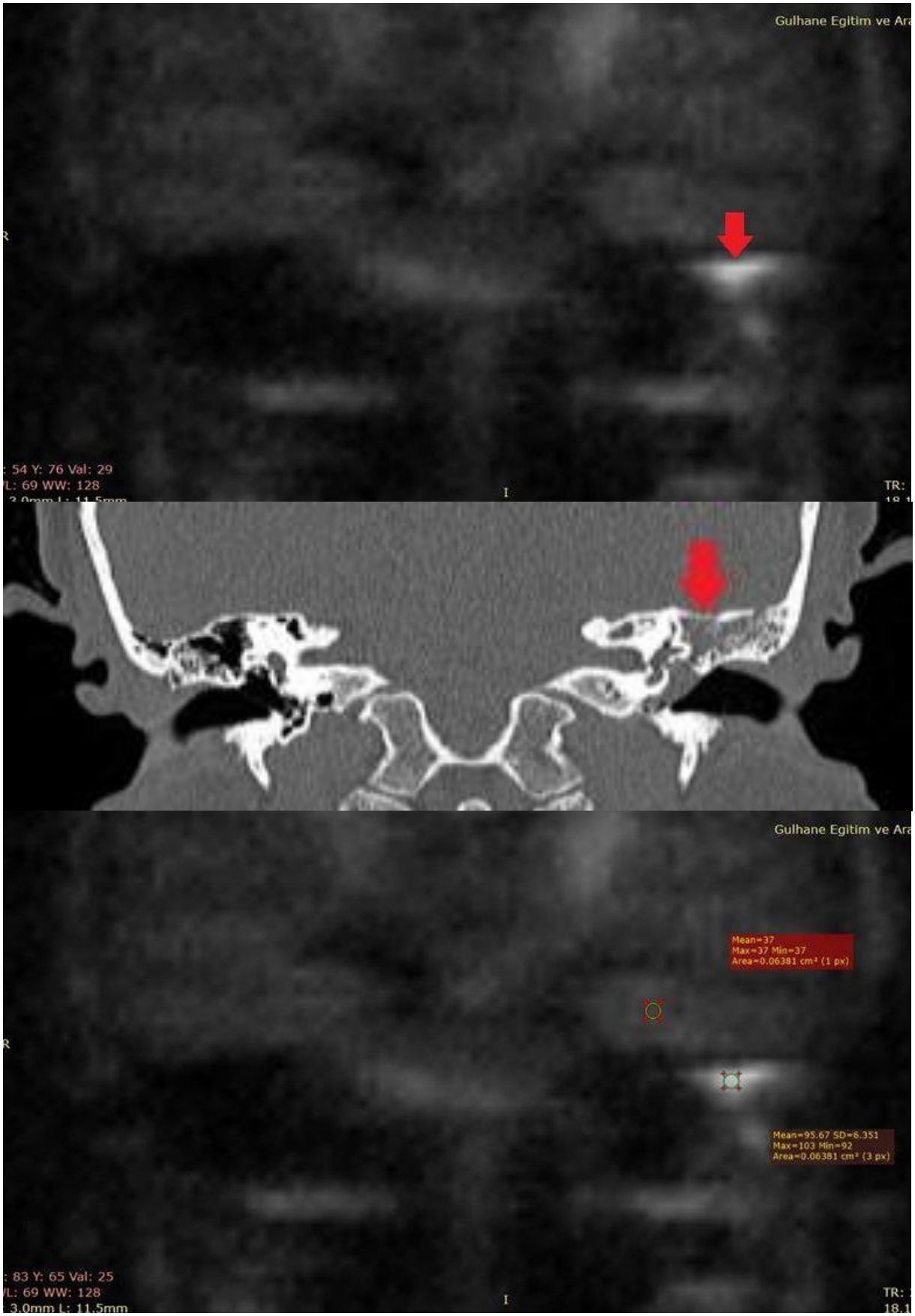

Figure 3. Carcinoid tumor (red arrow) confirmed by surgery. A. The lesion demonstrated very high signal compared with the brain parenchyma on HASTE DWI at b=1000 mm^2/s B.The soft tissue observed at the mastoid antrum on the coronal CT image. The tegmen tympani was intact. C. The SIR was 2.5.

Discussion

The current approach in the diagnosis of CL is the visual and numerical evaluation of the ADC map on diffusion MRI. To create an ADC map, at least two 'b' values of DWI images must be obtained. In our study, we aimed to perform a visual and numerical evaluation on a single 'b' value (b1000) of the DWI image. A significant difference in the visual assessment method was noted between CL and COM by the three observers by using only the b1000 value of DWI with a half short examination time in the present study ($p<0.001$). According to the sensitivity (82%–92%) and specificity (86%–96%) values reported in the literature with the same assessment method (2, 10, 13–17), the higher sensitivity found in our study was due to the presence of a single patient with a false negative result.

False-negative results, which can affect sensitivity rates, are often due to the technical incapabilities of the DWI. Small lesions and retraction pockets, referred to also as dry lesions, can be misdiagnosed on DWI (3). The mean lesion size in the present study was 12.71 mm, with the smallest lesion measuring 5 mm in size. Three of CL lesions measuring less than 3 mm that were removed with surgery were not included in the statistics due to lack of an abnormal signal on DWI. There was only one patient who was diagnosed false negatively due to an isointense sign with surrounding inflammation on DWI, and who was diagnosed with CL after surgery. There was no CL lesion located in the retraction pocket. Dry retraction pockets are lesions in which the keratin has disappeared, and only the surrounding epithelium with persistent aggressive potential is retained, making them undetectable on DWI (12, 18).

The eight patients in the COM group, who were mis-diagnosed as CL, thus reduced the specificity of visual SI assessment. In a visual qualitative assessment, a mildly hyperintense signal may not always indicate cholesteatoma and may decrease the uniqueness of the method with false-positive results (3). The most common reason for false positivity is chronic inflammatory lesions occurring at a rate of 88.9%, while other causes include cholesterol granuloma, abscess, fat grafts, and bone cement (7, 10–12, 19–21). Of the eight patients with a false-positive diagnosis of CL, seven had an inflammatory lesion, and one had carcinoid tumor, which was not reported in the literature previously (Figure 3).

To preclude a false-positive diagnosis of COM due to high signal intensity relative to the brain parenchyma, the visual SI was also evaluated in three grades as hyperintense, mildly hyperintense, and isointense. When a hyperintense lesion was considered CL and mildly hyperintense and isointense pathologies were considered COM, the sensitivity, specificity, PPV, and NPV were 82.14%, 90%, 88.46%, and 84.37%, respectively. The number of patients with false-positive results decreased from eight to three, and the specificity increased to 90%, although sensitivity decreased to 82.14% with the five false adverse effects of CL.

Studies have reported good interobserver agreement between experienced neuroradiologists in a visual qualitative SI assessment (12), while there is no data on the level of cooperation between general radiologists (8,20). A significant and robust positive correlation was identified between the visual assessment results of three physicians from different branches with varying levels of experience (Table 3).

As one of the objectives of the present study, a numerical evaluation was made of the SI and SIR values of the lesions, with the aim being to increase the sensitivity and specificity of DWI, despite those as mentioned above false negative and positive results (10, 11, 22). Özgen et al. reported a sensitivity and specificity of 100% using numerical values (3). In our study, the sensitivity and specificity of statistical SI assessment were 96.43% was 80% and showing no statistically significant difference to the visual SI assessment ($p<0.001$). The numerical SI values of the lesions that showed false positive or false negative results in the visual qualitative assessment were around the cut-off values considered for the diagnosis of CL (Table 4).

Limitations: The numerical SI results of the present study must be repeated and confirmed for different non-EPI DWI sequences and various magnetic fields, and each clinic must determine its cut-off value. A histopathological examination did not support the absence of CL in some patients who were negative based on clinical and radiological evaluation in the COM group.

Conclusion

There is a significant difference between cholesteatoma and chronic otitis media in the diagnosis made by visual and numerical signal evaluation only via b1000 valuable images. However, in false-positive cases, the ADC map is a problem solver and is required for high diagnostic accuracy.

Acknowledgments: **FC** conceived the idea and designed the study. **FC** and **HG** carried out the data analysis, which was overseen by **OS**, and interpreted the findings. **FC, BS, and MT** wrote the first draft of the report, which was critically reviewed and edited by all the authors. All the authors approved the final article. According to local regulation in place during the time of the study, retrospective studies did not require ethical approval. We thank **Lacin Ramazanlı** for image analysis. Financial Disclosure: The financial support for this study was provided by the investigators.

Conflict of Interest: The authors declare that they have no competing interests.

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Influence of hyperthyroidism on hepatic antioxidants and cytokines levels: An experimental study

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Abstract

Objective: Thyroid diseases greatly affect the liver. Hyperthyroidism can affect the function of the liver. This study aimed to investigate the possible change of antioxidant and pro-inflammatory cytokines levels in liver tissue in hyperthyroid rats.

Material and Methods: This study was carried out with 2 experimental groups. Hyperthyroid group was fed with 4 mg/kg L-thyroxine added standard fodder. Control group was fed with standard rat fodder. Liver selenium (Se) levels were measured by inductively coupled plasma optical emission spectrophotometer (ICP-OES). The antioxidant markers such as Selenoprotein P (SelP), and glutathione peroxidase (GPx), and the pro-inflammatory cytokines such as Interleukin (IL)-18, and Tumor necrosis factor-α (TNF-α) levels were studied in liver tissues by ELISA. All markers levels of liver samples were measured in tissue homogenates.

Results: Se, SelP, and GPx levels of the hyperthyroidism group were lower than the control group. (p=0.038, p=0.046, p=0.008 respectively). There was a significant increase in IL-18 and TNF-α levels in hyperthyroidism group when compared to control group (p=0.002, p=0.023 respectively). There was positive correlation between FT3 and FT4, IL-18 and TNF-α (r=0.761, r=0.843, and r=0.826 respectively), but there was negative correlation between FT3 and Se, SelP, and GPx (r=-0.833, r=-0.754, and r=-0.778 respectively).

Conclusion: Our findings showed that antioxidant marker levels were decreased, and pro-inflammatory cytokine levels were increased in liver tissues of hyperthyroid rats. These findings suggest that impaired antioxidant and pro-inflammatory status may play a role in liver pathogenesis due to hyperthyroidism.

Keywords: Hyperthyroidism, liver, antioxidant, pro-inflammatory cytokines

Introduction

Hyperthyroidism is a disease characterized by increased levels of thyroid hormones (thyroxine -T4 and/or triiodothyronine-T3), and decreased concentration of thyroid-stimulating hormone (TSH). The primary sites of conversion of T4 to T3 are the liver and kidneys. Thyroid disease is the most common disease that greatly affects the liver. Hyperthyroidism is known to be associated with abnormalities of the liver, including biochemical markers and histology. However, the mechanisms underlying the relationship between hyperthyroidism and hepatic dysfunctions are unclear (1).

Free radicals can oxidize a variety of cellular substances including DNA, proteins, and lipids, leading to changes in cell and tissue functions. Normally the oxidation process is minimized by the antioxidant defense system. However, oxidative stress develops when free radical production exceeds the antioxidant capacity of cells (2).

The high metabolic state in hyperthyroidism is associated with oxygen consumption and the production of reactive oxygen species (ROS), which causes oxidative stress. Oxidative stress decreases the effectiveness of the antioxidant defense system and causes oxidative damage in macromolecules, and tissue damage (3).

Cytokines are small molecular weight proteins that regulate the relationship between tissues and the immune system. These molecules are synthesized by many different cell types and have important roles in health and disease (4). Tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β are key inflammatory cytokines. They are essentially produced by active kupffer cells, neutrophils, and macrophages. TNF-α, and IL-1β production may lead to increased defense responses in parenchymal cells by activation of apoptosis. However, the crushing of these defensive responses may lead to necrosis death of the cells, and thus stimulate more inflammatory responses (1).

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It has been reported that thyroid hormone disorders increase inflammatory cytokine and ROS production (5, 6). Also, it is studied the liver functions in thyroid diseases. However, it was mostly taken into consideration circulating levels of liver function markers, when examined liver functions in hyperthyroidism (7, 8). Therefore, in our study, we assessed the antioxidant markers such as selenium (Se), Selenoprotein P (SelP), and glutathione peroxidase (GPx), and the pro-inflammatory cytokines such as TNF-α, and IL-18 in liver tissues of hyperthyroidism induced rats. Thus, it is aimed to evaluate the possible effects of these analyzed markers on liver function change due to hyperthyroidism.

Material and Methods

Study design: The experimental study was carried out with 16 Wistar-Albino male rats. Their body weight ranged from 200-250 g. All animals were kept under the same environmental conditions, i.e. at a room temperature of 25˚C, with an artificial light cycle (lights: 08:00–20:00 h), and were left for one week for adaptation. The rats were divided into two groups. The first group consisted of controls receiving standard rat fodder. The second group was fed with 4 mg/kg L-thyroxine added standard fodder during 30 days (9). All animals drank tap water. At the end of the 30-day experimental period, the blood samples were collected via cardiac puncture, and liver samples were taken. Serum samples were removed from blood samples after centrifugation at 3000xg for 20 min at 4°C. These samples were stored in polyethylene Eppendorf tubes at -80 ˚C until analysis.

Hormone Measurements: Serum thyroid stimulating hormone (TSH), free T4 (FT4), and free T3 (FT3) levels were measured by ELISA kits (Sunred biological technology, Shangai, China). All the procedures were carried out following the manufacturer's instructions.

Se Measurement in Liver Samples

Se levels were measured by inductively coupled plasma optical emission spectrophotometer (ICP-OES, Thermo iCAP 6000, Cambridge, UK). The tissue samples were weighed and transferred into metal-free glass tubes for digestion. The samples were first digested with 2 ml of concentrated nitric acid (HNO3) at 100 °C in the furnace (Merck, Darmstadt, Germany), and 1 ml of perchloric acid ($HClO_4$) was added to the cooled materials. The materials were then completely digested at 120 °C until the materials diminished to half of the original total volume. Digested materials were diluted with deionized water to 10 ml. Calibration standards were prepared using stock solution at a concentration of 1000 mg/L (Chem-Lab, Belgium). Elemental solutions of 0.0010, 0.0025, 0.0050, 0.0100, 0.0250 and 0.0500 ppm concentrations were prepared by using stock solution and distilled water (Millipore, Bedford, MA, ABD) containing 0.3% HNO3. Se element levels were determined by using 196.026 nm wavelength. Results were calculated as µg/g wet weight (µg/g tissue).

Preparation of Tissue Homogenates

Liver tissues were excised from all rats, rinsed with ice-cold saline and homogenized in 100 mMTris–HCl buffer (pH 7.4) using a homogenizer. The lysate was then cold-centrifuged at 12 000 x g for 30 min at 4 °C. Supernatants were collected and stored at -80°C until analyzed. Protein concentrations of supernatants were determined by Lowry's method (10).

Measurement of Antioxidant and Cytokine Levels in Liver Samples

The levels of SelP, GPx, TNF-α, and IL-18 in liver tissue samples were quantified according to the manufacturer's instructions and guidelines using the ELISA kits specific for the rat (Sunred biological technology, Shangai, China). Antioxidant and cytokine levels were determined in the supernatants of tissue homogenates.

Statistical analysis

Data are presented as mean ± the standard deviation (SD). Statistical analysis was performed using the Student t-test and Mann-Whitney U-test. $p<0.05$ was considered to indicate a statistically significant difference. In addition, the relationship between variables was investigated by the Pearson correlation test. All calculations were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, Inc., La Jolla, CA, USA).

Results

The Values of Thyroid Function Markers

Comparison of serum FT3 and FT4 levels of experimental groups showed that these parameters were higher in hyperthyroidism group than in control group (p=0.030, p=0.011, respectively) (Figure 1 A-B). Also, TSH values of hyperthyroidism group were lower than control (p=0.031) (Figure 1C).

Antioxidant and Cytokine Values of Liver Tissues

All antioxidant markers of liver samples were measured in tissue homogenates. Liver Se, SelP, and GPx levels of the hyperthyroidism group were lower than control group (p=0.038, p=0.046, p=0.008 respectively). (Table 1).

Also, cytokine markers of liver samples were measured in tissue homogenates. When experimental and control groups were compared for cytokine levels, it was seen that there was a significant increase in IL-18 and TNF-α levels in hyperthyroidism group when compared to control group (p=0.002, p=0.023 respectively) (Table 1).

Correlation analysis results of all studied markers in hyperthyroid group

When correlation of thyroid functions, antioxidants and pro-oxidant markers in hyperthyroid group were analyzed it was seen that there was positive correlation between FT3 and FT4, IL-18 and TNF-α (r=0.761, r=0.843, and r=0.826 respectively), but there was negative correlation between FT3 and Se, SelP, and GPx (r=-0.833, r=-0.754, and r=-0.778 respectively) (Table 2). Also, significant positive correlation was between Se and SelP, and IL-18 and TNF-α (r=0.922, and r=0.939 respectively), and negative correlation between FT4 and GPx values (r=-0.886) (Table 2).

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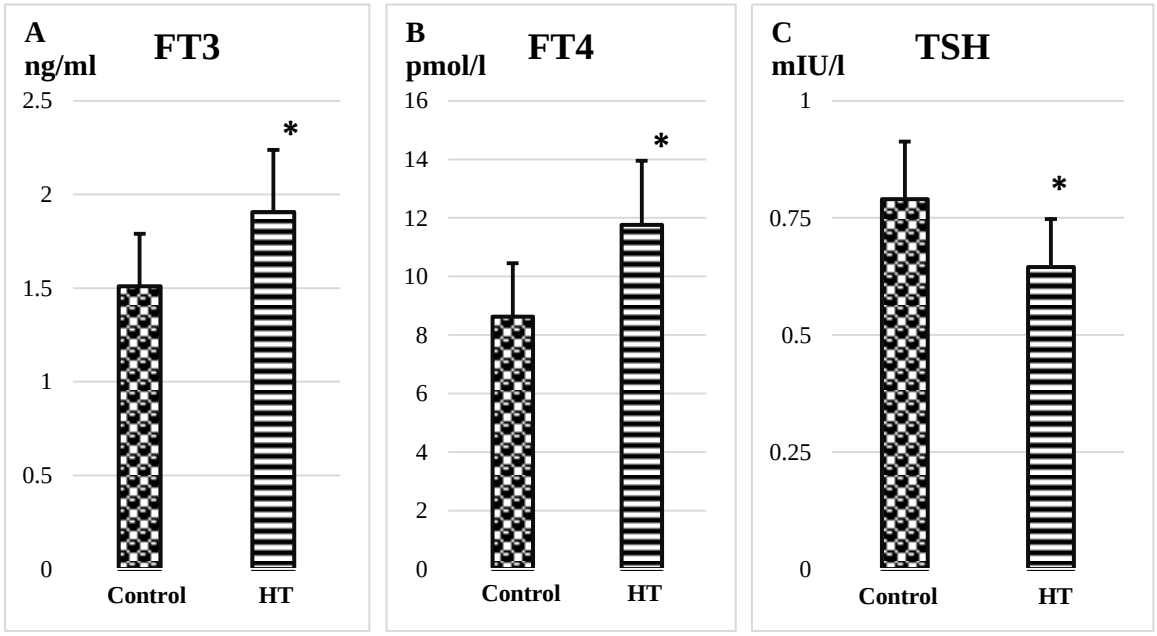

Figure 1A. Serum FT3 levels of studied groups. Values are presented as the mean ± the standard deviation. *p=0.030. **1B**. Serum FT4 levels of studied groups. Values are presented as the mean ± the standard deviation. *p=0.011. Figure **1C**. Serum TSH levels of studied groups. Values are presented as the mean ± the standard deviation. *p=0.031

Table 1. Antioxidant marker and pro-inflammatory cytokine levels of studied groups

	Control	HT	p
Se (µg/g wet tissue)	2.07±0.23	1.68±0.41	0.038
SelP (ng/mg protein)	14.67±2.03	12.59±1.77	0.046
GPx (ng/mg protein)	30.79±2.24	24.34±5.48	0.008
IL-18 (pg/mg protein)	8.72±1.54	12.74±2.44	0.002
TNF-α (pg/mg protein)	29.88±2.04	32.76±2.44	0.023

Values are presented as the mean ± the standard deviation

Table 2. Correlation of thyroid functions, antioxidant marker and pro-inflammatory cytokine levels in hyperthyroid group

	fT3	fT4	TSH	Se	SelP	GPx	IL-18
fT4	p=0.037 r=0.761						
TSH	p=0.151 r=-0.571	p=0.132 r=-0.595					
Se	p=0.015 r=-0.833	p=0.171 r=-0.547	p=0.058 r=0.714				
SelP	p=0.031 r=-0.754	p=0.059 r=-0.694	p=0.016 r=0.826	p=0.002 r=0.922			
GPx	p=0.025 r=-0.778	p=0.005 r=-0.886	p=0.275 r=0.443	p=0.177 r=0.538	p=0.095 r=0.638		
IL-18	p=0.013 r=0.843	p=0.140 r=0.578	p=0.358 r=-0.361	p=0.096 r=-0.626	p=0.155 r=-0.539	p=0.078 r=-0.648	
TNF-α	p=0.017 r=0.826	p=0.076 r=0.670	p=0.314 r=-0.395	p=0.143 r=-0.562	p=0.139 r=-0.566	p=0.069 r=-0.662	p=0.003 r=0.939

Discussion

Thyroid hormones are key determinants of cellular development, growth and metabolism. These hormones control various metabolic activities related to the anabolism or catabolism of carbohydrates, proteins, and lipids to protect homeostasis (11). Thyroid hormones are considered one of the main endocrine regulators of metabolic activity in cells, including hepatocytes (1). Imbalance of thyroid hormones in the body is associated with many chronic diseases, including diabetes mellitus, cardiovascular disease, and liver-related disorders (11). In the liver, the acceleration of aerobic metabolism by T3 enhances the generation of ROS at the mitochondrial, microsomal, and peroxisomal site (12).

Hyperthyroidism is a condition associated with overproduction and secretion of thyroid hormones. Liver dysfunction has been reported in 37% to 77.9% of patients with hyperthyroidism (13). A well-known negative effect of hyperthyroidism is hepatic toxicity, which is characterized by an increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (3, 13). However, the possible mechanism between hyperthyroidism and hepatic dysfunction has not been fully explained (13). In previous studies, some explanations have been proposed for liver dysfunction associated with hyperthyroidism. It has been found that oxidative stress and mitochondrial oxygen consumption increased in the liver of rats with hyperthyroidism (14, 15). It has been reported that apoptosis in the liver of rats due to hyperthyroidism occurs by activation of intrinsic and extrinsic pathways. These are two classic activation mechanisms of apoptosis. The intrinsic pathway involves the release of cytochrome c from the mitochondria, the production of ROS, and the loss of mitochondrial transmembrane potential. The extrinsic pathway contains cell surface death receptors such as TNF-α and Fas. Binding of TNF-α and Fas ligand to their respective receptors induces apoptosis via apical caspase-8 (16).

In our literature research, it has been seen that there have been many studies examined the relationship between hyperthyroidism and liver oxidant status. Venditti et al. (17) reported that hyperthyroidism was associated with the hepatic levels of oxidized lipids and proteins, leading to increased free radical production in the liver. Fernandez et al. (18) informed that hyperthyroidism increased the activity of the nitric oxide synthase enzyme, increasing the liver's pro-oxidant activity. Assaei et al. (3) reported that GPx and glutathione reductase activities decreased, and malondialdehyde (MDA) levels increased in the liver after T4 application to hyperthyroid rats. These findings have shown that hyperthyroidism increases the production of active oxygen species, hydroxyl radicals, which easily initiate free radical-mediated lipid peroxidation, and result in increased MDA production. Boisier et al. (20) informed that MDA levels were increased and GSH levels were decreased in the liver of hyperthyroid rats (19). Venditti et al. noticed that hyperthyroidism induced in rats by T3 daily injections for 10 days caused significantly increased MDA levels in the liver. Asayama et al. (21) found no change of MDA in liver homogenates from hyperthyroidism induced rats rendered hyperthyroid by administration of T4 to their drinking water over a 4-week period. But, they also found a low GPx concentration in the liver tissue. Thyrotoxicosis causes liver damage. Malik and Hodgson (22) were reported that the damage may occur due to hypoxia in the perivenular regions due to increased hepatic oxygen requirement.

Se is an important component of selenoproteins that play a role in many biological functions such as antioxidant defense, the formation of thyroid hormones, DNA synthesis, fertility and reproduction (23). SelP, which constitutes more than 50% of plasma Se reserves, is an extracellular glycoprotein. All tissues express SelP, but the liver is the primary source of SelP in plasma. SelP plays a role in carrying Se to tissues and is an important extracellular antioxidant. It eliminates peroxynitritis caused by the reaction of superoxide ions, which are produced in inflammation sites, with nitric oxide (23, 24). GPx is an important antioxidant enzyme. Its main function is to neutralize hydrogen peroxide (H2O2) and organic hydroperoxides in the intracellular and extracellular compartments (23).

In this study, we were examined the liver levels of Se, SelP, and GPx, which are known to have antioxidant roles, and were evaluated the effect of these antioxidant markers on the liver dysfunction of hyperthyroidism. Our findings showed that all of these antioxidant markers decreased in the hyperthyroid group. These findings may indicate that the liver antioxidant status is decreased due to hyperthyroidism.

Cytokines are small molecular-weighted peptides, which are located between tissues and the immune system. They either limit damage and suppress the activity and production of pro-inflammatory signals, or induce pro-inflammatory inflammation as a result of infection and injury (25). Some cytokines, such as IL-1 and TNF-α, regulate hepatocyte destruction. They stimulate the synthesis of acute-phase proteins in liver cells and mediate hepatocyte regeneration. In addition, TNF-α and IL-6 have been explained to have stimulating effects on hepatocyte growth (26). There is evidence to support the role of cytokines, including IL-1α, IL-6, and TNF-α in inflammatory liver disease. These cytokines are produced in the liver by kupffer cells and hepatocytes and play a role in hepatic inflammation (27).

IL-18, a member of the interleukin IL-1 family, is a cytokine with pleiotropic effects. IL-18 is produced by kupffer cells, macrophages, B cells and dendritic cells in lipopolysaccharide stimulation. It has been reported that acute liver damage due to IL-18 can occur through the upregulation of the Fas ligand (28).

TNF-α is a pro-inflammatory cytokine produced by activated macrophages and lymphocytes in response to tissue injury and infection. Serum TNF-α is increased in chronic inflammatory liver disease. In these patients, intrahepatic TNF-α levels are likely to be higher due to local TNF-α production in kupffer cells and hepatocytes. In addition, TNF-α has been associated with ROS production in hepatocytes (29). In our literature research, it has been

seen that studies examining the relationship between hyperthyroidism and liver cytokine level are limited. Tapia et al. (12) reported that the expression of TNF-α, IL-1β and IL-10 mRNA genes in liver tissues increase in hyperthyroid rats. Fernández et al. (6) observed increased TNF-a and IL-10 mRNA expressions in the liver of T3-treated rats.

In our study, when the findings of liver IL-18 and TNF-α related to hyperthyroidism were examined, it was observed that there were increased IL-18 and TNF-α levels in the hyperthyroid group compared to the control group. These findings may indicate that the liver pro-inflammatory cytokines are increased due to hyperthyroidism.

Conclusion

Our findings suggested that antioxidant marker levels were decreased, and pro-inflammatory cytokine levels were increased in liver tissues of hyperthyroid rats. These findings suggest that impaired antioxidant and pro-inflammatory status may play a role in liver pathogenesis due to hypothyroidism. Mechanisms of pathogenesis need to be investigated with further molecular studies.

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The association of axillary lymph node-positive breast cancer with metabolic parameters of 18F-fluorodeoxyglucose PET/CT

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Abstract

Objective: This study aims to examine the association between 18F-fluorodeoxyglucose PET/CT (18F-FDG PET/CT) metabolic parameters of lymph node-positive and lymph node-negative breast carcinomas.

Material and method: We included breast carcinomas patients who underwent 18F-FDG PET/CT imaging at our department between May 2018 and December 2019. A total of 108 female breast cancer patients were included (aged 48.8 ± 13.6years; range, 28-84 years). PET scanning was performed in 3D mode from the skull ceiling to the half of the thigh. According to pathology reports, we divided the patients into two groups: a lymph node-positive group of patients and a lymph node-negative group of patients. We calculated the sensitivity and specificity for determining the PET/CT pathological lymph node. Metabolic parameters like TLG (Total lesion glycolysis), MTV (Metabolic tumor volume), SUVmean, and SUVmax values were calculated.

Result: The lymph node-positive group's body weight and body mass index(BMI) were statistically higher than the lymph node-negative group (p=0,027,p=0,022 respectively). SUV max and SUV mean of the lymph node-positive group were statistically higher than the lymph node-negative group (p=0.008, p=0,009, respectively). Both TLG and MTV of the lymph node-positive group were statistically higher than the lymph node-negative group (p=0.01, P= 0.01, respectively). Ki-67(%) of the lymph node-positive group was not statistically different from the lymph node-negative group. We calculated the PET/CT's sensitivity and specificity as 78,57% and 59,09%, respectively. For the positive predictive value of PET/CT, we found 55%, and for the negative predictive value, it was 81.25%.

Conclusions: PET/CT metabolic parameters of patients with lymph node-positive breast cancer were higher than patients with lymph node-negative. High body weight and BMI appears to increase the possibility of metastases of lymph node. The sensitivity of PET/CT can be considered to be useful in determining the pathological lymph node, but the specificity of PET/CT is not very good.

Keywords: Breast cancer, 18F-FDG PET/CT, Lymph node

Introduction

Breast cancer, as the most deadly cancer for women, is the second most common cancer in the world, after lung cancer, with more than two million cases per year [1, 2].

A precise definition of the extent of the disease after a diagnosis of breast cancer is one of the most important measures to treat it. One of the most important early stages' prognostic factors of breast cancer is the evaluation of lymph node status. Conducting the evaluation by physical examination is not a reliable method for examining axillary lymph nodes' status, as in most cases, it is not possible to touch metastatic lymph nodes, and reactive lymph nodes might be mistakenly diagnosed as them [3,4,5].

About 85% of the breast's quadrants lymphatic drainage reaches the Axillary Lymph Nodes (ALNs), and the rest reaches the internal, supraclavicular and/or infraclavicular lymph nodes. The location and size of the tumor, the presence of lymph node invasion as well as the histologic grade are all associated with the possibility of ALN involvement [6,7].

There are several methods available today to diagnose ALN involvement, including axillary ultrasound, PET/CT, ultrasound-guided needle biopsy, and MRI.

Positron emission tomography/computed tomography (PET/CT) is combined imaging of anatomy and function.

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Not only does it show the morphological features of the lesion, but it may also report metabolic information of the lesion, bringing new opportunities for breast cancer's diagnosis, staging, re-staging, and treatment response assessment [8, 9].

The maximum standardized uptake value (SUVmax) and the mean standardized uptake value (SUVmean) measured with FDG PET in breast cancer are sensitive indicators for metabolic activity [10,11], which are usually used for examining the aggressiveness of tumor and is related with prognostic factors, such as the histological grade, histological type, proliferation index, and immunohistochemical factors [10,12-18]. Some parameters mixing volume and FDG intensity can also be used as metabolic parameters such as Total Lesion Glycolysis (TLG) and Metabolic Tumor Volume (MTV). In this study, we seek to find the relationship between 18F-fluorodeoxyglucose PET/CT (18F-FDG PET/CT) metabolic parameters of axillary lymph node-negative and axillary lymph node-positive of breast carcinomas and to determine PET/CT's sensitivity and specificity to evaluate the status of axillary lymph nodes.

Methods and Materials

The population of the study: In this study, we included patients who underwent 18F-FDG PET/CT imaging at our department having breast carcinomas between May 2018 and December 2019. A total of 108 female breast cancer patients were included (aged 48.8 ± 13.6years; range, 28-84 years). Before imaging, breast carcinoma was diagnosed by biopsy of all patients. Height and body weight of the patients were measured. BMI of patients was calculated (BMI=weight(kg)/[height(m)2). Pathological subtypes of breast cancer patients are infiltrative breast carcinoma (n = 83), invasive ductal breast carcinoma (n = 14), invasive lobular carcinoma (n = 6), invasive tubular carcinoma (n = 2), micropapiller carsinoma (n = 2) and cribriform carcinoma (n = 1).

Imaging procedure: After eight hours of fasting, patients were given 18F-FDG intravenously (blood glucose <200 mg / dL) and images of whole-body were taken from PET/CT scanner (Siemens 3D-TOF Siemens Medical Systems) 55 to 75 minutes after injection (19) low-dose CT scan (80mA, 120 kV) was conducted.

An intravenous injection 3,7MBq/Kg 18F-FDG was performed on the arm of the patient opposite to the primary breast tumor location. PET scanning was performed in 3D mode from the skull ceiling to the half of the thigh. Using an SUV of 2.5 as the threshold, MTV (ml) was assigned to the volume of the tumor with SUV $\geqslant$ 2.5.

In the delineated tumor volume, SUVmean was also defined as a mean SUV. TLG (SUVml) was defined as the product of the MTV multiplied by SUVmean. Metabolic parameters such as Metabolic Tumor Volume (MTV), Total Lesion Glycolysis (TLG), SUVmean, and SUVmax values were calculated.

Statistical Analysis: Via the Kolmogorov-Smirnov test, we tested the data normality. We summarized data for categorical variables as percentages and frequencies, and for continuous variables, we summarized them as mean and standard deviation (SD). For continuous variables, the Student t-test, and for categorical variables, the chi-square test was used to compare two groups. The positive and negative predictive values, sensitivity, and specificity of PET/CT for determination of lymph node positivity were calculated. An alpha level below 0.05 was considered for statistical significance. We conducted analyses by SPSS version 18 (Windows, Chicago, IL, USA).

Results

The body weight and BMI of the lymph node-positive group was statistically higher than the lymph none negative group(p=0,027, p=0,022 respectively). SUV max and SUVmean of the lymph node-positive group were statistically higher than the lymph node-negative group (p=0.008, p=0,009, respectively). Both TLG and MTV of the lymph node-positive group were statistically higher than the lymph node-negative group (p=0.01, P= 0.01, respectively).

The Ki-67(%) of the lymph node-positive group was not statistically different from the lymph node-negative group (figure-1 and table-1). The Sensitivity and specificity of PET/CT were 78.57% and 59.09%, respectively. The negative predictive value of PET/CT and Positive predictive value of PET/CT was 55% and 81.25%, respectively.

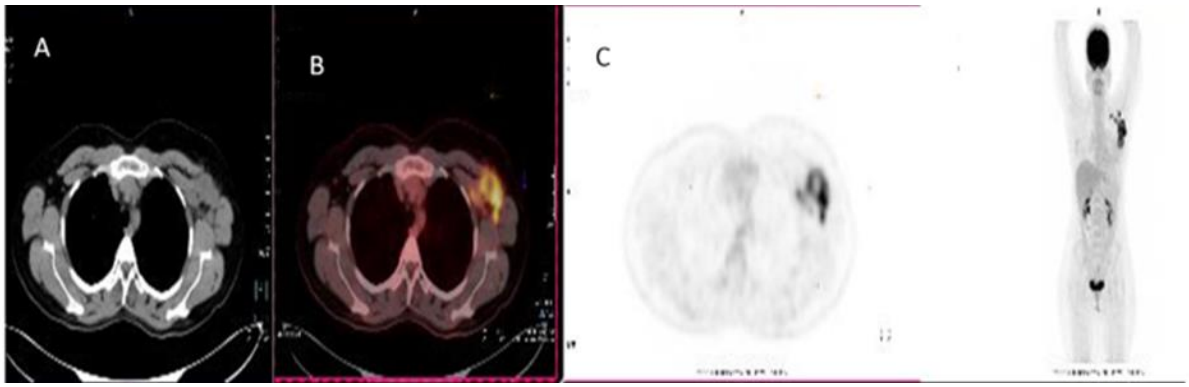

Figure 1: Left axillary lymph node metastasis of forty-five years older women with invasive ductal carcinoma in PET/CT. A= CT, B= PET/CT fusion, C=PET.

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Table 1: Comparison of axillary lymph node positive group and axillary lymph node-negative group.. (NS= Non Significant).

	Axillary lymph node negative(n=66)	Axillary lymph node positive(n=42)	p value
Age (year)	49,1 ±13,9	48,4 ±13,3	N.S
Weight (kg)	69,9 ±10,8	75,9± 14,8	0,027
Height(cm)	163±6	164±7	N.S
BMI(kg/m^2)	25,9±4,6	28,4±6,1	0,022
Suvmax	8,8 ±6,0	11,7 ±7,7	0,008
Suvmean	4,5 ±1,9	5,6± 2,4	0,009
TLG	23,8 ±28,3	46,5 ±49,3	0,01
MTV	7,3± 8,3	12,2± 15,5	0,01
Ki-67 (%)	22,2 ±22,8	32,3± 29,2	N.S

Table 2: Distribution of the patients according to pathological and PET/CT findings

		Pathologic findings (number of patients)	
		Positive	**Negative**
PET/CT findings (number of patients)	**Positive**	33	27
	Negative	9	39

Discussion

PET/CT has provided many benefits in developing a treatment plan for breast cancer. It can play an important role for breast cancer patients and identifying distant and occult nodal metastases provide benefits such as surveillance during and after neoadjuvant chemotherapy, preventing non-essential biopsies from a lymph node, and supporting patient treatment planning [20]. Fouster D et al stated that PET/CT have sensitivity (80% -94%) and specificity (86% -90%) in determining axillary lymph node metastases [21]. In a review article, PET/CT sensitivity was reported as 64% (59%-69%) and specificity as 93% (90%-95%) [22]. In contrast, some studies found that it has a 24-82% sensitivity for identifying metastasis of axillary node, and it was also stated that PET/CT has low sensitivity in determining axillary lymph nodes in the early stages of primary breast cancer.

In addition, studies have shown very low effectiveness of PET/CT for detecting micrometastases in breast cancer patients [23, 24]. The differences in results between these studies can be attributed to the population studied, the PET protocol, and the histopathological procedure applied. In our study, the sensitivity and specificity of PET/CT to show axillary lymph nodes in breast cancer cases were 78.57% and 59.09%, respectively. While the sensitivity values of PET/CT in determining the lymph node are compatible, the specificity value of our study is lower than the previous studies. The reason that the specificity values were lower than previous studies may be due to the micrometastases present in our patients. As stated in our study, the PET/CT sensitivity around 80% facilitates the detection of cases with lymph node metastasis.

Despite the fact that in determining ALN staging, the surgical approach reference standard has performed better than all other approaches, the volume-based metabolic PET/CT breast tumor parameters can be used to diagnose patients who do not require invasive procedures. This suggestion can have some clinical benefits for patients.

Volume-based parameters on 18F-FDG PET/CT, such as Total Lesion Glycolysis (TLG) or Metabolic Tumor Volume (MTV), represent total tumor burden as well as tumor metabolic activity. In our study, the SUVmax SUVmean, TLG, and MTV values of the axillary lymph node-positive group were significantly higher than the axillary lymph node-negative group. It was shown that SUVmax of breast tumors on 18F-FDG PET/CT had been associated with ALNM [25]. Young-Sil et al. [26] measured SUVmax SUVmean, MTV, and TLG. On univariate analysis, the authors showed that both SUVmax and SUVmean were associated with ALNM. Because MTV represents the total tumor burden, not merely the metabolic activity, MTV has been suggested as a prognostic factor for breast cancer [27, 28]. Ulaner et al. suggested that MTV may be related to lymph node and liver metastases and TLG associated with bone and lymph node metastases. Another study found TLG of the primary breast tumor as an independent predictor of ALN metastasis in invasive ductal cancer of breast [29]. In conclusion, in light of all findings, it can be said that parameters such as SUVmean, MTV, SUVmax, and TLG were associated with axillary lymph node metastases at specific rates. Especially in cases with very high metabolic parameters, clinicians are more likely to see lymph node metastases.

Considering that Ki-67(%) is one of the indicators of the bad prognosis, our expectation was that the ki-67(%) level was high in cases with positive axillary lymph node, In contrast, in our study, Ki-67 index of axillary lymph node-positive group and axillary lymph node-negative group was not different [30]. According to the results of our study, it can be said that the level of Ki-67(%) is not related to metastases of the axillary lymph node.

Obesity and low physical activity are related to a higher risk of breast cancer, and weight gain after diagnosis of breast cancer is related to a higher risk of recurrence. The mechanism between "energy excess" states and breast

cancer is likely multifactorial, including inflammatory cytokines and immune cells, adipocytokines, and excess hormones. Obese postmenopausal women have a higher risk of breast cancer with a relative risk of about 1.3 compared with the normal weight ones [31]. The Women's Intervention Nutrition Study (WINS) found that a dietary intervention associated with weight loss could decrease the risk of breast cancer recurrence by 24% at five years [32]. In the present study, the bodyweight and BMI of the patients with an axillary lymph node metastasis was higher than those without a lymph node metastasis. In many studies, it seems that this issue has not been clarified since the body weights of breast cancer cases are not specified. Bodyweight gain may indeed cause recurrences and lymph node metastases in breast cancers.

Conclusions

PET/CT metabolic parameters of axillary lymph node-positive breast cancer patients were higher than axillary lymph node-negative patients. High body weight and increased BMI levels appears to increase the possibility of axillary lymph node metastases. The sensitivity of PET/CT can be considered to be useful in determining the pathological axillary lymph node, but the specificity of PET/CT is not very good

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Conflict of Interest: The authors declare that they have no conflict of interest

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