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Original Article

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Investigation of Gasdermin-D and Pannexin-1 in Omental Adipose and Placental Tissues in Obese Pregnant Women

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Abstract

Aim: Gasdermin-D (GSDMD) and pannexin-1 (PANX1), which are proinflammatory proteins, may be involved in the pathogenesis of complications in pregnant women with obesity. We aimed to investigate the expression of GSDMD and PANX1 biochemically in samples of maternal omental and placental tissue obtained from pregnant women with or without obesity, and their correlation with maternal, obstetric, and fetal/neonatal variables.

Methods: The prospective observational study included 79 pregnant women who underwent elective cesarean sections with a pregnancy between 37 and 41 weeks from December 2021 to April 2022. They were divided into three study groups according to their body mass index (BMI): normal weight (with a BMI <25) (n=25), overweight (BMI between 25 and 30) (n=28), and obese (with a BMI >30) (n=26). Omental and placental GSDMD and PANX1 levels were measured by enzyme-linked immunosorbent assay between the groups.

Results: In the homogenate of omental tissue, the median levels of GSDMD and PANX1 protein in the overweight and obese groups were significantly lower than those in the normal weight groups (p=0.0154 and p=0.0184, respectively). In the placental tissue samples, the median levels of GSDMD and PANX1 proteins in the normal weight, overweight, and obese groups were similar.

Conclusion: GSDMD and PANX1 expressions were shown for the first time in the omentum and placenta together; which has the potential to be used as a predictive and diagnostic test panel after further studies in obese pregnant women.

Keywords: Gasdermin-D, pannexin-1, placenta, omental tissue, obesity, inflammation

Introduction

An increased prevalence of obesity in pregnant women may lead to an increased risk of pregnancy-related problems such as gestational diabetes, cesarean birth, hypertensive disorders, preeclampsia, neonatal hypoglycemia, shoulder dystocia, and macrosomia in newborns (1), as well as a long-term rise in the risk of obesity, diabetes, and cardiovascular diseases in children (2). However, the pathogenesis of the condition that affects the health of the mother and offspring caused by obesity during pregnancy remains elusive.

Adipose tissue is a dynamic endocrine organ that participates in a variety of processes, including inflammation (3). Because of the unfavorable secretion pattern of obesity, proinflammatory adipokine stimulates the production of reactive oxygen species, leading to increased oxidative stress (4). Additionally, the placenta secretes a variety of chemicals to sustain the physiology

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of pregnancy. Inflammation levels in the placenta could impact the health condition of the mother and her risk of developing gestational diabetes mellitus (GDM), along with the negative effects of obesity and GDM on the fetus (5). Pyroptosis is a rapid, proinflammatory form of cell death in which the pathways of gasdermin-D (GSDMD) and pannexin-1 (PANX1) proteins intersect through their interactions in cellular functions. These novel proteins are produced in cells from both adipose tissue and the placenta (6,7). GSDMD is a pore-forming protein that plays a critical role in inflammation, autoimmunity, and/or cell death. GSDMD is cleaved by activated caspase, releasing its N-terminal domain, which forms pores at the plasma membrane and induces pyroptosis (8). PANX1 hemichannels are expressed by a variety of cell types and form pores in the plasma membrane that allow the penetration of nonviable stains. In this manner, their signaling governs many physiological functions, but they have also been implicated in numerous pathological processes (9).

Understanding and preventing the underlying mechanisms can help prevent long-term pathologies caused by obesity in the mother and infant. Therefore, it is important to conduct further research to understand the etiology of pregnancy-related events in obese women. The expression of the proteins GSDMD and PANX1 has not been adequately investigated in maternal omental adipose tissue and placental tissue samples. We hypothesized that combining these proteins would enhance our understanding of their role in obesity-related pregnancy and perinatal outcomes. Therefore, we biochemically assessed the expression of GSDMD and PANX1 in samples of maternal omental adipose tissue and placental tissue obtained from pregnant women, dividing them into groups based on their body mass index (BMI).

Materials and Methods

Compliance with Ethical Standards

In this prospective observational study, omental adipose tissue and placental samples from mothers who delivered by cesarean section were examined histologically, and ELISA was performed. Ethical approval was obtained from the Institutional Ethics Committee of the University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital (dated: 24.11.2021, approval no.: 116-2021) and was registered in the ClinicalTrials.gov database (Registry no: NCT05475951, dated: 25.07.2022) according to the Helsinki Declaration and its later amendments or comparable ethical standards. Each woman was asked for written consent after being fully informed of the nature and intended use of the procedures. There was nothing about the study that could harm the health of the mother or the baby. The inclusion of mothers who underwent cesarean sections facilitated the collection of research materials.

Study Design

This study was conducted in accordance with appropriate clinical and experimental ethical guidelines between December 2021 and April 2022 at the Obstetrics and Gynecology Service. The study included 79 pregnant women, and they were divided into three study groups according to their BMI: normal weight (with a BMI<25) (n=25), overweight (BMI between 25 and 30) (n=28), and obese (with a BMI >30) (n=26) (Figure 1). The inclusion criteria were as follows: being between the ages of 20 and 35 years, having a term pregnancy (37-41 weeks), absence of abnormalities during pregnancy follow-up, having a planned cesarean section, absence of complications during surgery, being an appropriate gestational age newborn, and placenta with an anterior or posterior wall. Multiple pregnancies, pregnancies with oligohydramnios or polyhydramnios, preeclampsia, fetal growth restriction or premature birth, and intrauterine surgical procedures were all exclusion criteria.

The study included the basic clinical characteristics, sociodemographic properties, anthropometric measurements, cigarets, and comorbidities of the groups classified according to their BMI. The study also included the birth weight, APGAR values, pH values at birth, and gestational age of the infants born by cesarean section.

Sample Collection and Analysis

Following uterine repair during cesarean section, pieces of omental tissues were taken as adipose tissue, and placental samples were collected from four quadrants of the placentas, including the decidua and chorionic sides, 4-5 cm away from the umbilical cord entrance site for *in vitro* analyses in accordance with prior studies (10,11). Homogenization was applied to the omental and placental tissues collected for biochemical analysis. With ELISA tests, the expressions of GSDMD (12) and PANX-1 (13) proteins in omental and placental homogenates were measured, and the results were used in statistical analysis. The remaining omental tissue samples and placental tissues were fixed with 4% paraformaldehyde for more than 24 h; consequently, they were embedded to prepare wax blocks and cut into slices with a thickness of approximately 4 µm. The sections were stained with hematoxylin and eosin (H&E). Lastly, under-microscope images were collected and analyzed (12,13).

Statistical Analysis

IBM SPSS V26.0 (IBM, USA) software was used for statistical analysis. Graphical presentations were prepared using GraphPad Prism v9.x (GraphPad Software, USA).

Descriptive statistics are presented as average ± standard deviation, median value, 25-75 percentiles, frequency distribution, and percentage. The Kolmogorov-Smirnov test was used to determine whether the distribution of continuous variables obtained is normal or not. Continuous variables are analyzed by parametric ANOVA and Tukey's test if they fit into the normal distribution and by non-parametric Kruskal-Wallis and Mann-Whitney U test if they do not. For comparisons of categorical data, the chi-square test was used for categorical variables. The correlation among continuous variables was examined by Spearman correlation analysis. When the p-value was less than 0.05, the differences were considered significant.

Results

Table 1 presents the maternal and perinatal clinical characteristics of normal-weight, overweight, and obese pregnant women. There were no significant differences among the study groups regarding women's age, blood pressure, smoking status, economic status, gestational age at delivery, number of gravidities, parity, vaginal birth and cesarean birth, newborn's birth weight, Apgar scores at 1 and 5 min, cord blood pH, or the ratio of neonatal intensive care unit admission. Weight, height, and BMI measurements varied because the research groups were created based on the level of obesity.

In Figure 1, the omental tissue homogenate gasdermin-D and pannexin-1 levels of pregnant women

Table 1. Baseline clinical characteristic of study population

	Normal weight (n=25)	Overweight (n=28)	Obese (n=26)	p-value
Age (years)	26 (20-35)	31 (20-35)	28.5 (21-35)	0.163
Blood pressure (mmHg) Systolic Diastolic	 110 (90-150) 60 (60-80)	 110 (90-120) 65 (60-80)	 110 (100-140) 70 (60-80)	 0.51 0.661
Smoking, n (%) Yes No	 2 (8%) 23 (92%)	 0 (0%) 28 (100%)	 1 (3.8%) 25 (96.2)	 0.314
Weight (kg)	62 (48-74)	75 (66-89)	89 (74-123)	0.001
Height (cm)	160 (150-175)	162 (154-177)	158 (150-170)	0.015
Body mass index (kg/m²)	24.09 (20.5-24.8)	28.35 (26.56-29.53)	33.9 (30.9-43.07)	0.001
Comorbidities, n (%) Gestatitonal diabetes Gestatitonal hypertension Kidney diseases Asthma Hypothyroidism	 1 (4%) 1 (4%) 0 (0%) 1 (4%) 5 (20%)	 4 (60.7%) 1 (3.6) 0 (0%) 2 (7.1%) 4 (14.3%)	 3 (11.5%) 0 (0%) 1 (3.8%) 2 (7.7%) 1 (3.8%)	 0.645
Economic status, n (%) Low Low-medium Medium Medium-high High	 4 (16%) 11 (44%) 9 (36%) 1 (4%) 0 (0%)	 6 (21.4%) 13 (46.4%) 8 (18.6%) 1 (3.6%) 0 (0%)	 5 (19.2%) 16 (61.5%) 5 (19.2%) 0 (0%) 0 (0%)	 0.755
Gestational age (weeks)	38 (37-41)	39 (37-40)	39 (37-41)	0.419
Gravidity	3 (1-8)	3 (1-7)	3.5 (2-7)	0.246
Parity	2 (1-5)	3 (1-7)	3 (1-4)	0.408
Number of births Vaginal birth Cesarean	 1 (1-4) 0 (0-3)	 2 (1-4) 0 (0-4)	 2 (1-4) 0 (0-3)	 0.382 0.958
Birth weight (gr)	3240 (2235-4600)	3390 (2375-4410)	3545 (2150-4120)	0.105
Apgar score At 1 min. At 5 min.	 9 (4-9) 9 (5-10)	 9 (5-9) 10 (7-10)	 9 (5-9) 10 (6-10)	 0.997 0.969
Cord blood pH	7.33 (7.09-7.43)	7.36 (7.27-7.42)	7.32 (7.20-7.41)	0.233
NICU admission, n (%) Yes No	 11 (44%) 14 (56%)	 4 (14.3%) 24 (85.7%)	 8 (32%) 17 (68%)	 0.057

The presentation of the data in the table was made as median (minimum-maximum) and number (%). The comparisons of numerical variables were made by the Kruskal-Wallis ANOVA test. The analysis of the nominal data in this table was performed by the chi-square test
NICU: Newborn intensive care unit

with normal weight, overweight, and obesity are shown. The median levels of GSDMD protein in the overweight and obese groups were significantly lower than those in the normal weight groups (p=0.0116 and p=0.0154); however, the median levels of GSDMD in the overweight and obese groups were not comparable. The median levels of PANX1 protein in the overweight and obese groups were also significantly lower than those in the normal weight group (p=0.0101 and p=0.0184); however, no significant difference was found between the overweight and obese groups regarding the median levels of PANX1 protein.

The placental GSDMD and PANX1 levels of pregnant women with normal weight, overweight, and obesity are presented in Figure 2. There were no significant differences among the groups' placental GSDMD levels. In addition, the median levels of placental PANX1 proteins in the normal weight, overweight, and obese groups were similar.

In Figure 3, the serum lipid levels of the normal weight, overweight, and obese groups are shown. There were no significant differences among the groups' median serum levels of high-density lipoprotein, low-density lipoprotein, triglycerides, and cholesterol.

Histological examinations were also performed on placental and omental biopsy samples collected from pregnant women who participated in the study during a cesarean section. During the placental tissue tests, H&E staining revealed that four patients (one of them was normal weight, two of them were overweight, and one of them was obese) had a focal calcification infarction area in their placental tissue, and there were two cases of inflammatory placental tissue (a person was normal weight, a person was obese). All omental biopsy samples were found to be free of any pathological abnormalities.

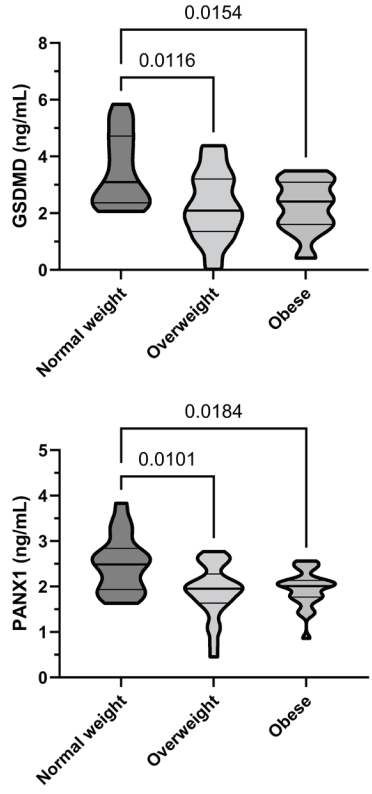

Figure 2. In the omental tissue samples, median levels of GSDMD and PANX1 proteins in the normal weight, overweight, and obese groups. Non-parametric data were presented as a median (dark line) with minimum and maximum values (smallest-largest limits), and comparisons were analyzed using the Kruskal-Wallis test. P-values representing significant differences are expressed on the graphs

GSDMD: Gasdermin-D, PANX1: Pannexin-1

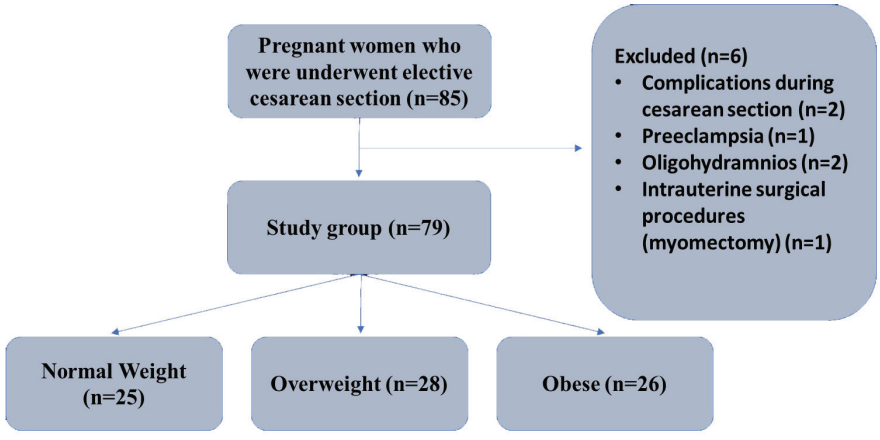

Figure 1. Flow diagram of the study design

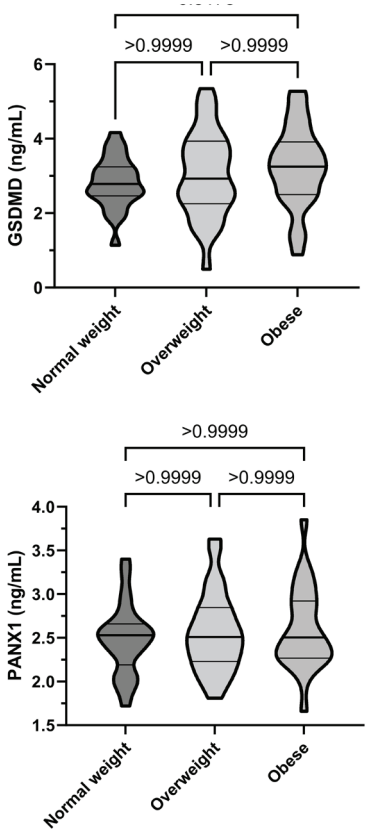

Figure 3. In the placental tissue samples, median levels of GSDMD and PANX1 proteins in the normal weight, overweight and obese groups. Non-parametric data were presented as a median (dark line) with minimum and maximum values (smallest-largest limits), and comparisons were analyzed using the Kruskal-Wallis test
GSDMD: Gasdermin-D, PANX1: Pannexin-1

Discussion

In this study, biochemical and histological changes in omental and placental tissues were observed to investigate the pathogenesis and impact of obesity on pregnancy. GSDMD and PANX1 proteins in omental tissue and the placenta were used to identify inflammation associated with maternal obesity. There was no significant difference in the concentrations of GSDMD and PANX1 in placental tissue among the study groups, although the concentrations of GSDMD and PANX1 in the overweight and obese groups were noticeably lower than those in the normal weight group in the omental tissue. These results would be expected to indicate higher levels of GSDMD and PANX1 in obese and overweight individuals. Obesity modifies metabolic states and may affect the expression of proteins implicated in pathways leading to cell death and signaling. Regarding the involvement of GSDMD and PANX1 proteins in pathways such as inflammation and cell death, their reduced levels may result from metabolic adaptations seen in women with obesity. Additionally, hormone levels are considerably altered during pregnancy, which can impact protein expression. Pregnancy hormones and obesity may interact differently in obese women compared with normal-weight women. The correlation of our findings in humans will be aided by additional clinical and epidemiological studies.

GSDMD and PANX1 proteins are linked to inflammation associated with maternal obesity. The pathogenesis of morbidities affecting mother and baby health caused by obesity during pregnancy is not well known. According to studies, if the stress in the adipose tissue's endoplasmic reticulum increases, obese pregnant women have increased inflammatory processes, which cause obesity and gestational diabetes to form morbidities during pregnancy (13). Studies that examined the pathophysiology of several pregnancy-related morbidities separately included GSDMD and PANX1. The extrinsic pathway of GSDMD is affected by caspase-1 and caspase-8, which also create pores in the cell membrane and result in lytic cell death. The cell membrane glycoprotein structure is triggered by PANX1 with the activation of caspase-3 and caspase-7, creating a channel (7). PANX1 and GSDMD are involved in various body parts. Lower levels of intracellular pyroptosis-related inflammatory factors, such as GSDMD, mitigate hippocampal neuronal injury (14). In a study, it was observed that spontaneous labor at term resulted in greater GSDMD levels in the amniotic fluid than delivery without labor. These findings offer proof that proptosis plays a role in the mechanisms that trigger the sterile inflammatory process of term parturition (12). GSDMD and other proptosis indicators were investigated in placental samples from pregnant women who had preeclampsia and participated in the study. Hypoxia intensifies stress in trophoblasts, considering the multifactorial structure of preeclampsia. Consequently, the GSDMD of the endoplasmic reticulum found in trophoblasts was much higher (15). In this study, according to the results of GSDMD in the homogenate of placental tissue, there was no significant difference in the expression of GSDMD. The underlying reason for this result may be that mothers with any complications, such as GDM and preeclampsia, were not included. On the other hand, when compared with GSDMD expression in the groups, omental adipose tissue was observed to be statistically significant. Although it was not observed beyond the placental tissue, the damage associated with obesity was detectable in the omental tissue. To better comprehend alterations in the placenta due to increased stress, conducting new studies that include pregnant women with complications will be beneficial.

PANX1 is located in many parts of the body; the brain and placenta are some places where it is located. They are found in the cell membrane and share structural similarities with connexins. The results of this study

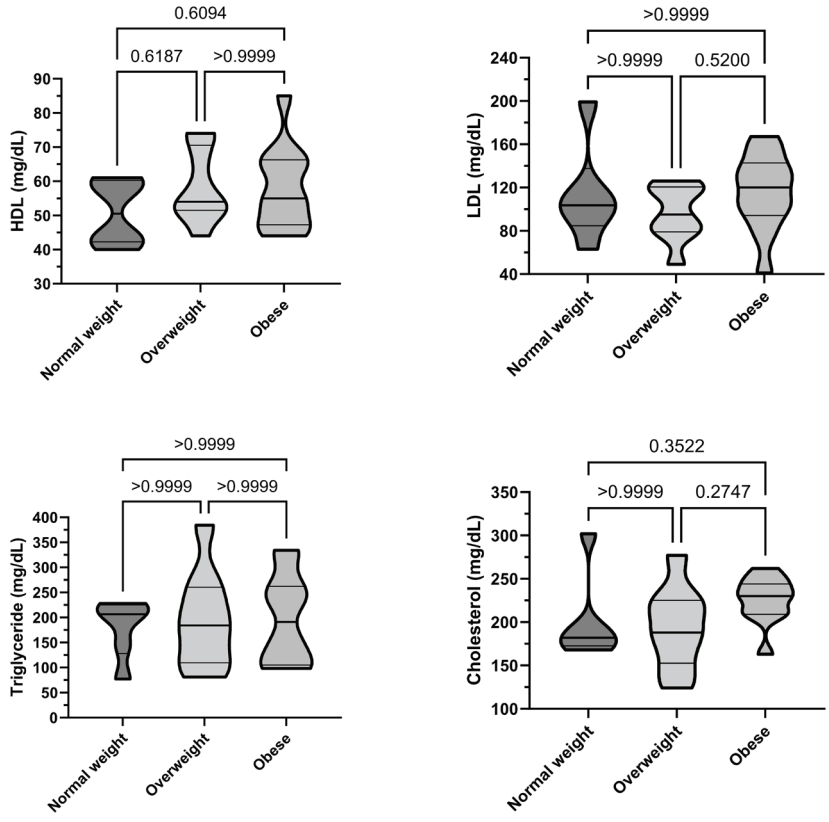

Figure 4. In the serum samples, median levels of HDL, LDL, triglyceride, and cholesterol in the normal weight, overweight and obese groups. Non-parametric data were presented as median (dark line) with minimum and maximum values (smallest-largest limits) and analyzed with the Kruskal-Wallis ANOVA test
HDL: High-density lipoprotein, LDL: Low-density lipoprotein

indicated that PANX1 is continually expressed within various cell types. Moreover, neuroepithelial growth is impeded in the absence of PANX1. This compromise stems from the dysregulation of both cell-cell and cell-matrix adhesion mechanisms, disruption of intracellular signaling pathways, and alterations in gene regulation. These findings highlight the crucial role of PANX1 in the coordination of important processes that regulate early brain development (16). According to some research, neurodevelopmental impairment in children caused by infection or vitamin D deficiency in mothers is linked to an increase in PANX1 suppression in infants (17). Another study was conducted to better understand the role of the PANX-1 protein in ensuring cell migration and purinergic communication. It has been claimed that PANX1 acts as a positive or negative regulator in various inflammatory events (18). It has been acknowledged that the insulin-pannexin-1-purinergic signal was activated with each other in adipocyte cell culture and that this path caused adipose tissue inflammation and type 2 diabetes (19). P2YX7 receptor and PANX1 synthesis in beta cells were shown to be affected by increased glucose levels in the environment in mice and rats. Increased purinergic signals regulate cell function and cell life (20). In this study, PANX1 and Toll-Like Receptor 4 were examined because they correspond to the inflammatory mechanisms involved in preeclampsia pathogenesis. PANX1 channels play a role in inhibiting ferroptosis, which is the reversal of cell death caused by reactive oxygen species produced by NADPH oxidase. Serum Panx1 has a high diagnostic power for ferroptosis in preeclampsia and correlates well with its placental expression (21). The PANX1 glycoprotein is crucial to cell type demise. Human placental cell culture was used to study the death mechanism of syncytiotrophoblasts, and it was discovered that the PANX1 glycoprotein was crucial to the cell type's death (22). There was not a significant difference in PANX1 protein expression within the groups in placental tissue homogenates, according to our data. This could be because the study groups did not include patients with preeclampsia, gestational diabetes, or other diseases. Past studies have revealed that PANX1 channels regulate the absorption of glucose by adipocytes

in response to insulin. Humans with obesity have higher levels of Panx1 expression in adipose tissue (23). An additional investigation revealed interactions within the purinergic and pannexin-1 signaling pathways in adipose tissue, and it was hypothesized that the dysregulation of this signaling may be a factor in type 2 diabetes and inflammation of the adipose tissue (19). The study results regarding the expression of PANX1 protein among the groups in omental tissue were consistent with prior studies; though there was a statistically significant difference.

Study Limitations

The results are more reliable because a prospective observational cohort study design was used. Comparative analysis was made possible by including a control group (normal weight) alongside the overweight and obese groups. Moreover, this study examines an essential relationship between pregnancy outcomes, inflammation, and maternal obesity. The emphasis on the inflammatory processes linked to GSDMD and PANX1 provides new insights into the pathophysiology of complications related to obesity during pregnancy. However, inflammatory processes involve numerous pathways and substances. One of the study's limitations is that additional inflammatory parameters like IL-6 and C-reactive protein were not evaluated. Additionally, due to a lack of adequate funding, immunohistochemical staining could not be performed, and research proteins were only biochemically evaluated by ELISA. Although we conducted the study in a single location, the generalizability of these findings may be limited. Larger, multicenter studies are necessary to generalize the results. Pregnant women with comorbidities such as GDM or preeclampsia were excluded from the study, which may restrict the ability to comprehend the distinctions in GSDMD and PANX1 expressions in these comorbidities.

Conclusion

We observed that maternal obesity during pregnancy increased proptosis, which may impact the health of the mother and infant. The results of this research contributed to the relevant literature in basic science. For both the mother and the newborn, obesity is a major contributor to the development of many problems and comorbidities during pregnancy. GDMD and PANX1 have the potential to be used as predictive and diagnostic test panels after further studies in obese pregnant women.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Institutional Ethics Committee of the University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital (dated: 24.11.2021, approval no.: 116-2021).

Informed Consent: Each woman was asked for written consent after being fully informed of the nature and intended use of the procedures.

Authorship Contributions

Concept: F.K., B.A.S., B.S.A., M.T.O., F.Y.G., A.C., Design: F.K., B.A.S., B.S.A., M.T.O., F.Y.G., A.C., Data Collection or Processing: F.K., B.A.S., B.S.A., M.T.O., F.Y.G., A.C., Analysis or Interpretation: F.K., B.A.S., B.S.A., M.T.O., F.Y.G., A.C., Literature Search: F.K., B.A.S., B.S.A., M.T.O., F.Y.G., A.C., Writing: F.K., B.A.S., B.S.A., M.T.O., F.Y.G., A.C.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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Epilepsy and Quality of Life in the Shadow of Stigmatization

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Abstract

Aim: Because of its chronic nature, epilepsy is a complex disease with psychosocial effects. This study aimed to investigate the stigma and quality of life of individuals with epilepsy.

Methods: This study was conducted between January 2022 and September 2022 in the epilepsy outpatient clinic of a university hospital located on the Anatolian side of Istanbul. In this descriptive and cross-sectional study, participants completed the Stigma Scale for Epilepsy (SSE), the Quality of Life in Epilepsy Inventory-10 (QOLIE-10), and a sociodemographic data form.

Results: The study included 130 patients (83 women and 47 men) with epilepsy. 56.2% of the participants had a medium stigmatization scores and 14.6% had a high stigmatization scores. It was determined that stigmatization was associated with age, education level, employment, and income. Total score of (SSE); participants aged 51 and over, primary school graduates, never worked, and receiving minimum wage were higher. The total SSE score did not differ according to gender, marital status, people with whom they lived together, date of diagnosis, duration of treatment, or date of the last seizure. Participants who had a seizure in the previous year without income had a lower quality-of-life score. It was determined that the QOLIE-10 scores did not differ in terms of gender, age, marital status, employment, education level, people they lived with, date of diagnosis, duration of treatment, and type of epilepsy.

Conclusion: The participants with high levels of stigma experienced a significant reduction in quality of life compared with the other groups. Therefore, the potential need for increased support and social rehabilitation for individuals affected by stigmatization should not ignored.

Keywords: Epilepsy, stigma, quality of life, cross-sectional study

Introduction

Throughout history, the interpretation of epilepsy in different cultures has varied. In some cultures, epilepsy was associated with evil spirits (djinns, demons, etc.) and paranormal powers, and non-scientifically valid methods (magic, witchcraft, etc.) were used in its treatment (1,2). Even though these negative attitudes are less prevalent today, individuals diagnosed with epilepsy still face stigma (3). Patients' social, educational, and professional lives have declined due to stigmatization, which has also negatively impacted their quality of life (4,5).

Numerous factors adversely affect the quality of life of individuals diagnosed with epilepsy. The physiological effects and chronic nature of epilepsy, along with regular hospital visits, legal restrictions (like not obtaining a driver's license or exemption from military service), protective family attitudes, negative cultural attitudes towards the disease, and stigma, all contribute to a decrease in quality of life, according to reports (6-8).

The concepts of quality of life and stigma can vary over time due to societal dynamics (9). Hence, it is important to assess the levels of stigma and quality of life of individuals diagnosed with epilepsy. This study was conducted with the purpose of investigating the relationship between stigma and quality of life in individuals with epilepsy.

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Methods

Complicance with Ethical Standards

For the conduct of this research, ethical approval was obtained from the Maltepe University Ethics Committee (approval no.: 2020/16-03, dated: 11.12.2020), and permission to conduct the study was also granted by the relevant institution (permission no.: 2022/02, dated: 25.01.2022). Written informed consent was obtained from all patients participating in the study. This study was conducted in accordance with the Helsinki Declaration. This article was derived from the master's thesis titled "Stigma and Quality of Life in Individuals with Epilepsy".

Study Design

This cross-sectional study was conducted between February and September 2022. Participants were 18 years of age or older, had primary school graduates as their lowest level of education, did not have any perception or communication problems, and visited the epilepsy clinic for routine appointments (Figure 1). Data were collected through face-to-face interviews in a suitable and quiet room within the clinic where participants and the researcher were present. The data collection process for each participant took approximately 15 minutes.

Data Collection Tools

The study employed the Stigma Scale for Epilepsy (SSE), developed by Baybaş et al. (11), to assess stigma levels in epileptic individuals in the Turkish population (10). The scale comprises 32 questions with a 4-point Likert-type answering system, with a minimum score of 25 and a maximum score of 100, with a cut-off value set at 50. Epileptic individuals scoring between 51 and 75 were considered moderately stigmatized, whereas those scoring between 76 and 100 were considered highly stigmatized (11). The Quality of Life in Epilepsy Scale-10 (QOLIE-10), the validity and reliability of which was performed by Mollaoğlu et al. (12), was used to determine the quality of life of Turkish epilepsy patients. The QOLIE-10 gives a total score ranging from 0 to 100, with a lower score indicating better quality of life (12).

Sample Size

The sample size was determined using G-Power 3.1.9.4 software, considering the correlation test (r=-0.324) in a reference study with a two-tailed hypothesis and a Type I error of 0.05. The calculated minimum sample size was determined to be at least 98, with a power of 0.95 (10). The study population consisted of patients who sought treatment at a university hospital's epilepsy outpatient clinic. This study had a sample size of 130 participants.

Statistical Analysis

Statistical analysis was conducted using SPSS 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, New York). The Kolmogorov-Smirnov test was used to assess the normal distribution's suitability. Independent t-tests and ANOVA tests were used to identify significant differences between scale scores and participants' socio-demographic data. In cases of significant differences between groups, the LSD post hoc test was performed. The significance level was set at 0.05.

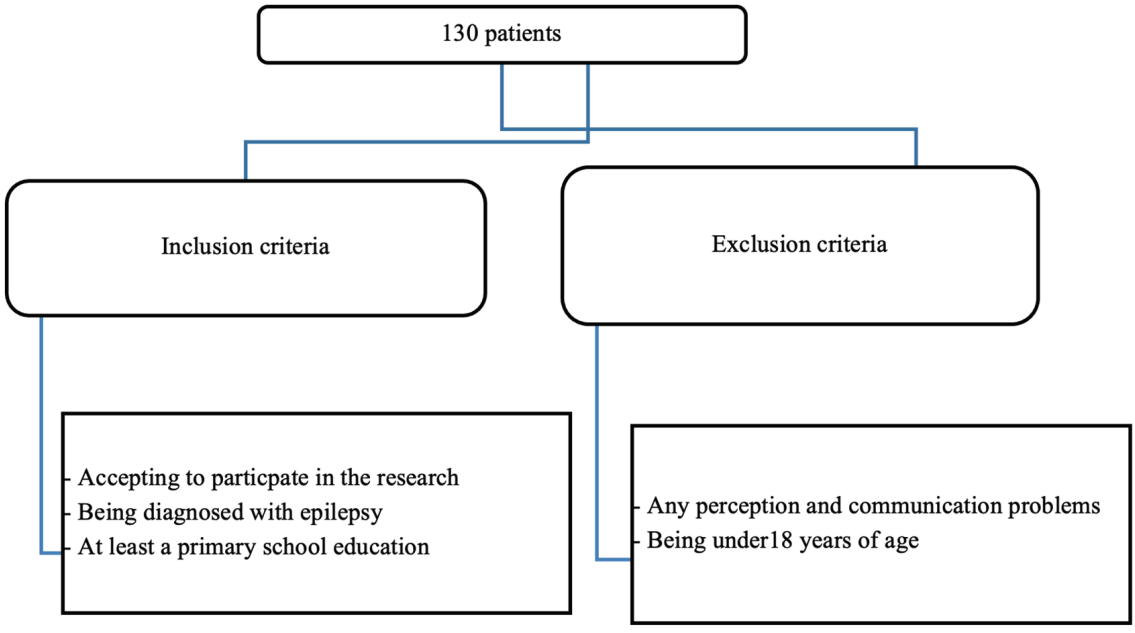

Figure 1. Study design

Results

Of the participants, 43.8% were in the age range of 31-50, and 26.9% had elementary education. 53.8% of the participants had no monthly income, and 38.5% had had a diagnosis of epilepsy for at least 16 years. Furthermore, 50.8% of the participants had experienced seizures in the past year, and 63.1% had focal seizures.

When the participants' SSE and QOLIE-10 scores were compared in terms of sociodemographic variables, a statistically significant difference was found between the SSE total score and age, employment status, educational level, and income. Participants "aged 51 and above" (p=0.015), those with an "elementary level" education (p<0.001), those who responded "no" (p=0.048) in terms of current employment status, and those with a "minimum wage" income (p=0.001) were statistically significant when compared to other groups (Table 1).

A statistically significant difference was found between the QOLIE-10 Total Score and monthly income level, as well as the date of the last seizure. In comparison with the other groups, those without income (p=0.015) and those who experienced seizures in the past year (p=0.001) were found to have higher scores (Table 2).

A statistically significant difference was found between the QOLIE-10 total score and stigma levels. The QOLIE-10 total score was found to be significantly higher in those with higher stigma levels compared to other groups (p<0.001). Regarding the relationship between quality of life and stigma levels in individuals diagnosed with epilepsy, it was determined that there is a positive correlation between the quality of life and stigma levels (r=0.454, p<0.001). However, since high QOLIE-10 scores indicate low quality of life, the relationship was indirectly found to be negative (13,14) (Table 3).

Discussion

In our study, we found that the stigma levels were higher in participants who were 51 years of age or older, had primary education, had never worked, and received the minimum wage. Sabatello et al. (13) reported higher stigma levels in participants aged 60 years and older and those not working. However, unlike our study, the researchers reported that stigma scores did not vary according to educational level (13). Contrary to Sabatello et al. (13), another study found similar results in the relationship between education and stigma level (14). Similar to our study, previous studies have reported that patients who are not working or have a low income level experience more stigma (15,16). In our study, the higher stigma experienced by participants aged 51 may be attributed to the fact that younger individuals can access information about epilepsy more easily and faster through online channels. It is also believed that some participants aged 51 years and older may have limited technological literacy, which may contribute to this trend.

In our study, participants who had no income and had a seizure within the last year were found to have a lower quality of life. In line with the current results, previous studies have consistently shown that patients with ongoing seizures and low income levels have a worse quality of life (17-20). Poor quality of life among participants with

Table 1. Distribution of participants' mean SSE and QOLIE-10 total scores according to socio-demographic characteristics (n=130)

Characteristics	n	QOLIE-10 $\overline{X}$ ± SD	SSE $\overline{X}$ ± SD
Gender			
Female	83	26.10±7.80	60.65±14.31
Male	47	24.94±8.99	60.53±16.61
t		0.770	0.043
p		0.442	0.966
Age			
1) Aged 18-30	50	26.62±7.92	57.96±14.57
2) Aged 31-50	57	24.95±8.11	59.68±14.69
3) Aged 51≤	23	25.43±9.33	68.65±15.25
F		0.557	4.343
		0.574	**0.015**
Post-hoc tests		-	**3-1, 2**
Employment Status			
Yes	93	25.51±8.56	58.96±14.83
No	37	26.11±7.47	64.76±15.23
t		-0.375	1.996
p		0.708	**0.048**
Level of Income			
1) No income	70	27.20±8.50	63.30±15.74
2) Minimum wage	26	26.04±8.32	63.96±14.99
3) Above minimum wage	34	22.26±6.68	52.50±10.64
F		4.354	7.286
p		0.015	**0.001**
Post-hoc tests		1>3	**1>3** **2>3**
Educational Level			
1) Elementary	35	27.51±9.09	70.74±15.39
2) Secondary	14	22.00±7.82	60.71±10.91
3) High school	45	25.69±8.22	59.02±13.61
4) Undergraduate and higher	36	25.31±7.31	52.69±12.83
F		1.555	10.647
p		0.204	**<0.001**
Post-hoc tests			1>2, 3, 4 3>4

t: Independent t-test, F: Anova test, post-hoc: LSD test
QOLIE-10: Quality of Life in Epilepsy Inventory-10, SSE: Stigma Scale for Epilepsy, SD: Standard deviation

frequent seizures may be attributable to feelings of inadequacy and exposure to social discrimination. In our study, it is noteworthy that patients with lower income levels experienced more stigmatization and a lower quality of life. It has been reported that low income has a strong relationship with stigmatization and quality of life for epilepsy patients (19).

Table 2. Distribution of participants' mean QOLIE-10 and SSE total scores according to disease-related characteristics (n=130)

Characteristics	n	QOLIE-10 $\overline{X}$ ± SD	SSE $\overline{X}$ ± SD
Duration of Epilepsy			
1) 0-5 yrs	19	24.95±8.30	61.79±17.61
2) 6-10 yrs	31	24.48±7.54	57.03±12.88
3) 11-15 yrs	30	26.47±7.97	61.73±13.90
4) 16 yrs ≤	50	26.22±8.90	61.70±16.16
F		0.425	0.755
p		0.735	0.522
Date of Last Seizure			
1) No seizures for 1-3 yrs	35	24.66±8.42	57.54±12.93
2) No seizures for 4-6 yrs	8	19.88±8.89	57.75±12.95
3) No seizures for 6 yrs 6≤	21	21.48±6.97	56.81±12.39
4) Seizures in the past year	66	28.26±7.55	63.79±16.73
F		6.137	2.038
p		**0.001**	0.112
Post hoc tests		**4>1, 2, 3**	-
Seizure Type			
Focal	82	26.13±8.71	62.18±15.34
Generalized	48	24.90±7.38	57.92±14.49
t		0.826	1.561
p		0.410	0.121

t: Independent t-test, F: Anova test, Post-hoc: LSD test
QOLIE-10: Quality of Life in Epilepsy Inventory-10, SSE: Stigma Scale for Epilepsy, SD: Standard deviation, yrs: Years

Table 3. Distribution of QOLIE-10 total and subscale mean scores according to the stigma levels of participants (n=130)

Characteristics	n	QOLIE-10 $\overline{X}$ ± SD
Levels of Stigma		
No Stigma	38	21.87±5.96
Moderate	73	25.93±8.21
High	19	32.32±8.14
F		12.023
p		**<0.001**
Post-hoc tests		**1>2, 3** **2>3**

F: Anova test, Post-hoc: LSD test
QOLIE-10: Quality of Life in Epilepsy Inventory-10, SD: Standard deviation

Participants with high levels of stigmatization were found to have a worse quality of life than those with low and moderate levels. Similarly, a study by Zhang et al. (21) found stigmatization to be negatively associated with quality of life, which is consistent with the findings of our study. In studies on quality of life and stigma associated with epilepsy, it is commonly observed that individuals diagnosed with epilepsy frequently experience stigmatization and tend to have a lower quality of life (16,22,23). This finding suggests that this finding arises from the difficulty that epilepsy patients experience in meeting their basic and social needs.

Study Limitations

The sample used in this study was obtained from a specific hospital. This could limit the results' generalizability. In addition, experimental studies are required to clearly reveal causal relationships. Despite these limitations, the study is important in terms of revealing the effect of stigma on the quality of life of patients with epilepsy. Furthermore, this study demonstrated that psychosocial support for patients with epilepsy should consider stigma-related factors to enhance their quality of life.

Conclusion

Our study showed that participants with high stigmatization levels had lower quality of life scores. Based on this information, it should be taken into consideration that the experience of stigmatization affects individuals' quality of life, and the potential need for more support and social rehabilitation for individuals affected by stigmatization should not be ignored.

Ethics

Ethics Committee Approval: For the conduct of this research, ethical approval was obtained from the Maltepe University Ethics Committee (approval no.: 2020/16-03, dated: 11.12.2020), and permission to conduct the study was also granted by the relevant institution (permission no.: 2022/02, dated: 25.01.2022).

Informed Consent: Written informed consent was obtained from all patients participating in the study.

Authorship Contributions

Surgical and Medical Practices: Z.A., Concept: Z.A., S.A., K.A.Y., Design: Z.A., S.A., K.A.Y., Data Collection or Processing: Z.A., S.A., Analysis or Interpretation: Z.A., S.A., Literature Search: Z.A., S.A., Writing: Z.A., S.A., K.A.Y.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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Evaluation of Serum and Salivary Lipid Peroxidation Levels According to Periodontitis Type

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Abstract

Aim: In this study, we aimed to investigate the role of malondialdehyde (MDA) in the pathophysiology of periodontitis by examining serum and salivary MDA levels in patients with advanced periodontitis and healthy individuals according to the periodontal disease classification revised in the workshop held by the American Association of Chartered Epidemiologists and the European Federation in 2017.

Methods: The study was designed as a cross-sectional study, and a total of 37 patients who applied to Karadeniz Technical University Faculty of Dentistry, Department of Periodontology, in 2022 for periodontal disorders or controls were included. A total of 37 individuals, aged 25-48 years, with stage III grade C periodontitis (Group 1; 13 patients), stage IV grade C periodontitis (Group 2; 12 patients), and a periodontally healthy group (Group 3; 12 individuals) were included in the study. After the demographic characteristics and body mass index (BMI) data were obtained, the clinical periodontal parameters and serum and saliva MDA values of the individuals were measured. All the obtained data were statistically analyzed.

Results: Although BMI was lower and education level was higher in the controls ($p<0.034$), other demographic characteristics did not differ between the groups. When the clinical periodontal parameters were examined, the lowest values were observed in the controls, whereas the highest values were observed in stage IV. The difference between all three groups was statistically significant ($p=0.000$). Although serum MDA levels did not differ between the groups, the highest MDA level was observed in stage III, and the lowest MDA level was observed in the controls. In addition, salivary MDA levels did not differ between the groups, with the highest MDA level observed in stage III and the lowest MDA level observed in stage IV.

Conclusion: The findings of our study showed that systemic and local lipid peroxidation levels increased/decreased in individuals with advanced periodontitis compared with the periodontal healthy group, but this change was not statistically significant. Our findings suggest that different oxidative stress mechanisms may also be involved in advanced periodontitis.

Keywords: Classification, lipid peroxidation, malondialdehyde, periodontitis, saliva, serum

Introduction

Periodontitis is a chronic inflammatory disease that can affect not only oral health but also the general health of individuals and can destroy the periodontal connective tissue and alveoli. Not only does this phenomenon manifest with varying destruction potentials and progression rates in both systemically healthy and sick individuals, but it also affects all age groups in society (1). Periodontal diseases, which are called chronic and aggressive periodontitis according to the periodontal disease classification established by the American Academy of Periodontology (AAP) in 1999, were combined under the name of "periodontitis" with the new classification published in 2017 by the AAP and the European Federation of Periodontology (EFP) (2,3). The 2017 classification of periodontal diseases also developed a staging and grading system, which facilitated the development of individual protocols for diagnosis, treatment planning, and patient response monitoring. The current classification, in addition to having many advantages over previous classifications,

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considers the disease's multifactorial etiology and the risk of disease recurrence or progression, revealing more comprehensive diagnostic and therapeutic approaches.

Thanks to current approaches, a different perspective on the etiopathogenesis of periodontal disease has emerged. Pathogenic organisms in plaque flora now establish the disease onset, and the host response plays a vital role in disease progression and severity. Periodontitis results from complex interactions between pathogenic bacteria and the host's immune response (4). The host's inflammatory and immune responses to bacteria colonizing periodontal and related tissues cover the systemic circulation path and ultimately all body systems (5). This process creates a complex, bidirectional array of host-microbe interactions involving networks of cellular and humoral factors, cytokines, chemokines, and growth factors (6). Although pathogenic organisms are the primary etiological agents, most periodontal tissue destruction is due to host responses to microorganisms and their products. It is known that reactive oxygen species (ROS) also play a role in the etiopathogenesis of periodontitis, in addition to activating the immune system (7).

ROS cause tissue damage via various mechanisms, such as deoxyribonucleic acid (DNA) damage, lipid peroxidation (LPO), protein damage (including hyaluronic acid and proteoglycans), and stimulation of pro-inflammatory cytokines, and they play an important role in the pathogenesis of many inflammatory diseases, including periodontitis (7). The destructive effects of ROS are neutralized by various protective antioxidant defense systems developed by cells. More specifically, a lack of balance between proteolytic enzymes, their inhibitors, ROS, and antioxidant defense systems causes oxidative stress. It is believed that oxidative stress causes cellular and molecular damage, which in turn leads to tissue destruction (7-9). Although the exact cause or result of inflammatory diseases remains unknown, determining oxidative stress is believed to be effective in revealing the pathogenic mechanisms of many diseases (7). Measuring LPO products such as malondialdehyde (MDA) was used to determine the level of oxidative stress (10).

Our hypothesis in this study was that MDA (serum and saliva) may play a role in the pathophysiology of different types of periodontitis, according to the 2017 revised classification.

This study aimed to investigate the role of MDA in the pathophysiology of periodontitis by examining serum and salivary MDA levels in patients with advanced periodontitis and healthy individuals, according to the periodontal disease classification revised in a workshop held by the American Association of Pediatricians and the European Federation in 2017 (3).

Methods

Compliance with Ethical Standards

The Scientific Research Ethics Committee of Karadeniz Technical University Faculty of Medicine approved the study (approval no.: 2022/8, date: 17.02.2022). Before starting the study, all participants were informed in detail about the contents of the study and provided consent.

Study Design

The study was designed as a cross-sectional study, and a total of 37 patients who applied to Karadeniz Technical University Faculty of Medicine, Department of Periodontology, in 2022 for periodontal disorders or controls were included in the study. According to the criteria determined by AAP and the EFP as a result of the joint study conducted at the end of 2017, the patients were divided into 3 groups (Figure 1), and the groups were formed as follows:

Group 1: Stage III, grade C periodontitis (n=13 patients).
Group 2: Stage IV, grade C periodontitis (n=12 patients).
Group 3: Control group (periodontally healthy individuals) (n=12 patients).

Stage III, Grade C Periodontitis

Patients were evaluated clinically and radiographically for stage III periodontitis according to the criteria adopted by AAP and EFP in 2017, and the stage III periodontitis group was formed according to the following criteria:

- Individuals who are systemically healthy and have a body mass index (BMI) <25.
- Patients with interdental clinical attachment loss ≥5 mm, radiographic bone loss extended to or exceeding the middle third, ≤4 tooth loss.

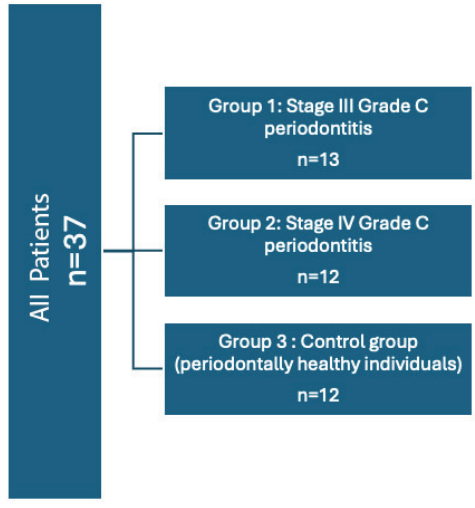

Figure 1. Diagram showing the distribution of groups

- Patients with probing depth ≥6 mm and vertical bone loss ≥3 mm.
- Patients with class 2 and 3 furcation problems and moderate alveolar defects.

Stage IV, Grade C Periodontitis

The patients were evaluated clinically and radiographically for stage IV periodontitis according to the criteria accepted by AAP and EFP in 2017, and the stage IV periodontitis group was formed according to the following criteria:

- Individuals who are systemically healthy and have a BMI <25.
- Patients with interdental clinical attachment loss ≥5 mm, radiographic bone loss extended to or exceeding the middle third, ≤5 tooth loss.
- Patients with probing depth ≥6 mm and vertical bone loss ≥3 mm.
- Patients with class 2 or 3 furcation problems, muscle dysfunction, secondary occlusal trauma, and severe alveolar defects.

Control Group

- Individuals who are systemically healthy and have a BMI <25.
- Individuals who do not have inflammatory features in their gums, with clinical parameters within the normal range, and who receive good oral care.

Exclusion Criteria

Patients with systemic disease, using medications that could affect periodontal health (calcium antagonists, anti-convulsant drugs, immunosuppressive agents, antioxidant drugs), having a BMI >25, smoking habits, and periodontal treatment in the last 6 months were excluded from the study.

Demographic and Clinical Periodontal Parameters

Separate patient follow-up forms were created for all participants, and demographic data such as age, gender, educational status, medical history, BMI, drug use, and smoking were recorded in these forms. The Periodontal Diseases Classification developed by AAP and EFP in 2017 was used for the clinical periodontal evaluation of the participants in the study. Probing pocket depth (PPD), clinical attachment level (CAL), gingival index (GI), probing bleeding index (PBI), and plaque index (PI) were measured to determine the clinical periodontal status of all individuals included in the study. Measurements were made by a single clinician using William's Periodontal Probe (Hu-Friedy, Chicago, IL). Orthopantomography and periapical radiographs of all individuals included in the study were evaluated and used as an aid in grouping patients with stage III and IV periodontal health.

Sampling Processes

Saliva Samples

Saliva samples from the participants were collected in the early morning hours. Participants were instructed not to consume liquids other than water, not to eat, and to come to their morning appointments without daily oral care at least 12 hours before giving the sample. It was confirmed that they followed this before the sample was taken. After the patients were kept with their mouths open, saliva samples were collected without stimulation using a dropper and then transferred to Eppendorf tubes. The collected samples were stored at 80 °C until examination.

Serum Samples

Blood samples from the participants were collected in the same session as saliva samples. To ensure standardization of the samples, all participants were placed in a sitting position. After blood sampling, serum was obtained via centrifugation at 3000 rpm for 10 min. Serum samples were transferred to Eppendorf tubes after centrifugation and stored at 80 °C until examination.

Laboratory Studies

Saliva and serum MDA levels were measured at the Karadeniz Technical University Faculty of Medicine, Department of Medical Biochemistry. The determination of salivary MDA levels is based on measuring the absorbance at 532 nm of the color of the complex formed by MDA and thiobarbituric acid (TBA) in an acidic medium (11). The amount of MDA in serum samples was analyzed using the TBA-reactive substance method developed by Yagi (12). The red formed as a result of the reaction between MDA and TBA was measured using spectrophotometry. Serum lipids and proteins were precipitated using a phosphotungstic acid/sulfuric acid system to remove water-soluble substances that react with TBA and give the same color (12-15).

Statistical Analysis

After collecting data for the study, the Statistical Program for Social Sciences version 20.0 was used for the statistical evaluation of the data. The results of the biochemical analysis of periodontal and laboratory parameters were statistically evaluated. The conformity of the data to a normal distribution was examined using the Shapiro-Wilk test. The comparative measurement of clinical parameters between stage III and stage IV periodontitis and the control group was performed using the Kruskal-Wallis test between all groups and the Mann-Whitney U test between the paired groups. In all these evaluations, $p<0.05$ was considered statistically significant.

Results

Demographic Findings

The participants in our study were aged between 25 and 48. The mean age was 38.1±6.4 years. The patients' ages were 39.7±4.4 in stage III periodontitis, 38.9±7.7 in stage IV periodontitis, and 35.6±6.6 in periodontal health. Of the 37 patients included in our study, 27 (73%) were female and 10 (27%) were male. When the educational status of the individuals included in the study was examined, 24.3% (n=9) were primary school graduates and 43.2% (n=16) were university graduates. The BMI of all 37 individuals (100%) included in our study was <25. The patients' mean BMI was 23.5±1.3. Table 1 presents the demographic characteristics of the patients.

Clinical Findings

Table 2 presents comparisons of the clinical periodontal parameters among all patients and the paired groups. A statistically significant difference was found in the comparison of the clinical periodontal parameters of the patients in all groups and in the comparison between the paired groups (p=0.000).

Laboratory Findings

Table 3 presents the salivary and serum MDA levels of all groups included in our study. No statistically significant difference was observed in the salivary and serum MDA levels between the groups.

Table 1. Comparative analysis of demographic characteristics of individuals participating in the study

	All patients	Stage III (G1)	Stage IV (G2)	Control (G3)	p-value (G1-G2-G3)
Age	38.1±6.4	39.7±4.4	38.9±7.7	35.6±6.6	>0.05
Gender F. M.	 73% 27%	 84.6% 15.4%	 66.7% 33.3%	 66.7% 33.3%	>0.05
Educational status Primary Middle High Associate degree University	 9 (24.3%) 1 (2.7%) 9 (24.3%) 2 (5.4%) 16 (43.2%)	 4 (30.8%) - 5 (38.5%) 1 (7.7%) 3 (23.1%)	 5 (41.7%) 1 (8.3%) 3 (25%) - 3 (25%)	 - - 1 (8.3%) 1 (8.3%) 10 (83.3%)	**0.034**
BMI (kg/m^2)	23.5±1.3	24.1±0.8	23.9±0.9	22.5±1.4^	**0.004**

^Statistical significance was found in the comparative analysis of G3 with G1 and G2 (p<0.05), F.: Female, M.: Male, BMI: Body mass index

Table 2. Intergroup comparison of clinical periodontal parameters

Clinical parameters	Groups	N	X ± SD	Median	Chi-square	p-value[&]
PPD	Stage III	13	3.3±0.53	3.1	32.009	**0.000**
	Stage IV	12	4.5±0.25	4.6		
	Control	12	1.8±0.19	1.8		
CAL	Stage III	13	3.4±0.48	3.3	32.025	**0.000**
	Stage IV	12	4.6±0.22	4.7		
	Control	12	1.9±0.23	1.9		
PBI	Stage III	13	0.92±0.02	0.9	26.636	**0.000**
	Stage IV	12	0.95±0.02	0.9		
	Control	12	0.06±0.03	0.5		
GI	Stage III	13	1.9±0.06	1.9	32.059	**0.000**
	Stage IV	12	2.6±0.21	2.7		
	Control	12	0.06±0.03	0.05		
PI	Stage III	13	1.9±0.15	1.9	32.074	**0.000**
	Stage IV	12	2.6±0.18	2.5		
	Control	12	0.05±0.03	0.05		

[&]Kruskal-Wallis test
PPD: Probing pocket depth, CAL: Clinical attachment level, PBI: Probing bleeding index, GI: Gingival index, PI: Plaque index, SD: Standard deviation

Table 3. Intergroup comparison of saliva and serum malondialdehyde concentrations

Clinical parameters	Groups	N	X ± SD	Median	Chi-square	p-value[&]
Saliva MDA level (nM)	Stage III	13	8.3±1.7	7.4	0.396	0.820
	Stage IV	12	7.8±0.9	7.5		
	Control	12	7.9±0.8	8.1		
Serum MDA level (nM)	Stage III	13	8.9±5.2	6.7	0.753	0.686
	Stage IV	12	7.9±2.0	7.3		
	Control	12	7.1±0.9	7.0		

[&]Kruskal-Wallis test
MDA: Malondialdehyde, SD: Standard deviation

Discussion

The 1999 classification of periodontal diseases has remained valid for approximately 20 years and has been extremely effective in determining diagnosis and treatment planning, supportive periodontal treatment, and, most importantly, establishing a nonsurgical periodontal treatment protocol (16). However, the 1999 classification's inability to distinguish between chronic and aggressive periodontitis in diagnosis and the inapplicability of certain treatment protocols necessitated its revision over time. Also, progress in molecular biology has shown how important host susceptibility is in diagnosis and prognosis, as shown by many immunological studies. Personalized medicine has also been helped by many biomarkers, such as oxidative stress parameters. To sum up, periodontal disease is a multifactorial disease where different immune-inflammatory mechanisms are at work, host sensitivity is a big part of how the disease will progress, and systemic and environmental factors like smoking, stress, and diabetes are big parts of how the disease starts.

The various details mentioned above have revealed the necessity of a more comprehensive classification of periodontal disease. The 2017 classification is a comprehensive classification that distinguishes periodontal health, gingivitis, and suspected periodontitis, establishes the differential diagnosis between periodontitis and decreased periodontitis, improves the staging system to assess the severity and complexity of periodontitis, and improves the grading system to assess the risk profile. According to the current classification, performing different immunological or molecular studies will shed light on the formation of new information in the literature that can help determine diagnosis and treatment planning. Based on this concept, our study aimed to contribute to the existing literature on rapidly progressive forms of periodontitis by measuring LPO, a measure of oxidative stress, in individuals with advanced stage and grade C periodontal disease. This study is the first to examine the systemic and local mechanisms of LPO in individuals with advanced periodontitis, according to the 2017 classification.

In our study, serum samples collected from patients were examined to assess systemic responses, and total unstimulated saliva samples were analyzed to assess local responses. Stimulation of saliva increases the flow of gingival crevicular fluid (GCF) from the periodontal pocket during chewing. As a result, it can increase the number of antioxidant and plasma-related molecules in saliva (10). It has been reported that changes in the salivary flow rate of individuals with periodontitis significantly affect the concentrations of markers in saliva (17-19).

The relationship between oxidative stress and aging has been demonstrated by various studies for many years (20,21). On the other hand, over-reactive aldehyde production and protein oxidation caused by oxidative stress induced by LPO and glycoxidation reactions have been shown to play a role in aging and various age-related chronic diseases (22). Considering the findings of the abovementioned studies and the fact that oxidative stress and LPO increase with age, patients in similar age groups were included in the study to avoid age differences between the groups. In addition, there were no gender differences between the three groups in this study. Since it is known that vascular oxidative stress affects sex and hormones, attention was paid to the fact that the sex ratios between the groups were similar in the study (23). As a result, there was no difference between the three groups in terms of age and sex, and it was appropriate to ensure standardization between the groups and not to be affected by age and sex in the oxidative stress picture. It has been known for many years that obesity causes oxidative stress and plays a role in numerous chronic inflammatory diseases, including cancer, hypertension, diabetes... and periodontitis (24-26). Therefore, the BMIs of individuals were also measured in this study, and it was observed that the values obtained were between 20 and 25 kg/m^2 in all groups.

Many studies have shown an increase in oral health-related quality of life with increasing education levels (27,28). In the literature, these data have been associated

with an increase in the level of education of individuals, the attention shown to personal oral care and awareness of oral health, as well as the regular visits of individuals to their routine dental examinations (29). Owing to the increase in income and education level, there is an increase in the number of dental visits made not only for the complaints of individuals but also for routine checkups (30-32). When the results of our study are evaluated together; stage III and stage IV periodontitis patients, whose socio-economic and socio-cultural levels are lower than the controls, seem to have progressed in the stage of periodontal disease because the importance they attach to oral-dental health is less than that of the healthy group, their oral hygiene practices are inadequate, and they do not go to the dentist regularly. Our findings are consistent with those of the literature.

In our study, clinical periodontal parameters were also measured along with a radiographic examination to diagnose periodontitis in healthy individuals. Probing pocket depth, CAL, GI, PPI, and PI values measured within the scope of clinical periodontal parameters form the basis of periodontal clinical examination. According to the "2017 Current Periodontal Disease Classification", which follows the measurements of clinical periodontal patients, dividing patients diagnosed according to periodontal examination into 3 separate groups as stage III and stage IV periodontitis, periodontal health improves the clinical periodontal effect. Despite observing the lowest values in the control group and the highest values in stage IV, there was a statistically significant difference across all three groups.

Periodontal disease consists of active periods, in which destruction is evident and severe, and silent inactive periods, in which the inflammatory response of the host decreases and destruction slows or even almost stops. The burst hypothesis refers to this process, which persists in the form of active and inactive periods (33). The active phase of periodontal disease begins with the formation of gram-negative dental plaque. In this process, active periodontal destruction occurs with attachment loss, resulting in a periodontal pocket or deepening of the existing periodontal pocket. In this period, gingival bleeding may occur due to probing or spontaneous bleeding, and an increase in inflammatory fluid may be observed in the gingiva. Further biochemical, immunological, genetic, and microbiological studies on samples such as saliva, serum, GCF, gingival tissue, and plaque are the most appropriate diagnostic criteria for determining periodontal disease activity (34). However, in addition to clinically measuring PPD and CAL levels, GI and PBI parameters are also important in determining periodontal disease activity. Furthermore, our findings confirmed the idea that inflammation was present in both groups, but more severe inflammation was observed in parallel with the severity of destruction in the Stage IV group.

LPO is a very harmful mechanism that destroys the structure of cells through chain reactions that cannot be intervened with (7,35,36). Measuring LPO products readily determines tissue destruction due to ROS. MDA is the most studied end-product of polychasia unsaturated fatty acid peroxidation (37). In our serum MDA levels, although there was no statistical difference between the groups with periodontitis and healthy controls, the highest value was in Stage III, and the lowest value was in healthy controls. When our salivary MDA findings were examined, it was observed that the highest value was in Stage III and the lowest value was in Stage IV, although there was no significant difference between the periodontitis and healthy groups. Although the results of the studies examining the serum, salivary, GCF, and gingival tissues MDA levels of patients with periodontitis differ from each other, it has been shown that GCF and salivary MDA levels are significantly higher than those without periodontitis in general, whereas systemic MDA levels do not change or increase compared with controls (10,14,18,38-42). When we evaluate the findings of the abovementioned studies together, even though it is revealed that systemic and local LPO increases or does not change in periodontitis, it is widely believed that LPO, as an oxidative stress marker, may be associated with periodontal inflammation and bone loss and may play an important role in the pathogenesis of periodontal disease.

Study Limitations

When we evaluated our LPO findings under the guidance of the above studies, we observed that systemic and local LPO did not change according to periodontal health in periodontitis and in Stage III periodontitis compared with Stage IV periodontitis. However, the highest LPO value was detected in patients with stage III periodontitis. We recognized that the small number of patients was a major limitation. However, despite this limitation, our results suggest that various oxidative stress markers may play a part in the harmful effects of advanced periodontitis, taking into account the current system for grading and staging the disease. To learn more about how oxidative stress changes in different stages of periodontitis, it will be helpful to measure markers like total antioxidant capacity and oxidative stress index, as well as protein oxidation and DNA damage. Instead, studying enzymatic antioxidants like glutathione peroxidase, superoxide dismutase, and catalase, as well as non-enzymatic antioxidants, may help figure out how oxidative stress affects the development of periodontitis at different stages.

Conclusion

Simultaneously, the observation of the lowest salivary MDA values in stage IV of our study implies that an increase in inflammation may trigger the activation of adaptive mechanisms like antioxidants, which could potentially mitigate oxidative damage resulting from LPO. Further studies examining more individuals and more comprehensive parameters to shed light on this issue will be beneficial in terms of understanding the place of oxidative stress in the pathogenic mechanisms of periodontitis at different stages and developing new treatment approaches.

Ethics

Ethics Committee Approval: The Scientific Research Ethics Committee of Karadeniz Technical University Faculty of Medicine approved the study (approval no.: 2022/8, date: 17.02.2022).

Informed Consent: Before starting the study, all participants were informed in detail about the contents of the study and provided consent.

Authorship Contributions

Surgical and Medical Practices: G.O.D., E.B., Concept: G.O.D., A.A., E.B., Design: G.O.D., E.B., Data Collection or Processing: G.O.D., A.A., Analysis or Interpretation: G.O.D., A.A., E.B., Literature Search: G.O.D., E.B., Writing: G.O.D., E.B.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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Prevalence of Sarcopenic Obesity and Associated Factors in Older Adults

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Abstract

Aim: Sarcopenia is a significant contributor to morbidity and mortality, with unfavorable consequences. The present study investigated the prevalence of sarcopenia associated with obesity, referred to as sarcopenic obesity, and the associated factors.

Methods: This was a cross-sectional study, which was a type of retrospective observational study. The study included patients aged ≥65 years who were treated outpatiently between May 2020 and February 2022. The study population was divided into two groups: the SO group and the SO group alone. In the univariate analyses, we investigated the relationship between age, sex, number of medications, polypharmacy, comorbid conditions, frailty, and undernutrition in the two groups. The parameters found to be related to each group in the univariate analyses were further evaluated using multivariate analyses.

Results: Among the 367 participants, the median age was 71 years (65-97). The proportion of cases with sarcopenic obesity was 15.3% (n=56), whereas the proportion of individuals with sarcopenia but without obesity was 16.6% (n=61). In the multivariate analysis, age (p=0.001) and frailty (p<0.001) were found to be factors associated with SO.

Conclusion: The prevalence of sarcopenia in older adults with obesity is similar to that in those with obesity, although female sex and frailty stand out as factors with a particular association with sarcopenic obesity.

Keywords: Sarcopenia, obesity, elderly

Introduction

The aging process has been shown to lead to alterations in body composition, including increased visceral fat deposition and decreased muscle mass (1-4). In older adults, a decrease in muscle mass is associated with sarcopenia, whereas an increase in visceral fat is associated with a tendency toward obesity (1-4). Sarcopenia has been identified as a significant geriatric health problem associated with adverse consequences, such as falls, frailty, physical disability, institutionalization, and an elevated risk of mortality (5,6).

The European Working Group on Sarcopenia in Older People 2 (EWGSOP 2) was published in an attempt to describe sarcopenia using various definitions and methods (1) and recommends the use of the SARC-F test for sarcopenia screening (1). Recent studies have introduced the novel concept of sarcopenic obesity to the lexicon, referring to sarcopenia combined with obesity (1-4). The rapidly increasing prevalence of SO and its serious consequences have become critical public health problems in the aging population (1-4). Sarcopenia and obesity share numerous pathophysiological mechanisms, and the two conditions can exacerbate each other (1,2).

We aimed to investigate the prevalence of SO and the potential associated factors using screening methods in patients aged 65 years or older who presented to internal medicine outpatients.

Methods

Compliance with Ethical Standards

This study was conducted in accordance with the principles outlined in the revised form of the 2013 Declaration of Helsinki. The Clinical Researches Ethics Committee of the University of Health Sciences Turkey,

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Kartal Dr. Lutfi Kirdar City Hospital approved the study (approval no.: 2022/514/224/28, date: 27.04.2022). Written informed consent was obtained from all participants.

Participants

The data of patients aged 65 years or older who presented to the internal medicine outpatient department for routine control visits between May 30, 2020, and February 1, 2022 were retrospectively reviewed. Patients who had records that showed how many other conditions were found during exams, how many medications they were taking, their height and weight, and whether they had any geriatric syndromes (like sarcopenia, frailty, and malnutrition), were included in the study. Those who declined to undergo screening tests during routine control visits and those who were unable to respond to the questions due to cognitive problems were excluded from the study (Figure 1).

Data Collection

The number of comorbid conditions, number of medications, and height and weight measurements of the participants were recorded during the examinations. Polypharmacy was defined as the consumption of four or more medications (7), and obesity was defined as a body mass index (BMI) of 30 kg/m^2 or greater (8). Sarcopenia screening was performed using the SARC-F questionnaire, in which a score of 4 or higher was considered to indicate sarcopenia (4); frailty was identified as a score of 3 or higher on the FRAIL questionnaire (9); malnutrition was screened using the mini nutritional assessment-short form (10), in which a score of 11 or lower was considered indicative of undernutrition; and sarcopenic obesity was identified as a BMI of 30 kg/m^2 or greater, combined with a SARC-F score of 4 or higher (4,8), whereas a BMI lower than 30 kg/m^2 and a SARC-F score of 4 or greater was identified as sarcopenia without obesity (4).

Statistical Analysis

IBM SPSS Statistics software was used for the statistical analysis of the study data. Study data were expressed in a suitable statistical format depending on their fitness to a normal distribution. The data distribution was determined using the Kolmogorov-Smirnov test. For paired comparisons, a chi-square test was employed for the analysis of nominal data, while a Mann-Whitney U test was used for variables without a normal distribution. In the paired analyses, multicollinearity was assessed among variables that exhibited a significant association with SO, and factors without multicollinearity that demonstrated a significant association were further

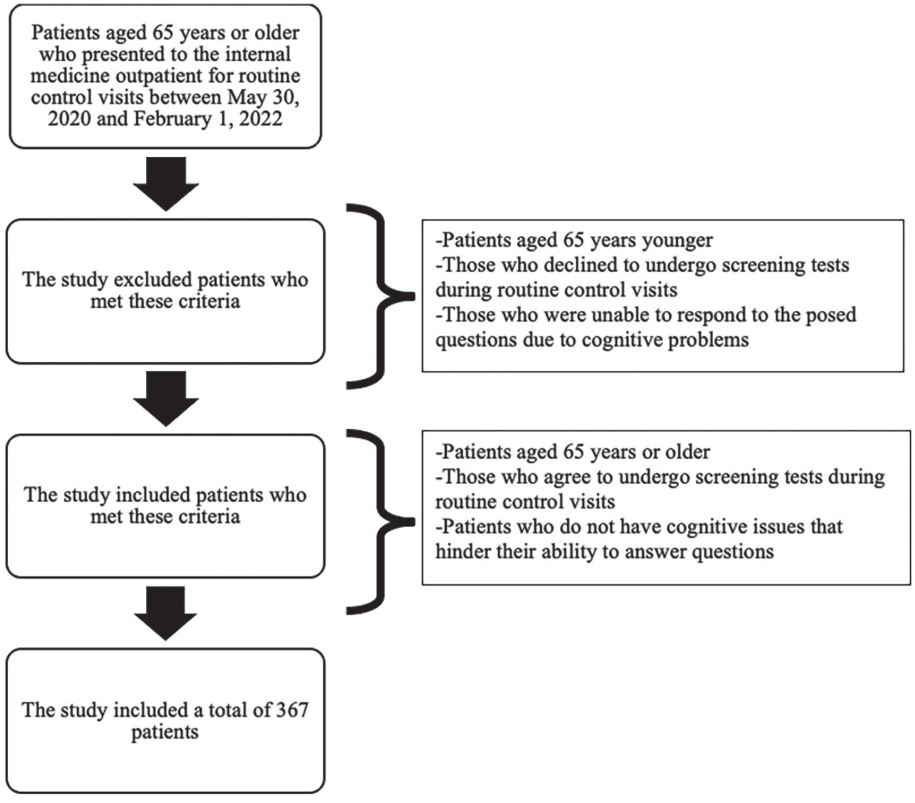

Figure 1. Flowchart of patient selection

examined using regression analysis. Factors demonstrating a significant association are expressed using odds ratios with corresponding confidence intervals. The statistically significant value was determined as p<0.05.

Results

Included in the study were 367 patients, of whom 212 (57.8%) were women, and the median age was 71 years (65-97 years). Of the participants, 200 (54.5%) had diabetes and 160 (43.6%) had polypharmacy. The prevalence rates of geriatric syndromes such as obesity, sarcopenia, frailty, and undernutrition were 41.4% (n=152), 31.9% (n=117), 29.4% (n=108), and 42.5% (n=156), respectively. In the study population, 15.3% had both obesity and sarcopenia, 16.6% had sarcopenia without obesity, 26.2% had obesity without sarcopenia, and 42% were neither obese nor sarcopenic (Table 1).

In the paired analyses, age, sex, number of medications, polypharmacy, number of comorbid conditions, frailty, and undernutrition were found as factors associated with sarcopenic obesity in older adults (p-values in respective order: p=0.037, p<0.001, p=0.003, p=0.012, p=0.009, p≤0.001, p=0.035), while age, polypharmacy, frailty, and undernutrition were identified as factors associated with sarcopenia without obesity (p-values in respective order: p<0.001, p=0.036, p<0.001, p=0.002) (Table 2).

Table 1. Baseline data's of study population

Age*	71 (65-97)
Sex	
Female	212 (57.8%)
Male	155 (42.2%)
Number of medications	4 (0-15)
Polypharmacy	160 (43.6%)
Number of chronic diseases	3 (0-7)
Diabetes mellitus	200 (54.5%)
Presence of obesity	152 (41.4%)
BMI (kg/m^2)*	28.7 (16.8-66.2)
Sarcopenia Screening (SARC-F4)	117 (31.9%)
Frailty (FRAIL scale ≥3)	108 (29.4%)
Undernutrition (MNA-SF ≤11)	156 (42.5%)
Body phenotype	
Obesity and sarcopenia	15.3% (56)
Non-obese and sarcopenic	16.6% (61)
Obese and non-sarcopenic diet	26.2% (96)
Non-obese and nonsarcopenic	42% (152)

Data are presented as mean ± standard deviation or number (percentage) as applicable.
*Data are presented as median.
MNA-SF: Mini nutritional test-short form, BMI: Body mass index

In the multivariate analysis, sex [odds ratio (OR)=0.261, 95% confidence interval (CI) (0.122-0.558), p=0.001] and frailty (OR=4.958, 95% CI (2.541-9.675), p<0.001) were found as factors associated with SO (Table 3), whereas age (OR=1.117, 95% CI (1.061-1.176), p<0.001) and frailty (OR=7.741, 95% CI (3.964-15.119), p<0.001) were found as factors associated with sarcopenia without obesity (Table 4).

The multivariate analysis revealed that frailty was associated with both SO and sarcopenia without obesity, and SO was associated with female sex (Tables 3 and 4).

Discussion

In the present study, a prevalence of sarcopenia of 31.9% was identified in the sample, of which 15.3% of the cases had SO and 16.6% had sarcopenia without obesity. Female sex and frailty were identified as factors associated with SO.

In a cross-sectional study conducted by Öztürk et al. (11) involving a comprehensive geriatric assessment of 423 outpatients aged 65 years or older in the Southeastern region of Turkey, a prevalence of sarcopenia alone (sarcopenia without obesity) of 15% was reported based on the EWGSOP1 criteria (combining decreased muscle mass with decreased performance status), whereas the prevalence of SO was 11% (11). The study by Öztürk et al. (11) did not include an assessment of frailty; however, sarcopenic obesity was found to be associated with lower

Table 2. Related factors with sarcopenic obesity and alone sarcopenia in univariate analyses

	Obesity and sarcopenia (SARC-F4)	p-value	Non-obese sarcopenia (SARC-F 4)	p-value
	56 (15.3%)	N/A	61 (16.6%)	N/A
Age$^{\beta}$	72 (66-90)	0.037$^{\Theta}$	76 (65-92)	<0.001$^{\Theta}$
Sex$^{\beta}$ Female Male	46 (82.1%) 10 (17.9%)	<0.001$^{\Theta}$	34 (55.7%) 27 (44.3%)	0.725
Number of medications	5 (0-14)	0.003$^{\Theta}$	5 (0-15)	0.060
Polypharmacy$^{\beta}$	33 (58.9%)	0.012$^{\Theta}$	34 (55.7%)	0.036$^{\Theta}$
Number of chronic diseases	3 (0-7)	0.009$^{\Theta}$	3 (0-6)	0.098
Presence of diabetes mellitus$^{\beta}$	35 (62.5%)	0.191	33 (54.1%)	0.949
Frailty (FRAIL scale 3)$^{\beta}$	37 (66.1%)	<0.001$^{\Theta}$	44 (72.1%)	<0.001$^{\Theta}$
Undernutrition (MNA-SF 11)$^{\beta}$	31 (55.4%)	0.035$^{\Theta}$	37 (60.7%)	0.002$^{\Theta}$

Data are presented as mean ± standard deviation or number (percentage) as applicable.
*Data are presented as median, $^{\Theta}$Significant p-value, Mann-Whitney U test, $^{\beta}$Chi-square test
MNA-SF: Mini nutritional test-short form

mini-mental state assessment scores, higher geriatric depression scores, and an increased risk of falls, while no association with malnutrition was identified. The study by Öztürk et al. (11) is consistent with the present study in its reporting of the prevalence of SO and the predominance of females in this cohort, although the two studies differ in terms of the assessment of different variables for their association with sarcopenia.

In a prospective observational study of 350 older patients hospitalized in the geriatrics unit, Atmis et al. (12) investigated the association between two-year mortality and both sarcopenia without obesity and sarcopenic obesity, defining sarcopenia based on the EWGSOP 1 criteria (combining decreased muscle mass with either decreased muscle strength or low performance status) and obesity using body fat percentage. They reported a prevalence of 21.1% for sarcopenic obesity and 11.4% for sarcopenia without obesity (6). The prevalence rates reported by Atmis et al. (12) differed from those in the present study, which can be attributed to the different definitions of obesity and sarcopenia adopted, as well as the specific focus on inpatients in their study.

de Lima et al. (13) conducted a cross-sectional study that examined 106 older adults aged 60 years and over regarding malnutrition, frailty, and sarcopenia; they defined frailty by FRAIL, sarcopenia by SARC-F, and obesity by BMI. Their study found that frailty was associated with sarcopenia but not obesity. We believe that this difference may be attributable to the different prevalences of frailty in obese older adult patients. Additionally, unlike our results, de Lima et al. (13) found that malnutrition, which they evaluated using the Simplified Nutritional Appetite Questionnaire, was associated with sarcopenia in their study. The results of their study differ from our study's results. This difference is due to the different methods they used to screen for malnutrition and the fact that they did not adjust for important factors such as polypharmacy.

Yang et al. (14) conducted a retrospective cross-sectional study to examine the relationship between SO and frailty in 2372 older adults. In their study, they found a relationship between SO and frailty, similar to the results of our study (14). Similarly, Frisoli et al. (15) found in the results of their cross-sectional study, which included 371 elderly participants aged 60 years and over, that there was a significant relationship between sarcopenic obesity and frailty, similar to the results of our study.

Table 3. Factors related to SO in multivariate analysis[X]

	p-value	OR	95 %CI	
			Lower	Upper
Age	0.297	1.029	0.975	1.087
Sex	0.001[Θ]	0.261	0.122	0.558
Polypharmacy	0.108	1.690	0.891	3.204
Frailty	<0.001[Θ]	4.958	2.541	9.675
Undernutrition	0.710	1.132	0.589	2.174

[X]Regression analysis
[Θ]Significant p-values
CI: Confidence interval, OR: Odds ratio

Table 4. Factors associated with alone sarcopenia in multivariate analysis[X]

	p-value	OR	95% CI	
			Lower	Upper
Age	<0.001[Θ]	1.117	1.061	1.176
Polypharmacy	0.927	1.031	0.539	1.974
Frailty	<0.001[Θ]	7.741	3.964	15.119
Undernutrition	0.132	1.655	0.859	3.187

[X]Regression analysis, [Θ]Significant p-values
CI: Confidence interval, OR: Odds ratio

Study Limitations

In a study of 1,366 outpatients aged 60 years, Ozkok et al. (4) reported a prevalence rate of 7.5% for SO and 2.8% for sarcopenia alone, defining obesity based on body fat percentage and sarcopenia based on handgrip strength, and identified an association between frailty and both SO and sarcopenia (4). The higher prevalence rate reported in the present study can be attributed to the use of the SARC-F questionnaire, which is a fundamental test for screening sarcopenia, although both sarcopenia phenotypes exhibit similar characteristics in terms of frailty. In a recent meta-analysis, the prevalence of sarcopenic obesity was noted to vary significantly due to the use of different definitions, although both studies reported an association between SO and frailty (16).

The present study's strengths include its status as one of the few studies of its kind to date that involved a substantial number of patients, allowing an accurate determination of the prevalence of SO and its associated factors among older outpatients in our catchment area. The retrospective nature of the study prevents the establishment of any cause-and-effect relationship due to the lack of follow-up data on patients.

Conclusion

This study revealed that SO is a prevalent geriatric syndrome among older adult outpatients. We concluded that female sex and frailty contribute to SO.

Ethics

Ethics Committee Approval: The Clinical Researches Ethics Committee of the University of Health Sciences Turkey, Kartal Dr. Lutfi Kirdar City Hospital approved the study (approval no.: 2022/514/224/28, date: 27.04.2022).

Informed Consent: Written informed consent was obtained from all participants.

Authorship Contributions

Surgical and Medical Practices: T.O.V., Concept: M.E.B., Design: M.E.B., Data Collection or Processing: T.O.V., Analysis or Interpretation: M.E.B., Literature Search: M.E.B., T.O.V., Writing: M.E.B.

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Original Article

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The Significance of Hounsfield Unit and Tumor Diameter in the Differentiation of Malignant and Benign Adrenal Masses

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Abstract

Aim: The Hounsfield unit (HU) used in non-contrast computed tomography (CT) imaging can predict adrenal masses. In the literature, a HU measurement of >10 on non-contrast CT has been reported to have a wide range of sensitivity (33-72%) in detecting malignancy in patients with adrenal masses, and the size of malignant masses is >4 cm in approximately 90% of cases. The current study investigated the role of the HU value and tumor diameter measured on preoperative CT imaging in the differentiation of benign and malignant masses.

Methods: Data analysis was conducted on patients undergoing adrenalectomy for adrenal masses at two different tertiary care centers between January 1, 2019 and January 1, 2023. Patients who underwent an adrenalectomy non-contrast CT scans were assessed for HU and tumor size. The patients were divided into two groups according to histopathologically confirmed benign or malignant masses. Statistical analysis, including receiver operating characteristic curve assessment, was performed to evaluate the diagnostic accuracy.

Results: The study included 108 patients, of whom 66.7% (n=72) were female and 33.3% (n=36) were male. The mean age was 51.01±14.01 years. The laparoscopic technique was used in 72 patients, the robotic technique in 17, and the open technique in 19. The mean length of hospital stay was 4 (2-37) days. The mean tumor size was 55 (10-230) mm. The mean operative time was 80 (50-180) minutes. The mean amount of intraoperative blood loss was 40 (20-300) milliliters. The surgical method, tumor diameter, operative time, amount of intraoperative blood loss, and HU value of the mass statistically significantly differed between the groups ($p<0.001$). We found that the cut-off values of HU and tumor diameter for distinguishing malignant masses from benign masses were 30.5 and 72.5 mm, respectively. At a cut-off value of 30.5 or above, HU had a sensitivity of 100% and a specificity of 81.6% in identifying malignant masses, whereas a sensitivity of 100% and a specificity of 88.7% were determined for malignant masses with a tumor size of 72.5 mm or above.

Conclusion: The HU value and tumor diameter were crucial for distinguishing between benign and malignant adrenal masses, enhancing diagnostic accuracy, and informing treatment decisions.

Keywords: Adrenal mass, Hounsdield unit, diameter of tumor

Introduction

Although adrenal masses are not very common in clinical practice, their prevalence increases with age, reaching 10% at the age of 70 years (1,2). On the other hand, benign adrenal masses are common, with a prevalence of 2-9% depending on the population (3). Malignant adrenal masses represent rare cancers, with an annual incidence estimated to vary between 0.5 and 2/1,000,000 cases (4,5). Approximately 10-25% of malignant adrenal masses are diagnosed incidentally (6-8). The major difficulty in diagnosis is identifying whether the lesion is benign or malignant. In the diagnostic

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evaluation of patients with adrenal masses, there are two important issues: the evaluation of hormonal activity and the determination of the possibility of malignancy (9).

Evaluation of hormonal status in adrenal masses is an important step in guiding the decision to excise the mass. Therefore, the measurement of hormones and hormone metabolites in the blood and urine has diagnostic value. In hormone-active cases, treatment is mostly surgery. The presence or suspicion of malignancy is another surgical indication. Many diagnostic imaging methods have been used to evaluate the malignant status of adrenal masses. However, despite the availability of these methods, the negative excision rate is not yet zero. Non-contrast computed tomography (CT) imaging can evaluate the condition based on the lipid content of the adrenal mass, which forms the basis for the evaluation of malignancy. The Hounsfield unit (HU) is a relative quantitative measurement of radio intensity used by radiologists in the interpretation of CT images. The absorption/attenuation coefficient of radiation within a tissue is used to create a grayscale image during CT reconstruction. HU, also known as the CT unit, is calculated based on the linear transformation of the fundamental linear attenuation coefficient of the X-ray beam (10-12). Early studies showed that HU depended on various CT parameters (13). However, the standardization of these parameters is necessary to ensure that HU becomes a reliable diagnostic measurement tool (14). HU is specific for lipid-rich lesions; therefore, it has a high specificity for adenomas (15). Nevertheless, certain benign masses poor in lipids may be misdiagnosed with the use of HU (16). Accurate diagnosis and appropriate treatment are crucial for preventing unnecessary adrenalectomies, which occur in more than 40% of cases. In a previous study, it was suggested that a tumor diameter of 4 cm and HU of 20 on non-contrast CT could have diagnostic value for malignant adrenal masses (17).

This study aimed to investigate the role of tumor diameter and HU values measured on CT in differentiating malignant and benign masses by retrospectively examining the data of patients who underwent adrenalectomy.

Methods

Compliance with Ethical Standards

The study was initiated after receiving approval from the University of Health Sciences Turkey, Antalya Training and Research Hospital, Clinical Research Ethics Committee (approval no.: 16/21, date: November 23, 2023) and administrative approval from our institution. The data of patients who underwent surgery for adrenal masses at two different tertiary care centers from January 1, 2019, to January 1, 2023 were retrospectively screened.

Study Design

Patients who underwent adrenalectomy and had adrenal masses proven to be benign or malignant by histopathology were included in the study. Our study includes data from patients who underwent adrenalectomy at two centers between January 1, 2019 and January 1, 2023. Nevertheless, patients with metastatic masses, those receiving intensive care, and those aged 18 years were excluded from the study. Further excluded from the study were patients with additional malignancies, pregnant and breastfeeding women, and patients with a current or recent (<6 months) history of taking drugs known to alter steroid synthesis or metabolism. All demographic and clinical data, including sex, age, surgical procedure, and postoperative outcomes, were obtained from the institutional database. All patients were screened in terms of 24 h urine metanephrine, normetanephrine, pheochromocytoma, and hypercortisolism (1 mg dexamethasone test and adrenocorticotropic hormone test). Hypertensive patients were additionally screened for excess aldosterone production (aldosterone-to-renin ratio and 24-hour urinary aldosterone). CT findings were re-evaluated by a radiologist. The size of the adrenal gland masses was measured. To determine the HU values, a circular area was placed on the adrenal mass, and the average value was recorded. The longest diameter of the adrenal mass was measured on an image showing the maximum cross-sectional area.

A total of 119 patients were evaluated. Eight patients under 18 years of age and three patients who died in the intensive care unit were excluded from the study. The sample consisted of 108 patients. The patients were divided into two groups: benign and malignant.

Statistical Analysis

The normality of the distribution of the data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Parametric data are expressed as mean ± standard deviation, and non-parametric data as median (minimum-maximum). The independent samples t-test was used to compare parametric data, whereas the Mann-Whitney U test was used to compare non-parametric data. A chi-square or Fisher's exact test was used to analyze categorical data. The optimal cut-off values of the predictive factors were determined using receiver operating characteristic (ROC) curves. In the ROC analysis, the value with the highest sensitivity and specificity was deemed the cut-off value.

Results

Patients' Demographic Characteristics

The mean age of the patients was 51.01±14.01 years. Thirty-six (33.3%) patients were male, and 72 (66.7%) were female. The pathology result was benign

in 98 (90.7%) patients (benign adrenal adenoma in 49.1%, benign pheochromocytoma in 17.6%, benign myelolipoma in 9.3%, and other benign pathologies in 14.7%) and malignant in 10 (9.3%) patients (malignant pheochromocytoma in two and malignant adrenocortical carcinoma in eight). Right adrenalectomy was performed on 54 (50%) patients, open adrenalectomy on 19 (17.6%), laparoscopic adrenalectomy on 72 (66.7%), and robotic adrenalectomy on 17 (15.7%). While 55 (50.9%) patients had no comorbidities, nine (8.3%) patients had Cushing's syndrome, 21 (19.4%) had hypertension, 14 (13%) had diabetes mellitus, and nine (8.4%) had multiple comorbidities (diabetes mellitus, hypertension, and chronic renal failure). The mean length of hospital stay was 4 (2-37) days. The mean tumor size was 55 (10-230) mm. The mean operative time was 80 (50-180) minutes. The mean amount of intraoperative blood loss was 40 (20-300) milliliters. The laboratory data of the patients is shown in Table 1.

Radiological Evaluation

In the non-contrast abdominal CT sections of the cases in which a mass was detected in the adrenal gland, a region of interest (ROI) was placed in the most homogeneous area of the lesion in the gland, and density (HU) measurements were performed by placing a ROI in the middle portion of the spleen (Figures 1, 2). Preoperative CT images were evaluated by a specialist radiologist (HP). Tumor size was defined as the maximum axial plane diameter. Hounsfield units were measured from non-contrast images by placing a single circular ROI over the tumor. The ROI covered the largest possible area of the tumor plane while avoiding necrosis, hemorrhage, and calcifications.

Group Comparisons

When comparing the groups, no statistically significant differences were found in age, sex, tumor side, presence of comorbidities, glucose, potassium, neutrophil count, lymphocyte count, or length of hospital stay ($p>0.05$). Although the neutrophil-to-lymphocyte ratio, which is known to be a link between inflammation and cancer, was higher in the malignant group, the difference was not statistically significant ($p>0.05$). Surgical method, tumor diameter, operative time, amount of intraoperative blood loss, and the HU value of the mass significantly differed between the malignant and benign groups ($p<0.05$) (Table 2).

ROC Analysis of Variables

ROC curve analysis was performed for HU and tumor diameter to differentiate between benign and malignant masses. In this analysis, the area under the curve value of HU was found to be 0.863 [95% confidence interval (CI): 0.796-0.931] ($p<0.001$), and that of tumor diameter was 0.978 (95% CI: 0.950-1) ($p<0.001$) (Figure 3). The cut-off value was determined to be 30.5 mm for mass HU and 72.5 mm for tumor diameter. After dichotomizing the data according to these cut-off values, malignancy was identified in 10 of the 28 patients with a mass density >30.5 HU and in 10 of the 21 patients with a tumor diameter >72.5 mm. Upon combining the two cut-off values, all 10 patients with malignancy were observed to

Table 1. Demographic and laboratory data of the patients

Variables		n=108 (100%)
Age (years)		51.01±14.01
Sex	Male	36 (33.3%)
	Female	72 (66.7%)
Tumor side	Right	54 (50%)
	Left	54 (50%)
Surgery type	Open	19 (17.6%)
	Laparoscopic	72 (66.7%)
	Robotic	17 (15.7%)
Comorbidity	None	55 (50.9%)
	Cushing's syndrome	9 (8.3%)
	HT	21 (19.4%)
	DM	14 (13%)
	DM + HT/CRF	9 (8.4%)
Pathology	Benign	98 (90.7%)
	Malign	10 (9.3%)
Tumor histology	Malignant pheochromocytoma	2 (1.9%)
	Malign adrenocortical carcinoma	8 (7.4%)
	Benign adrenal adenoma	53 (49.1%)
	Benign pheochromocytoma	19 (17.6%)
	Benign myelolipoma	10 (9.3%)
	Others	16 (14.7%)
Hospital stay (days)		4 (2-37)
Tumor diameter (mm)		55 (10-230)
Glucose (mL/dL)		106.5 (69-356)
K+		4.3±0.83
Lymphocyte count		1.9 (0.55-4.77)
Neutrophil count		5.64 (1.3-19.72)
NLR		2.27 (0.66-23.33)
Operative time (min)		80 (50-180)
Blood loss (mL)		40 (20-300)
Mass HU		16.37 (-95.36-85)
Spleen HU		48.99 (20.14-140)

K+: Potassium, DM: Diabetes mellitus, HT: Hypertension, CRF: Chronic renal failure, NLR: Neutrophil-to-lymphocyte ratio, HU: Hounsfield unit

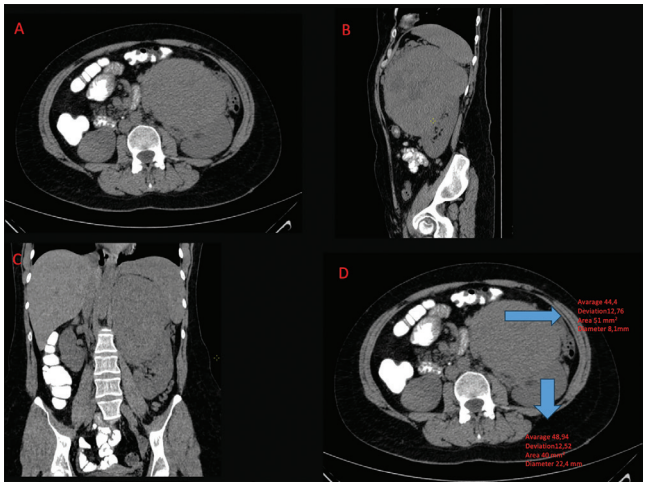

Figure 1. The diagnostic assessment of a malign lesion located in the left adrenal gland is supported by axial (A), sagittal (B), and coronal (C) plane images. Additionally, measurements of Hounsfield units from both the adrenal lesion and the spleen are provided in the axial section (D)

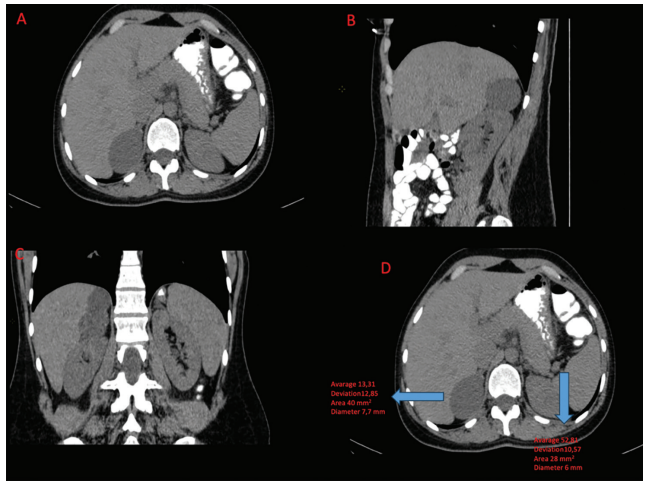

Figure 2. The diagnostic assessment of a benign lesion located in the right adrenal gland is supported by axial (A), sagittal (B), and coronal (C) plane images. Additionally, measurements of Hounsfield units from both the adrenal lesion and the spleen are provided in the axial section (D)

Table 2. Comparison of groups in terms of demographic and laboratory data

Variables		Benign group (n=98)	Malignant group (n=10)	p-value
Age (years)		51.32±13.53	48±18.81	0.479
Sex	Male	35	1	0.160
	Female	63	9	
Tumor side	Right	50	4	0.507
	Left	48	6	
Surgery type	Open	10	9	**<0.001**
	Laparoscopic	71	1	
	Robotic	17	0	
Comorbidity	None	50	5	0.833
	Cushing's syndrome	9	0	
	HT	19	2	
	DM	12	2	
	DM + HT/CRF	8	1	
Tumor diameter (mm)		50 (10-140)	140 (75-230)	**<0.001**
Glucose (mL/dL)		105.5 (69-356)	133.5 (95-326)	**0.040**
K+		4.3±0.56	4.33±0.82	0.902
Lymphocyte count		2 (0.55-4.77)	1.37 (0.92-3.5)	0.092
Neutrophil count		5.64 (1.3-19.72)	5.59 (3.2-10.64)	0.791
NLR		2.59 (0.66-23.33)	3.05 (1.71-9)	0.118
Operative time (min)		80 (50-140)	95 (90-180)	**<0.001**
Blood loss (mL)		40 (20-150)	85 (40-300)	**<0.001**
Hospital stay (days)		4 (2-37)	5.5 (2-15)	**0.049**
Mass HU		15.74 (-95.36-85)	38 (31-60.21)	**<0.001**
Spleen HU		47.72 (20.14-140)	67.5 (37-120)	**0.011**

K+: Potassium, DM: Diabetes mellitus, HT: Hypertension, CRF: Chronic renal failure, NLR: Neutrophil-to-lymphocyte ratio, HU: Hounsfield unit

have a density of >30.5 HU and a diameter of >72.5 mm (Table 3). None of the patients with benign masses had a density >30.5 HU or a tumor diameter >72.5 mm.

Discussion

Imaging methods must be used to distinguish between malignant and benign lesions (18). However, adenomas often contain sufficient intracytoplasmic fat to produce lower attenuation values. Consequently, the density decreases with an increasing lipid mass ratio. Although the lipid ratio is high in benign cases, it is lower in malignant cases. In the literature, no cases of adrenal malignancies have been reported to have a density value of 0 HU. Cases with a density of 4-20 HU should raise strong suspicion (19). The size of the lesion and a history of cancer are important factors in determining whether the mass is benign. While the incidence of carcinoma is 2% in masses smaller than 4 cm, it reaches 6% in masses larger than 4-6 cm and 25% in those larger than 6 cm (20). According to various guidelines and recent publications, a small mass size and high lipid content (<4 cm size and <10 HU attenuation value) are considered markers of a benign lesion. Nevertheless, up to 30% of adrenal masses fail to satisfy well-established criteria for a benign lesion, and new approaches are needed. Recently, image-based texture analysis on CT has been employed to differentiate between benign and malignant tumors by obtaining quantitative parameters that can be useful for measuring the presence of necrosis, hemorrhage, calcification, and intracellular lipid content (20-22). It is considered that tissue analysis of adrenal masses on CT will obviate the need for contrast material injection in these patients, thereby reducing the risk of allergic reactions and negative effects on renal function (23,24).

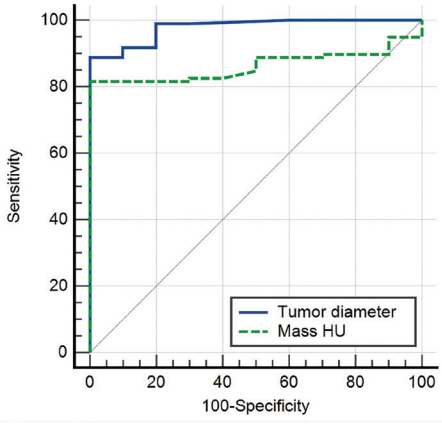

Figure 3. Results of receiver operating characteristic analysis of Hounsfield unit and tumor diameter for the differentiation of benign and malignant masses

HU: Hounsfield unit

The precise and effective characterization of adrenal masses using noninvasive imaging is a crucial element in the algorithm for assessing cancer or malignancy risk (15,25-28).

A German study concluded that a HU threshold greater than 21 provides the highest diagnostic accuracy for identifying adrenocortical carcinoma, with a sensitivity of 96% and a specificity of 80% in statistical tests (29). The radiological features of early, small adrenocortical carcinomas are rarely reported in the literature and can easily be overlooked (30) In the research conducted by Schloetelburg et al. (31), it was observed that more than 20% of benign lesions exhibited dimensions exceeding 4 cm, while over 45% of malignant lesions were measured to be less than 4 cm, including a mere 1.7 cm adrenocortical carcinoma.

In a comprehensive study conducted in Korea, ROC curve analysis was used to distinguish malignant lesions from benign lesions, and the optimal cut-off value of mass size was determined to be 3.4 cm (sensitivity: 100%; specificity: 95.0%), whereas that of pre-contrast HU was

Table 3. Characteristics of the data dichotomized according to cut-off values for malignant and benign masses

Variable			Benign group	Malignant group	
Mass HU	<30.5		80	0	Sensitivity: 100% Specificity: 81.6% PPV: 35.7% NPV: 100%
	≥30.5		18	10	
Tumor diameter	<72.5		87	0	Sensitivity: 100% Specificity: 88.7% PPV: 47.6 NPV: 100%
	≥72.5		11	10	
Mass HU and tumor diameter	<30.5	<72.5	69	0	
		≥72.5	11	0	
	≥30.5	<72.5	18	0	
		≥72.5	0	**10**	

HU: Hounsfield unit, PPV: Positive predictive value, NPV: Negative predictive value

19.9 HU (sensitivity: 100%; specificity: 67.4%). The authors suggested that a diameter of 3.4 cm and a density of 20 HU could be used for benign-malignant differentiation in patients with non-functional adrenal masses, regardless of the change in mass size (32).

Kostiainen et al. (33) found that on non-contrast CT, adrenal malignant tumors were >20 HU and the tumor diameter was 92 (20-196) mm, suggesting that malignancy could not be excluded only based on the small size of the tumor. In another study, Torresan et al. (34) reported the mean mass size to be 62.3 (±35.2) mm for adrenal carcinomas and 56.6 (±42.4) mm for adrenal adenomas, and the mean HU values to be 33.4 and 20.2, respectively. In other studies conducted to differentiate between benign and malignant masses, a cut-off value of 20 HU has been recommended (35,36).

Study Limitations

There were some limitations to this study. First, the retrospective design may introduce selection and information biases, affecting the generalizability of the findings. Second, the sample size is relatively small, which may limit the statistical power and robustness of the conclusions. Third, the study was conducted at only two centers, which may not represent broader clinical practices. In addition, in developing countries, malignant diseases may be delayed due to social reasons and differences in CT scanning protocols, which may affect the comparison of results. Variations in CT imaging protocols and radiological assessments could influence the consistency of HU measurements. Finally, due to the rarity of malignant adrenal masses, there is an inherent imbalance in the number of benign versus malignant cases, potentially skewing the results. Future prospective studies with larger, more diverse cohorts and standardized imaging protocols are necessary to validate these findings.

Conclusion

Parameters such as the HU value and tumor diameter can differentiate benign from malignant adrenal masses, thereby aiding in diagnosis and treatment. However, there is a need for prospective studies with higher patient volumes.

Ethics

Ethics Committee Approval: The study was initiated after receiving approval from the University of Health Sciences Turkey, Antalya Training and Research Hospital, Clinical Research Ethics Committee (approval no.: 16/21, date: November 23, 2023).

Informed Consent: The data of patients who underwent surgery for adrenal masses at two different tertiary care centers from January 1, 2019, to January 1, 2023 were retrospectively screened.

Authorship Contributions

Surgical and Medical Practices: H.O., M.Y., S.Y., Concept: H.O., A.S.A., Design: R.C.C., Data Collection or Processing: T.C.Y., R.C.C., M.Y., Analysis or Interpretation: R.C.C., S.C., M.Y., S.Y., Literature Search: H.O., O.C., A.S.A., Writing: H.O.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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Investigation of 50 g Oral Glucose Challenge Test Efficacy in Pregnant with and Without Risk Factors in Gestational Diabetes Screening

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Abstract

Aim: Who should be screened for gestational diabetes mellitus (GDM) is still a matter of debate. This study was to investigate the results of administering the 50 g oral glucose loading test to pregnant women with risk factors for GDM instead of all pregnant.

Methods: In this retrospective study, pregnant women were divided into two groups according to whether they had at least one of these risk factors. Eighty-four pregnant women had no risk factor for gestational diabetes, while 120 of the 204 pregnant women had at least one risk factor. The 50 and 100 g oral glucose challenge test (OGCT) results were recorded. The prevalence of GDM and predictive values of OGCT were statistically analyzed.

Results: The positive predictive value of 50 g OGCT was 20% for pregnant women without risk factors and 48.39% for pregnant women with at least one risk factor. The difference between these two groups was statistically significant.

Conclusion: The positive predictive value of 50 g of OGCT in pregnant women without risk factors was 20%, whereas that in pregnant women with at least one of the risk factors was 48.39%, a statistically significant difference. According to our study, if a selective population is screened, 2.38% of pregnant women without risk factors will not be diagnosed. Therefore, we emphasize the importance of universal screening.

Keywords: Pregnancy, diabetes, 50 g glucose challenge test, risk factor, efficiency

Introduction

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance at different levels that is first detected during pregnancy and is a common endocorinologic disorder in pregnancy (1,2). The established risk factors for GDM are: being over 35 years, body mass index >27 kg/m^2, history of macrosomic babies in previous pregnancies, poor obstetric history (recurrent pregnancy loss, unexplained intrauterine death, history of pre-eclampsia, birth of babies with unexplained anomalies), ethnicity (more in blacks), history of diabetes in first-degree relatives, and history of GDM in a previous pregnancy (3). Despite more than three decades of research, there is no consensus on the screening and diagnosis of GDM. Sensitivity, specificity, reproducibility, non-physiologicity for pregnant women, and cost-benefit calculation of glucose tolerance tests used for screening have always been a matter of debate, and no consensus has been reached on this issue (4-7). We hypothesized that GDM screening would only be sufficient for pregnant women with risk factors.

The American Diabetes Association (ADA) and the International Association of Diabetes and Pregnancy Working Groups have recommended one-step screening, whereas the American College of Obstetricians and Gynecologists (ACOG) has adopted the Carpenter-Coustan

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screening approach of 2-step screening (6). The one-step test is the 75 g oral glucose tolerance test (OGTT). Another method includes a 2-stage approach consisting of a 50 g oral glucose challenge test (OGCT) and a 100 g OGTT. The one-hour 50 g glucose test is frequently used for screening purposes worldwide, and its success in detecting GDM ranges between 60% and 89% (8). More pregnant women are diagnosed with diabetes with single-stage 75 g OGTT, and the fasting period of pregnant women must be sufficient for this test to be performed. The 50 g OGCT, which can be performed at any patient visit, is often sufficient to terminate screening and prevent unnecessary reuterin of patients. Specialized professional societies generally accept these two methods. In 20% of patients undergoing a one-step test, a three-hour second-step test on an empty stomach is required (9). However, perinatal outcomes were similar between the two tests (8). There are different opinions on whether 50 g OGCT should be performed in all pregnant women or in pregnant women with risk factors for GDM. There are organizations that support screening only pregnant women with risk factors, as well as those that recommend screening all pregnant women (7).

The fact that different guidelines have different diagnostic approaches and threshold values leads to unfavorable results in terms of cost and efficiency worldwide. Different diagnostic approaches also lead to more or fewer diagnoses (10). However, the applicability of these diagnostic methods can be challenging for pregnant women. Although there is generally acceptance of the necessity of GDM screening worldwide, there is uncertainty about how such screening should be performed. Some organizations advocate GDM screening for all pregnant women, and others advocate GDM screening only for pregnant women with risk factors (10). If screening tests are performed in all pregnant women, problems such as a significant increase in cost and patient non-compliance may occur, whereas performing screening tests only in pregnant women with risk factors may cause concerns that some patients may be missed (1,10,11). Although National Institute for Health and Clinical Excellence (NICE), Australasian Diabetes in Pregnancy Society (ADIPS), and International Diabetes Federation support screening of selected populations with risk factors, international organizations such as ADA, WHO, and ACOG are more inclusive and advocate screening tests for the entire pregnant population (12,13).

The aim of this study was to investigate whether it would be sufficient to perform the 2-step test only in pregnant women with risk factors instead of all pregnant women.

Methods

Compliance with Ethical Standards

Informed consent for the use of hospital records was obtained from the patients. Institutional Review Board approval was obtained for this study (approval no.: 2010-88107).

Study Design

The data used in this study were obtained by retrospectively reviewing the records of 204 pregnant women approximately 24-28 weeks of gestation at gestation at Istanbul Sisli Hamidiye Etfal Health Practice and Research Center, Department of Obstetrics and Gynecology between January and April 2010. Patient information was accessed using files and patient records. Patient registration forms were examined, and characteristics such as age, height, and weight, body mass index, number of births, history of gestational diabetes in previous pregnancies, date of last menstrual period, personal and family history, medications used, history of macrosomic babies in previous pregnancies, history of preeclampsia, premature birth, abortion, and anomalous babies were questioned. Fetal weight and amniotic fluid index were also measured. Risk factors for gestational diabetes were included in the patient identification forms as follows (14,15).

1. Obesity (Body mass index >30 kg/m^2).
2. Anamnesis of gestational diabetes in previous pregnancies.
3. Type 2 diabetes in first-degree relatives.
4. History of macrosomic babies in previous pregnancies (>4000 kg).
5. Polyhydramnios in previous pregnancies (amniotic fluid >24 cm).
6. Polyhydramnios in the current pregnancy (amniotic fluid >24 cm).
7. Advanced-age pregnancy (>35).
8. Adverse obstetric anamnesis (preeclampsia, abortion, and preterm birth).
9. Presence of glucosuria.
10. Fetal size in the current pregnancy (>95th percentile).

Pregnant women were divided into two groups according to whether they had at least one of these risk factors. Eighty-four pregnant women had no risk factor for gestational diabetes, while 120 of the 204 pregnant women had at least one risk factor. Patients with type 1 or type 2 diabetes mellitus or carbohydrate intolerance at any level before pregnancy, those with multiple pregnancies, those with diagnosed endocrinopathy, renal and hepatic diseases, pregnant women under the age of 18 years, and those using drugs that may affect insulin secretion or sensitivity were excluded from the study. In addition, values ++ and above (approximately 5.6 mmol/L) were

considered indicative of glycosuria in pregnant women. Pregnant women were included and excluded in our study, and their numbers are presented in Figure 1.

Laboratory records of pregnant women were divided into groups, and screening test results were obtained. The ADA, ACOG, and the Fourth International Gestational Diabetes Study Group recommended a plasma glucose threshold value of 140 mg/dL. In our study, we accepted 140 mg/dL as the threshold value. The laboratory results of patients who underwent 50 and 100 g OGCT and OGTT were recorded.

Statistical Analysis

Statistical evaluation was performed using the SPSS 19.0 package. Categorical data were compared using the chi-square test. Logistic regression was used to analyze the relationship between binary results and the screening method applied according to the categorical variables of the pregnant woman. Positive predictive values of 50 g OGTT were calculated in pregnant women with and without risk factors. Descriptive statistics for numerical variables were presented as positive predictive values. $P<0.05$ was considered significant.

Results

The mean age of pregnant women was 25.5 years. Of the 204 pregnant women included in the study, 84 (41.18%) had none of the risk factors and 120 (58.82% of the cases) had at least one risk factor. Accordingly, obesity and a family history of DM ranked first among the risk factors (Table 1).

Gestational diabetes mellitus was detected in 32 of the 204 pregnant women included in the study, and its prevalence was calculated as 15.69%. The prevalence of GDM in pregnant women without risk factors was calculated to be 2.38%.

While the positive predictive value of 50 g OGTT was 20% in pregnant women without risk factors, it was 48.39% in pregnant women with at least one of the

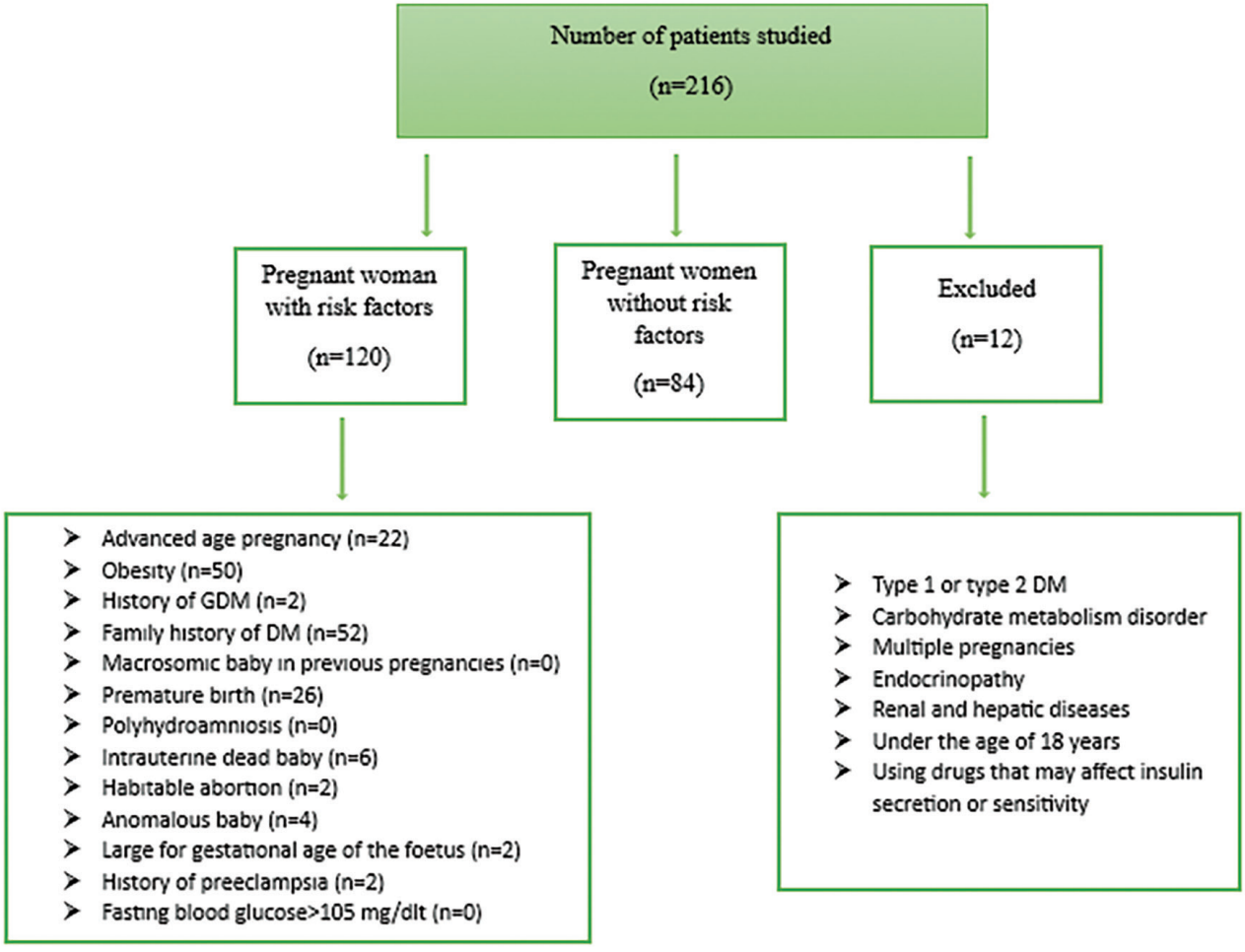

Figure 1. Consort flow diagram of the study

GDM: Gestational diabetes mellitus, DM: Diabetes mellitus

risk factors. The difference between these two groups was statistically significant ($p<0.05$). Although 94% of pregnant women diagnosed with GDM had at least one risk factor, the remaining 6% did not.

Discussion

Screening for GDM has become a routine practice in prenatal follow-up because the problems it causes for mother and fetus can be prevented with appropriate treatment (16). With good glycemic control, malformation and perinatal mortality rates decrease. The aim in pregnant women with diabetes is to maintain the blood glucose profile at the optimum level and thus minimize poor perinatal outcomes. For this purpose, within the framework of the GDM screening program, a 1 h 50 g glucose tolerance test is recommended for all pregnant women between 24 and 28 weeks (17). One of the most controversial issues is whether the 50 g OGTT used in GDM screening should be performed in all pregnant women or in pregnant women with risk factors for developing gestational diabetes. Although screening for gestational diabetes is strongly recommended, there is no consensus among the WHO, ADA, ADIPS, NICE, and ACOG regarding screening recommendations for this disease (18). The ADA advocates the idea that performing this screening test in all pregnant women is unnecessary and costly, and there are studies supporting this (19-22). One such study, by Jiménez-Moleón et al. (23) in 2000, found GDM in 7 of 1138 pregnant women without risk factors.

The ADA does not recommend screening pregnant women with low-risk factors. In addition, it recommends screening pregnant women with high risk factors at the first visit and pregnant women with moderate risk factors at 24-28 weeks of gestation. NICE recommends screening only pregnant women with risk factors (13). In many countries around the world, GDM screening is routinely recommended to be performed between 24 and 28 weeks. In this case, all pregnant women were tested after glucose loading without differentiating between low-risk and high-risk GDM populations. We face many problems, such as negative feedback from patients, non-compliance of patients with the test, difficulties related to national economies such as cost-effectiveness, unnecessary additional tests due to different threshold values related to the tests, and low prevalence of GDM in pregnant women without risk factors. For these reasons, the idea of performing the test only in pregnant women with risk factors has emerged (24).

The risk factors for GDM identified in our review of the literature are as follows; Obesity (body mass index >30 kg/m^2), Anamnesis of gestational diabetes in previous pregnancies, type 2 diabetes in first-degree relatives, History of macrosomic babies in previous pregnancies (>4000 kg), Premature birth (births before 24-37 weeks of gestation), Polyhydramnios in previous pregnancies (amniotic fluid >24 cm), Polyhydramnios in the current pregnancy (amniotic fluid >24 cm), Intrauterine dead

Table 1. Distribution of risk factors in pregnant women

Risk factor	Number of patients	%
Advanced-age pregnancy	22	18.3
Obesity	50	41.7
History of GDM	2	1.7
Family history of diabetes mellitus	52	43.3
Macrosomic baby in previous pregnancies	0	0.0
Premature birth	26	21.7
Polyhydroamniosis	0	0.0
Intrauterine dead baby	6	5.0
Habitable abortion	2	1.7
Anomalous baby	4	3.3
Large for gestational age of the fetus:	2	1.7
History of preeclampsia:	2	1.7
Fasting blood glucose level >105 mg/dlt	14	11.7
Glucosuria	0	0.0

GDM: Gestational diabetes mellitus

Table 2. Diagnostic efficacy of the 50 g oral glucose challenge test (28)

Threshold	Screening	Criteria	Sensitivity	Specificity	LR+ (95% CI)	LR- (95% CI)
≥140	50 g OGCT	CC	85 (76-90)	86 (80-90)	5.9 (4.2-8.3)	0.18 (0.11-0.29)
≥140	50 g OGCT	ADA	88 (76-97)	84 (79-87)	6.0 (5.1-7.0)	0.16 (0.06-0.45)
≥140	50 g OGCT	NDDG	85 (73-92)	83 (78-87)	5.1 (3.9-6.6)	0.18 (0.10-0.34)
≥140	50 g OGCT	CDA	81 (58-95)	69 (59-79)	2.6 (1.8-3.8)	0.27 (0.11-0.67)
≥140	50 g OGCT	WHO	70 (43-85)	89 (73-94)	6.5 (5.1-8.3)	0.33 (0.22-0.52)
≥130	50 g OGCT	CC	99 (95-100)	77 (68-83)	4.2 (3.0-5.9)	0.02 0.003-0.08)
≥130	50 g OGCT	NDDG	88 (67-90)	66 (47-84)	2.7 (1.8-3.9)	0.14 (0.34-0.55)
≥220	-	CC	17 (12-24)	100 (99-100)	-	0.83 (0.78-0.89)

ADA: American Diabetes Association, CC: Carpenter Coustan, CDA: Canadian Diabetes Association, IADPSG: Association of the Diabetes and Pregnancy Study Groups, OGCT: Oral glucose challenge test, WHO: World Health Organization, LR+: Positive-likelihood ratio, LR: Negative-likelihood ratio, NDDG: National Diabetes Data Group, CI: Confidence interval

baby, Anamnesis of three or more abortions, History of an anomalous baby, Advanced age pregnancy (>35), Presence of glucosuria, Fetal size in the current pregnancy (>90 percentile), History of toxemia in previous pregnancies (3,21). The sensitivity, specificity, reproducibility, non-physiological, and cost-benefit calculations of glucose tolerance tests used for screening have always been a matter of debate, and no consensus has been reached on this issue (25).

The generally accepted view is that screening tests are expected to have a low false-negative rate. Typically, high specificity is expected in diagnostic tests. As with other screening tests, the aim of GDM screening is to ensure that false-negative and false-positive test results are close to 0. Although screening strategies with different options and cut-off values have been tried for this purpose, these objectives have not been achieved yet. Therefore, pregnant women who do not have GDM are often misdiagnosed with GDM, and some pregnant women are not diagnosed with GDM (10). The Table 2 shows the diagnostic yields of the 50 g OGCT test from different organizations (26).

In this study, we aimed to investigate whether it is sufficient to perform 50 g OGCTT alone in pregnant women with at least one risk factor for the development of GDM. However, performing OGTT on all pregnant women to detect GDM incurs significant costs. The difficulty in repeatability of the two-stage test, which is the most commonly used test in the world, side effects, such as nausea, vomiting, and feeling of weakness during the procedure, and the stress it causes in pregnant women until the second test, have mobilized researchers for alternative methods. Therefore, selective screening has attracted increasing attention in recent years (27-29). According to these views, if pregnant women with defined risk factors for GDM are screened instead of all pregnant women, screening costs can be significantly reduced, and unnecessary screening can be avoided because of the selective population (30).

In a study by Naylor et al. (29) (Toronto Trihospital Gestational Diabetes Project), 3131 pregnant women were categorized into low, intermediate, and high risk groups according to the presence of defined risk factors (age, pre-pregnancy body mass index, and ethnicity). In this manner, the low-risk group was not screened, whereas all patients in the intermediate- and high-risk groups underwent routine testing (31). Thus, 34.7% of pregnant women were protected from unnecessary testing and stress. It has been demonstrated that the incidence of GDM in pregnant women with no risk factors is 2%. This approach did not result in a significant decrease in the number of pregnant women diagnosed with GDM but protected a statistically significant number of pregnant women from unnecessary OGCT. In this study, we found that the prevalence of GDM among pregnant women without risk factors was 2%. Similar to Naylor et al. (29), when we excluded pregnant women with no risk factors, we found that approximately 41% of pregnant women did not need to undergo screening.

In another study, it was emphasized that 10% of GDM cases were missed as a result of not including the low-risk group in screening (30,31). In our study, we found that approximately 6% of GDM cases that should have been diagnosed would not have been diagnosed if pregnant women without risk factors for GDM were not screened (32,33). In a study by Weeks et al. (34), there was no statistically significant difference in the rates of macrosomia, cesarean delivery, and shoulder dystocia in pregnancy outcomes between groups with and without risk factors. In a study by Benjamin et al. (35) which included 341 pregnant women, the results of 50 and 100 g OGCTT along with fasting blood glucose levels and risk factors were comparatively analyzed. Sensitivity, positive and negative predictive values, positive and negative likelihood ratios, and false positive and false negative values were analyzed. As a result, fasting blood glucose levels together with risk factors were found to be superior to the 50 g glucose tolerance test due to its diagnostic efficacy, easy applicability, and low cost. In this study, we examined the positive predictive values of 50 g OGCT in pregnant women with and without risk factors for GDM and found that the difference was statistically significant. In this study, we found that the positive predictive value of 50 g OGCT increased to 60% in pregnant women with more than one risk factor. Similar to our study, de Sereday et al. (36) reported that a single-stage 100 g or 75 g loading test may be preferred for pregnant women with more than one risk factor. Although the fact that GDM incidence is below 5% in many populations suggests that screening is unnecessary, considering that it increases perinatal risks fourfold, this disease seems to be worth screening. We, like many expert professional organizations and health organizations, think that GDM screening should be performed (18). In our study, 204 pregnant women were admitted to our antenatal clinic, and all of them underwent a 50 g OGTT. Pregnant women with a 50 g OGTT >140 mg/dL underwent a 100 g OGTT. In our study, the prevalence of GDM was 15.69% among pregnant women. The prevalence of GDM in our clinic was found to be higher than that in many studies, which is due to the fact that our clinic is a tertiary health center. In our study, the prevalence of GDM was calculated as 2.38% in pregnant women without risk factors and 25% in pregnant women with at least one risk factor. In our study, as the number of risk factors increased, the prevalence of GDM also increased. In pregnant women with two

risk factors simultaneously, the positive predictive value of 50 g of OGTT increases to 60%, and the prevalence of GDM increases to 37.50%. Glucose loading without a 50 g glucose screening test in pregnant women with more than one risk factor may be a different method that should be investigated. According to the results of our study, the positive predictive value of 50 g OGTT was 20% in pregnant women without risk factors and 48.39% in pregnant women with at least one of the risk factors, and there was a statistically significant difference between them ($p<0.05$). In our study, 120 of the 204 pregnant women had at least one risk factor, and 84 did not have any risk factors. In 62 (51.6%) pregnant women with at least one risk factor, the blood glucose level was >140 mg/dL at the end of the 1st hour, and 30 (25%) of them were diagnosed with GDM. In 10 (11%) pregnant women without risk factors, the blood glucose level was >140 mg/dL at the end of the 1st hour, and 2 of them had GDM (25%). In this case, if only pregnant women with risk factors had been screened, 84 pregnant women would not have been screened, and 6.25% of the pregnant women who should have been diagnosed with GDM would not have been diagnosed. Carpenter and Coustan (37) reported in a selective study in which only pregnant women with risk factors were screened that 50% of pregnant women could not be screened, and in this case, 1/3 of pregnant women could not be diagnosed.

Study Limitations

There are various limitations to this study. This study was conducted retrospectively and has a small sample size. The study was conducted at a solitary center.

Conclusion

According to our study, if only pregnant women with risk factors are screened, 2.38% of pregnant women may not be diagnosed. Although selective screening, as opposed to universal screening, is an alternative for low-resource countries, the exclusion of pregnant women with risk factors is far from being generally accepted. However, although it is a controversial issue to perform GDM screening only in pregnant women with risk factors instead of all pregnant women, it may not be an erroneous approach to offer such an option according to our study.

Ethics

Ethics Committee Approval: Necessary permissions were obtained from the hospital management Institutional Review Board for this study (approval no.: 2010-88107).

Informed Consent: Informed consent for the use of hospital records was obtained from the patients.

Authorship Contributions

Surgical and Medical Practices: E.E., S.O., Concept: E.E., S.O., Design: E.E., S.O., Data Collection or Processing: E.E., Analysis or Interpretation: E.E., Literature Search: E.E., S.O., Writing: E.E.

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Original Article

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Serum FGF-21 Levels During COVID-19 Infection Recovery Period

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Abstract

Aim: Mitochondrial dysfunction causes oxidative stress, which triggers the release of proinflammatory cytokines, which play an important role in the immune response. One of these cytokines, fibroblast growth factor-21 (FGF-21), has demonstrated an increase in its level in severe coronavirus disease-2019 (COVID-19) infection. In this context, this study aimed to investigate whether FGF-21 can be used in the follow-up of COVID-19 infection.

Methods: This study was conducted as a cross-sectional design between January 1, 2022, and December 31, 2022. This study included women and men over 18 years old who had recovered from the COVID-19 infection (n=27). The data regarding hospitalization place (internal medicine ward, internal medicine ward + intensive care unit), comorbidities, vital signs, acute respiratory distress syndrome development, and applied treatments were obtained from hospital records. Fibroblast growth factor-21 levels were specifically studied for this study.

Results: The FGF-21 level was found to be 254 pg/mL at the beginning of the study and increased to 454 pg/mL at the end of the study. The difference was found to be statistically significant (p=0.004).

Conclusion: Considering the increasing level of FGF-21 compared to the beginning of the infection, it is thought that FGF-21 plays a role in the healing process in the COVID-19 infection.

Keywords: Fibroblast growth factor-21, COVID-19, acute respiratory distress syndrome, risk factors, hospitalization, and intensive care unit

Introduction

The fibroblast growth factor (FGF) family comprises polypeptides consisting of five paracrine subfamilies and one endocrine subfamily. Paracrine subfamilies play important roles during embryonic development. The endocrine subfamily members are FGF-19, FGF-21, and FGF-23, which are hormones that help regulate the metabolism of bile acid, lipids, glucose, vitamin D, and minerals. They act by binding to tyrosine kinase receptors (1). One of the FGFs is FGF-21. Fibroblast growth factor-21 levels increase in cases of inflammation such as obesity, metabolic syndrome, and stress. This increase protects the organism from the effects of inflammation and oxidative stress. It is known that FGF-21 levels increase in the early stages of illnesses and are related to healing. Li et al. (2) studied FGF-21's effects on the post-myocardial infarction healing term and the development of fibrosis and reported that FGF-21 levels increased during the healing process.

In addition, FGF-21 increases in cases of acute inflammation, such as bacterial infections. FGF-21 mice were more likely to die because of endotoxemia (3). The observation of more deaths after lipopolysaccharide injection in mice without FGF-21 suggested that FGF-21 protects the organism against sepsis. A possible underlying cause is that FGF-21 inhibits macrophage activation (4). It is thought that a high FGF-21 level creates a protective mechanism against sepsis. However, it was determined that the increase during bacterial infection occurs in the late stage of inflammation (5). Similarly, FGF-21 levels change during viral infection, and it helps to follow-up on the infection (6).

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Coronavirus disease-2019 (COVID-19) is an important viral infection that caused a global pandemic. Because it can be asymptomatic or mild, it can also lead to pneumonia, acute respiratory distress syndrome (ARDS), respiratory failure, multiple organ failure, and even death. The risk of hospitalization and death is higher in individuals with DM, obesity, and cardiovascular disease (7). The inflammatory response during the disease is the underlying cause of this condition. The immune response enters a vicious cycle when a cytokine storm adds to this low-grade inflammation (8).

In COVID-19 infection, RNA and proteins belonging to the virus settle in the mitochondria of the host and disrupt the functioning of the mitochondria, which have an important role in the immune response. Mitochondrial dysfunction leads to oxidative stress, which leads to the release of proinflammatory cytokines, which have an important role in the immune response. Fibroblast growth factor-21, also known as cytokines, is one of these cytokines, and its level has been shown to increase in severe COVID-19 infection (9). In this context, this study aimed to investigate whether FGF-21 can be used in the follow-up of COVID-19 infection.

Materials and Methods

Compliance with Ethical Standards

The ethical approval was obtained from the Tokat Gaziosmanpasa University Faculty of Medicine, Clinical Research Ethics Committee (approval no.: 21-KAEK-165, date: 01.07.2021).

Study Design

This study was conducted as a cross-sectional design between January 1, 2022, and December 31, 2022. Women and men who were over 18 years old and recovered from the COVID-19 infection were included in this study. Pregnant women, lactating women, those younger than 18 years of age, and substance abusers were excluded from the study. Forty hospitalized patients were reviewed, and 32 were included in this study. Five individuals died during the study period, and the process continued in 27 patients (Figure 1). They reported complaints of cough, fever, and lower respiratory tract infection and were diagnosed with COVID-19 pneumonia on the basis of the results of polymerase chain reaction and lung tomography at admission. Anamnesis, demographic data, and routine laboratory results were obtained from the hospital database. Oxygen saturation, blood pressure, fever, and pulse values of the patients were measured daily.

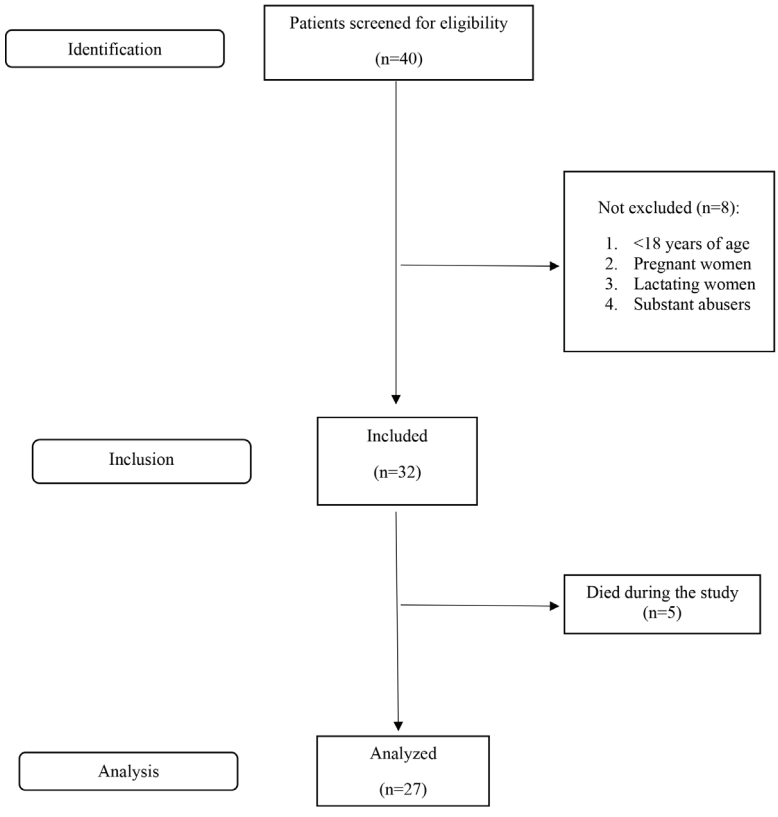

Figure 1. Patient flow diagram

Test values, which were routinely sent from the patients, such as hemogram, fasting plasma glucose (FPG), lipid profile [total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride], C-reactive protein (CRP), D-dimer, ferritin, and kidney and liver function tests, were obtained from the hospital database for the days of hospitalization and discharge. Serum specimens were analyzed for FGF-21 levels on the days of hospitalization and discharge.

Patients with FPG ≥126 mg/dL and HbA1c level ≥6.5 were considered diabetic, and those with FPG 100-125 mg/dL and HbA1c level 5.7-6.4 were considered pre-DM.

Collection of Blood Samples

Cells from blood samples were rapidly separated using a centrifuge at 3000 g for 10 min and then stored at 80 °C until analysis.

FGF-21 Measurements

FGF-21 levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biological Technology Company, Cat. No. E-EL-H0074, USA) following the manufacturer's instructions. The measurement range of the FGF-21 ELISA kit was 31.25-2000 pg/mL. After the serum samples were diluted by X^2 using a dilution buffer, two wells were studied for each sample. Plates were scanned using a Thermo Scientific microplate reader (USA) at 450 nm. Fibroblast growth factor-21 levels were calculated in pg/mL using the 4-parameter standard curve. The final concentrations were determined by multiplying the results by the dilution factor.

Statistical Analysis

Statistical analyses were conducted using IBM SPSS for Windows Version 24.0 software. Numerical variables are summarized as mean ± standard deviation (SD) and median (minimum-maximum), whereas categorical variables are expressed as numbers and percentages. The differences between the groups in terms of categorical variables were analyzed using the χ^2 test or Fisher's exact test. The Kolmogorov-Smirnov test, histograms, SD/mean ratios, skewness, and kurtosis were used to determine if the numerical variables were distributed normally. The homogeneity of variance was tested using the Levene test. The t-test was used for normally distributed parameters, and the Mann-Whitney U test was used for non-normally distributed parameters. Pearson's correlation analysis was used to determine the relationships between the parameters. The significance level was set at $p<0.05$

Results

This study involved 27 patients. Only 7 of the patients were followed up in the general ward, whereas the remaining 20 patients who developed acute respiratory distress syndrome (ARDS) were followed up in the ICU and were taken to the general ward when there was no need for the ICU anymore (Table 1). Methylprednisolone, favipiravir, proton pump inhibitors, antibiotics (piperacillin-tazobactam or moxifloxacin), and low-molecular-weight heparin were administered to the patients for treatment.

Table 2 compares the patients' vital signs and laboratory parameters from the first to the last days of hospitalization. When compared to the moment of admission, there was an improvement in oxygen saturation, as well as a significant increase in lipid parameters (blood urea nitrogen, alanine aminotransferase, hemogram, and FGF-21), and a significant decrease in creatinine and CRP at the moment of discharge.

A correlation analysis was performed between the change in the FGF-21 level (ΔFGF-21) and the change in other numerical data. There was a significant negative correlation between HDL change (Table 3), whereas there was no correlation between other parameters and ΔFGF-21.

Twenty of the patients were followed in the ICU, whereas seven patients were followed in the ward. Considering glucose metabolism, although there were more DM and preDM patients and fewer normal ones in the ICU (p=0.027), it was observed that the distribution was not affected after COVID-19 infection (Table 4). It was thought that being diabetic or pre-DM increased the risk of hospitalization in the ICU.

Table 1. Basal characteristics of the patients

Parameter	Data
Age, mean ± SD	56.8±16.3 (34-93)
Gender (F/M) n (%)	14 (51.9)/13 (48.1)
Systemic disease, n (%)	
HT	3 (11)
ASHD	2 (0.7)
RA	1 (0.3)
DM	13 (48.1)
preDM	8 (29.6)
Patient's ward, n (%)	
General ward	7 (25.9)
General ward + ICU	20 (74.1)
Number of patients receiving pulse steroids during hospitalization, n (%)	26 (96.3)
Pulse steroid dose (mg), mean ± SD	119.25±103.32
Number of patients who developed ARDS, n (%)	23 (85.2)
Number of patients with antibiotic indication, n (%)	24 (88.9)

SD: Standard deviation, F/M: Female/Male, HT: Hypertension, ASHD: Atherosclerotic heart disease, RA: Rheumatoid arthritis, DM: Diabetes mellitus, ARDS: Acute respiratory distress syndrome, ICU: Intensive care unit

Table 2. Comparison of parameters in all patients according to the beginning and end of COVID			
	At the beginning	**At the end**	**p-value**
Fever (°C)	36.2 (35.8-37.9)	36 (36-36.7)	0.143
Heartbeat rate (beat/min)	83.6±15.2	84±12.8	0.903
Systolic pressure (mmHg)	112.6±13.4	114.8±13.4	0.471
Diastolic pressure (mmHg)	69.2± 10.3	70.3±8.5	0.502
Saturation	**94.6±3.4**	**96.7±1.7**	**0.005***
FPG (mg/dL)	138 (84-369)	115 (82-439)	0.885
HbA1c	6 (5.4-11.9)	6 (5.3-11.9)	0.066
Triglyceride (mg/dL)	**115 (35-386)**	**180 (83-426)**	**0.001***
HDL (mg/dL)	**41.9±14.3**	**46.7±12.1**	**0.041***
LDL (mg/dL)	**103.2±30.1**	**121.1±31.9**	**0.001***
Total cholesterol (mg/dL)	**159.3±34.8**	**178.7±39.8**	**0.048***
Creatinine (mg/dL)	**0.97 (0.47-6.70)**	**0.83 (0.34-5.8)**	**0.025***
BUN (mg/dL)	**32 (15-177)**	**48 (96-160)**	**0.022***
ALT (U/L)	**20 (10-82)**	**38 (10-199)**	**0.003***
AST (U/L)	28 (9-102)	19 (10-106)	0.160
CRP (mg/L)	**49.88 (3.56-219)**	**2.56 (0.26-142)**	**<0.001***
D-dimer (mcg/mL)	0.37 (0.04-8.46)	0.28 (0-6.94)	0.279
Ferritin (mcg/L)	229 (32-1629)	158 (14.1-7738)	0.254
Fibrinogen (mg/dL)	479.2±118.2	500.5±180.2	0.810
Hb (g/dL)	12.28±3.2	12.69±2.1	0.328
Leucocyte (10^3/μL)	**6550 (1760-14250)**	**9680 (1677-25220)**	**0.024***
Lymphocyte (10^3/μL)	**1010 (250-8520)**	**2240 (720-3610)**	**<0.001***
Neutrophil (10^3/μL)	**5098.1±2858**	**7639.6±3451.4**	**0.003***
Platelet (10^3/μL)	**232000 (123000-554000)**	**347000 (148000-659000)**	**<0.001***
FGF-21 (pg/mL)	**254 (32-861)**	**454 (33-1888)**	**0.004***

*: Statistically significant
COVID: Coronavirus disease, FPG: Fasting plasma glucose, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, PreDM: Pre-diabetes mellitus, DM: Diabetes mellitus, BUN: Blood urea nitrogen, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C-reactive protein, Hb: Hemoglobin, FGF-21: Fibroblast growth factor-21

Table 3. Correlation between ΔFGF and variation in numerical parameters		
	R-value	**p-value**
ΔHDL (mg/dL)	-0.424	0.044*

*: Statistically significant
FGF: Fibroblast growth factor, HDL: High-density lipoprotein

Table 4. Relationship between the ICU and glucose metabolism			
	Only ward (n=7)	**Ward + ICU (n=20)**	**p-value**
Glucose metabolism (before COVID), n (%) Normal PreDM DM	 4 (57.1) 2 (28.6) 1 (14.3)	 2 (10) 6 (30) 12 (60)	**0.027*** **All subgroups were statistically significantly different (post-hoc analysis)**
Glucose metabolism (after COVID), n (%) Normal PreDM DM	 2 (28.5) 4 (57) 1 (14.5)	 2 (10) 5 (25) 13 (65)	0.079

*: Statistically significant
ICU: Intensive care unit, COVID: Coronavirus disease, PreDM: Pre-diabetes mellitus

Discussion

All patients recovered from the infection and were discharged. Therefore, there was a positive change in the parameters, indicating inflammation. The fact that all the patients recovered and the FGF-21 levels increased compared with the onset of infection indicates that FGF-21 metabolism increases during the recovery period of COVID-19 infection. Following the COVID infection, there was an increase in all lipid parameters. Additionally, there was an increase in HDL. Lipid metabolism is active during the viral infection process (10). It meets the need for lipids, which increases because of viral replication. In the literature, it has been reported that the lipid pathway plays an important role in the progression of viral infection, that there is an increase in activity of this pathway during the infection process, and that trying to break this pathway with anti-lipid drugs such as statins contributes to the infection process (11,12). Furthermore, the increase in the lipid profile in this study can be explained by this. The fact that patients had to be administered steroids may also be a factor in this increase.

Fibroblast growth factor-21 levels are high in obese people with dyslipidemia and diabetes. It has been identified as a potential biomarker for metabolic syndrome and diabetes (13,14). In a study conducted by Gawlik et al. (15), FGF-21 levels were higher than normal in patients with type 2 diabetes, whereas a negative correlation with HDL was found in these patients. A negative correlation between FGF-21 and HDL was also observed in another study in which FGF-21 levels were investigated in individuals with a high metabolic risk like type 2 DM, metabolic syndrome, atherosclerosis, and smoking (16). We also found a negative correlation between FGF-21 and HDL levels in our study, which supports the literature (Table 3).

In this study, DM and preDM were detected more frequently at discharge than at disease onset. Besides, the need for the ICU was observed more in DM and preDM patients. There was no relationship between FPG, HbA1c, and FGF-21 levels.

Steroids, or glucocorticoids, are drugs of common use. Both benefits and harms are associated with steroids. Some of the negative effects include osteoporosis, diabetes, dyslipidemia, cardiovascular disorders, and neurological dysfunction. Hyperglycemia is a frequently occurring condition. Lipolysis and proteolysis promote gluconeogenesis in the liver, which generates a substrate for gluconeogenesis in muscle and adipose tissue. This process inhibits insulin synthesis and secretion in the pancreas, resulting in insulin resistance in peripheral tissues and eventually causing hyperglycemia, also known as diabetes (17,18). Steroids are one of the most important weapons used for inflammation caused by the virus in the COVID-19 infection. The fact that high amounts of pro-inflammatory cytokines were detected in the serum and respiratory samples of patients showed that immune modulation, i.e., suppression of the immune system, is important in the fight against disease. In fact, the use of immunosuppressive drugs like steroids has made significant contributions to reducing COVID-related morbidity and mortality (19). Therefore, steroids, which are widely used all over the world during COVID-19, have also been widely used in our country and in our hospital. Almost all of our patients have taken pulse steroids. As a result, the number of patients with dysglycemia at the time of discharge was determined to be higher than that at the beginning. This may be due to steroid use, and it may have developed due to β-cell damage and cytokine storms caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) virus in the pancreas. In addition, the fact that the immune system is already impaired in uncontrolled diabetes has led to diabetic patients being more affected by SARS-CoV-2 and more hospitalizations (20). Given this information, it was thought that COVID-19 infection and steroid usage are important risk factors for DM and preDM development, and DM and preDM further increase the need for ICU, but there is no relationship between DM and FGF-21. However, the small number of patients may have limited the detection of a significant relationship.

Examining the results, it can be seen that there was a significant increase in the creatinine level and a significant decrease in lymphocytes and CRP. These findings are expected in hospitalized COVID-19 patients (21). There was no relationship between the FGF-21 level and gender. There is no evidence of this in the literature, either.

Ajaz et al. (9) investigated mitochondrial functions in healthy individuals, COVID-19-infected patients, and patients with pulmonary infection. They examined the FGF-21 and IL-6 levels in these participants. They have shown an increase in the utilization of glucose and glycolysis in COVID-19-infected patients. It was determined that there was a correlation between the severity of the COVID-19 infection and the increase in FGF-21 and IL-6 levels. Furthermore, it was reported that the HbA1c level was remarkably high in the COVID-19-infected group. However, no relationship was found between FGF-21 and glucose metabolism in this study, which may be because of the limited number of patients. Mitochondrial functions are the main factor in the natural immune response to viral infections (22).

Yan et al. (23) studied 193 patients with severe COVID-19 infection and found that the clinical course

was worse and mortality was higher in those with DM. Similarly, it was determined in this study that patients hospitalized in the ICU had more DM and preDM than patients hospitalized in the general ward.

In some previous studies, it was shown that FGF-21 cytokine levels increased in both diabetic patients and individuals with other metabolic diseases (24,25). Level is observed to increase in cases of insulin resistance, DM, and obesity. In the study, the increasing level of FGF-21 at the moment of discharge indicates that the action of this molecule promotes the functioning of the immune system and its fight against COVID-19.

Study Limitations

The presented study has some limitations. The small number of patients is the most important limitation, which restricts more specific results. Because the patients were hospitalized, their general condition was critical. Therefore, almost all of them had to be administered steroids. This may have affected the results achieved regarding glucose metabolism. Despite the limitations mentioned above, this study shows that there is a new parameter that can be used when fighting COVID infection, which is FGF-21. Fibroblast growth factor-21 is more specific than markers used in infection monitoring, such as CRP. Therefore, it is easy to track the COVID infection.

Conclusion

Fibroblast growth factor-21 metabolism is closely related to COVID-19 infection. It is activated in the fight against COVID-19. It can play an effective role in the healing process. The increase in FGF-21 can be used as a parameter to indicate that the COVID infection is healing.

Ethics

Ethics Committee Approval: The ethical approval was obtained from the Tokat Gaziosmanpasa University Faculty of Medicine, Clinical Research Ethics Committee (approval no.: 21-KAEK-165, date: 01.07.2021).

Informed Consent: Informed consent was taken from all the participants.

Authorship Contributions

Concept: A.D.D., Z.Ç., F.M.Z., Design: A.D.D., Z.Ç., F.M.Z., Data Collection or Processing: A.D.D., Z.Ç., F.M.Z., Analysis or Interpretation: A.D.D., Z.Ç., F.M.Z., Literature Search: A.D.D., Z.Ç., F.M.Z., Writing: A.D.D., Z.Ç., F.M.Z.

Conflict of Interest: No conflicts of interest were declared by the authors.

Financial Disclosure: This study received no financial support.

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Case Report

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Nasogastric Tube Placement as an Unusual Cause of Iatrogenic Hemopneumothorax in a Geriatric Patient: A Case Report and Current Literature Review

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Abstract

Iatrogenic hemopneumothorax is the presence of air and hemorrhagic fluid in the pleural space. It is a complication of invasive procedures performed for diagnosis and treatment. A 75-year-old woman with a history of cerebrovascular disease and aspiration pneumonia underwent wire-guided nasogastric tube (NGT) on the first day of palliative care follow-up because of the risk of aspiration with oral feeding. Posterior-anterior chest radiography was performed after the procedure because the patient had chest pain and tachypnea. Posterior-anterior chest radiography showed that the NGT guidewire was in the right costophrenic sinus and was associated with pneumothorax. Iatrogenic hemopneumothorax may develop secondary to many invasive procedures, as well as secondary to NGT, which is a simple clinical procedure. We aim to present an unprecedented case in which a new-generation NGT with a guidewire caused a hemopneumothorax during insertion.

Keywords: Iatrogenic hemopneumothorax, nasogastric tube, palliative care

Introduction

Iatrogenic hemopneumothorax is the presence of air and hemorrhagic fluid in the pleural space, a complication of invasive procedures performed for diagnosis and treatment (1). It often occurs after interventional procedures, such as subclavian vein catheterization, transthoracic biopsy, pleural biopsy, and intercostal nerve block (1,2). Cases of iatrogenic pneumothorax after nasogastric tube (NGT) insertion have been reported. We aim to present an unprecedented case in which a new-generation NGT with a guidewire caused a hemopneumothorax during insertion.

Case Presentation

A 75-year-old woman with a history of cerebrovascular disease and aspiration pneumonia underwent wire-guided NGT on the first day of palliative care follow-up because of the risk of aspiration with oral feeding. Posterior-anterior chest radiography (PACG) was performed after two hours of the procedure because the patient had chest pain and tachypnea. Posterior-anterior chest radiography showed that the NGT guidewire was in the right costophrenic sinus and was associated with pneumothorax (Figure 1A). The patient underwent a 28 French (Fr) chest tube and chest tube connected to a single chamber underwater drainage system. (Figure 1B). Hemorrhagic drainage of 300 cc was observed. The NGT was terminated, and the lung was expanded in the PACG. Hemodynamic, hemogram, and drainage monitoring were closely performed. The patient was followed up for 10 days with a drain with a total drainage of 800 cc. The chest tube was terminated after the drainage and air leaks were stopped (Figure 1C). After that, the patient was followed up in the intensive care unit for 64 days. She died of sepsis due to pneumonia progression.

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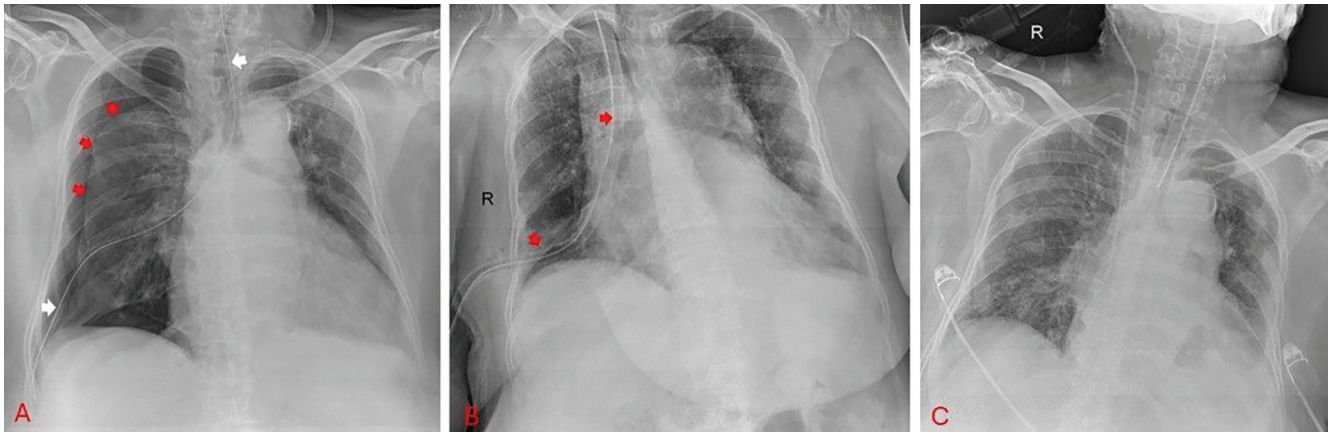

Figure 1. A) PACG after NGT insertion. Red arrows indicate the boundaries of the collapsed lung due to pneumothorax. White arrows indicate NGT. **B)** PACG after tube thoracostomy. Red arrows indicate the thoracic drain extending from the basal to the apex. **C)** PACG after termination of tube thoracostomy.

Discussion

The overall reported complication rates after NGT administration ranged from 0.3% to 8.0%. Various thoracic (bronchial placement and intravascular penetration) and non-thoracic (enteral and intracranial) complications have been reported. Misplacement of the trachea or bronchial tree occurs in approximately 0.2-0.3% of patients (3). Various complications, including atelectasis, lung abscess, bronchial perforation, pulmonary hemorrhage, pneumothorax, and empyema, are frequently observed after tracheobronchial placement. We wanted to contribute to the literature by presenting our case of hemopneumothorax after NGT placement, which is a very rare presentation in the literature.

Iatrogenic pneumothorax rarely develops after NGT for decompression of intestinal obstruction and feeding in patients with impaired oral feeding (4). In one study, NGT was performed in 740 patients who were followed-up in the intensive care unit, and pleuropulmonary complications developed in 14 patients (2%). Hemopneumothorax was found in only one patient with complications (5). Iatrogenic hemopneumothorax can occur after many invasive procedures and can also occur after NGT, which is considered a simple clinical procedure. In our case, unlike those in the literature, wire-guided NGT was performed, and hemopneumothorax occurred because the guide wire in the tube came out of the tube and perforated the lung parenchyma due to misplacement of the NGT.

Control with PACG after NGT placement is the gold standard, but in practical application, control is performed in the absence of cough or respiratory distress during placement; aspiration of gastric contents with or without litmus test, and positive auscultation of air injected through the epigastrium. However, these are unreliable, and Rassius et al. showed that misplaced tubes cannot be predicted using bedside tests (5,6). We did not perform PACG control immediately after NGT administration in our case. We performed PACG when the patient developed chest pain and tachypnea. The onset of symptoms was approximately 2 hours after the procedure. The absence of distress during insertion is particularly unhelpful because patients requiring NGT, usually due to poor swallowing, have reduced pharyngeal sensation and may not show any symptoms (7).

In treatment, follow-up with oxygen support is recommended if the patient is asymptomatic and the pneumothorax is less than 20%, and tube thoracostomy is recommended if the patient is symptomatic or has a pneumothorax of more than 20% (2). We performed tube thoracostomy in our patient because she had chest pain and tachypnea after NGT application, and a pneumothorax of >20% was detected on PACG.

In conclusion, although very rare, iatrogenic hemopneumothorax may occur after NGT placement. PACG should be performed before the active use of NGT to verify NGT placement under the diaphragm.

Ethics

Informed Consent: Informed consent was obtained.

Authorship Contributions

Concept: B.O.C., M.C., N.S., F.S., Design: B.O.C., N.S., F.S., Data Collection or Processing: B.O.C., M.C., N.S., F.S., K.A., Analysis or Interpretation: M.C., N.S., F.S., K.A., Literature Search: B.O.C., M.C., N.S., F.S., K.A., Writing: B.O.C., N.S., F.S.

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

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Case Report

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Prostatosymphyseal Fistula and Pubic Osteomyelitis after Transurethral Resection of the Prostate: A Challenging Complication and Current Literature Review

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Abstract

Transurethral prostate surgery can lead to a rare and late complication known as a prostatosymphyseal fistula. In the literature, there are only a limited number of reported cases of this type of fistula. This case underscores the complexities involved in disease management, as well as the challenges in establishing an initial diagnosis, given the inadequacy of conventional imaging methods to detect prostate-symphyseal fistulas. The presence of non-urological symptoms in patients often impedes the recognition of prostate-symphyseal fistulas, leading to a delay in diagnosis. In this case report, a patient who presented to the emergency department with complaints of sudden-onset difficulty walking and fever in the second month after bipolar transurethral resection of the prostate is presented. The diagnosis of pubic osteomyelitis and prostatosymphyseal fistula was made using advanced examinations such as contrast-enhanced pelvic computed tomography and magnetic resonance imaging. In the treatment, transperitoneal fistula tract excision and repair with an omental flap were performed. No complaints were observed during the 1-year postoperative follow-up. The surgeon's vigilant approach is paramount for promptly identifying this rare complication.

Keywords: Osteomyelitis, transurethral prostate resection, prostatosymphyseal fistula

Introduction

Benign prostatic hyperplasia is a prevalent condition among older males, impacting millions of people annually. Various medical and surgical therapeutic approaches are available for the treatment of these conditions. Among surgical interventions, transurethral resection of the prostate (TURP) is the gold standard procedure (1). TURP can lead to a rare and late complication known as prostatosymphyseal fistula (PSF). In the literature, there are only nine reported cases of this type of fistula after TURP (2). The case depicts the intricacies of disease administration, along with the intricacies of making the preliminary diagnosis because conventional imaging methods were inadequate to discern PSF. Prostatosymphyseal fistula recognition is often impeded by the presence of non-urological symptoms, which can lead to a delay in diagnosis. Hence, the surgeon's acute discernment plays a pivotal role in promptly recognizing this uncommon complication.

Case Report

A 56-year-old male patient was admitted to the emergency department with complaints of nausea and vomiting for 3 days, right inguinal pain that started when he woke up in the morning, and an inability to stand and walk. No dysuria or lower urinary tract symptoms were present.

His fever was 38.9 °C. He was using acetylsalicylic acid at 100 mg/day because of coronary artery disease. He had bipolar TURP 2 months ago. There was no capsule perforation or suspicion during the surgery, and there were no intraoperative or immediate postoperative complications. In routine blood tests, the hemoglobin level was 11.9 g/dL, the white blood cell count was

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15.9×10^9/L, C-reactive protein level was 14.2 mg/L, and the procalcitonin level was 108.8 ng/L. Serum urea and creatinine levels were within normal limits. *Escherichia coli* growth was positive in blood culture, whereas midstream urine culture was negative. Contrast-enhanced pelvic computed tomography (CT) showed an infective collection area of 80-18 mm with air densities extending from the right obturator fossa to the right inguinal region and air densities compatible with osteomyelitis adjacent to the symphysis pubis in both pubic rami (Figures 1 and 2). Pelvic magnetic resonance imaging (MRI) showed a fistula tract extending from the anterior of the prostatic urethra to the symphysis pubis (Figure 3). Piperacillin-tazobactam 4x4.5 g intravenous treatment was initiated, and urinary drainage was performed using suprapubic bladder catheterization. The fistula tract was removed by median laparotomy and closed with an omental flap. The abscess area was drained. The patient started to mobilize on the first postoperative day. In the first month postoperatively, cystourethrography revealed no extravasation, and the suprapubic catheter was removed. The patient voided normally and was on the continent. He did not have any complaints during the first year of follow-up.

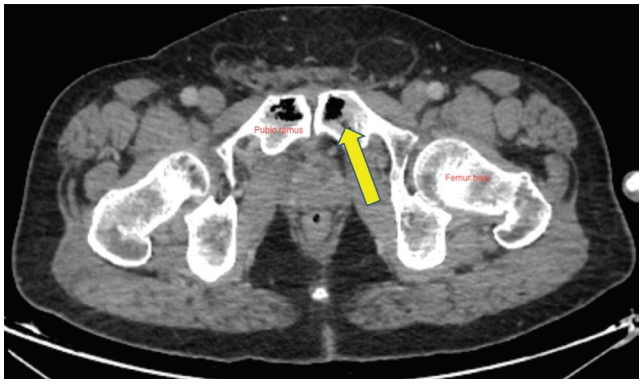

Figure 1. Pelvic CT. Air densities consistent with osteomyelitis were observed adjacent to the pubic symphysis in both pubic rami (arrow)
CT: Computed tomography

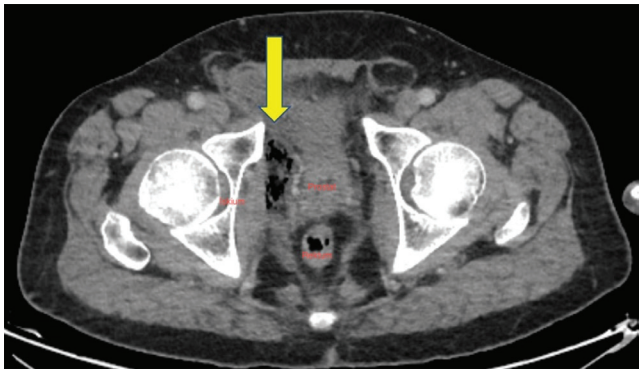

Figure 2. Pelvic CT. Infective collection area with air densities extending from the right obturator fossa to the right inguinal region (arrow)
CT: Computed tomography

Discussion

Transurethral resection of the prostate is generally a safe method; however, it has known complications such as bleeding, urinary incontinence, urethral stricture, and retrograde ejaculation (1). Fistula formation from the prostatic urethra to the symphysis pubis, defined as a PSF, is a very rare complication. PSF has been reported not only after TURP but also after transrectal prostate biopsy, photoselective vaporization of the prostate, radical prostatectomy, radiotherapy, and salvage cryotherapy (3-8).

The underlying cause of PSF is believed to be an injury in the anterior prostatic capsule following therapeutic approaches, possibly due to tissue weakening from previous RT or fortuitous perforation in the course of surgery (9). During the procedure, a hypovascular zone may be created beyond the visible area, which may ultimately lead to necrosis and tissue sloughing. Bleeding resulting from capsular perforation, along with the subsequent application of prolonged coagulation, can intensify tissue necrosis and contribute to the pathogenesis of the condition. The use of indwelling catheters may additionally precipitate secondary bacterial infections, seeding the pubic symphysis and culminating in pubic osteomyelitis, ultimately predisposing patients to PSF.

When patients present with pain in the pubic region and/or groin following prostate procedures, particularly during ambulation, it should prompt clinicians to initiate further investigations. Complementary diagnostic tools such as CT and MRI play pivotal roles in evaluating patients suspected of having PSF (10). CT scans, particularly during the excretory phase of contrast injection, aid in identifying

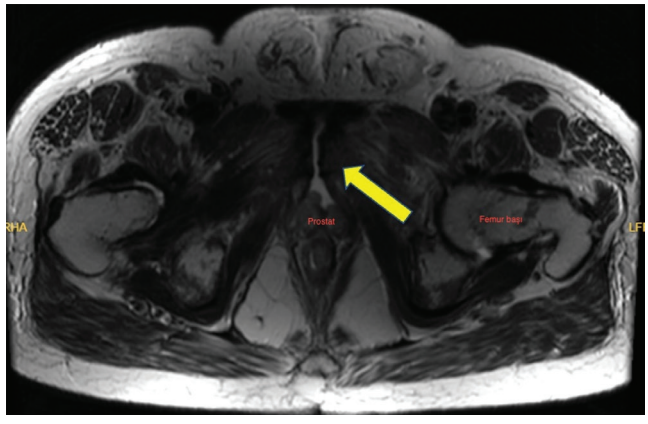

Figure 3. Pelvic MRI. Axial T2 image demonstrates the prostatosymphyseal fistula (arrow)
MRI: Magnetic resonance imaging

the presence of urine within the joint space, a hallmark of PSF. On the other hand, MRI exhibits higher sensitivity in detecting inflammatory changes in the pubic bone or adjacent soft tissues in patients with PSF. Therefore, a combination of these imaging techniques proves invaluable in diagnosing and managing PSF.

Surgical repair methods such as debridement of the symphysis pubis and fistula closure using the rectus abdominis muscle, omental or peritoneal interposition flaps, radical prostatectomy, and urinary diversion are the main approaches in treatment (2-4). At present, the available evidence does not suffice to ascertain the most effective invasive approach for PSF, whether through radical retropubic prostatectomy coupled with symphysis pubis debridement to remove the entire fistula tract or via fistula debridement with an interposition flap such as omental or peritoneal. Further research with extended follow-up periods is imperative to develop definitive treatment guidelines. It has recently been reported that PSF secondary to TURP, radical prostatectomy, and RT was successfully treated with urinary diversion and intravenous vancomycin, as well as oral ciprofloxacin and clindamycin (3).

Prostatosymphyseal fistula is an uncommon complication that may arise because of the variety of therapeutic options available for treating prostate disease. This condition can result in significant disability for affected patients. However, it poses a clinical quandary because of the limited role of diagnostic methods and delayed presentation. Furthermore, effective management of this complication requires a multidisciplinary approach involving specialists in urology, infectious disease, and orthopedic surgery. As a result, it is critical for healthcare providers performing procedures to be aware of the possibility of PSF, particularly when patients exhibit orthopedic symptoms or persistent infective symptoms upon follow-up.

Ethics

Informed Consent: Informed consent was obtained from the patient for the publication of this case at the time of discharge.

Authorship Contributions

Surgical and Medical Practices: - Concept: - Design: - Data Collection or Processing: - Analysis or Interpretation: - Literature Search: - Writing: Both authors contributed equally.

Conflict of Interest: No conflicts of interest were declared by the authors.

Financial Disclosure: This study received no financial support.

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Case Report

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EDTA-Dependent Pseudothrombocytopenia Associated with Hashimoto's Thyroiditis: A Case Report and Current Literature Review

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Abstract

Pseudothrombocytopenia (PTCP) can be an analytical error in the automatic blood cell count. Blood samples containing autoantibodies against platelets collected in ethylenediaminetetraacetic acid (EDTA) tubes can lead to platelet accumulation at room temperature. Agglutinated platelets are detected as larger cells using automated counters, which incorrectly leads to falsely low results. Hence, when a low platelet count is noted, PTCP must also be considered. In this study, we report a case of EDTA-dependent PTCP in a patient who has Hashimoto's thyroiditis with the current literature review.

Keywords: Pseudothrombocytopenia, Hashimoto's thyroiditis, EDTA

Introduction

Gowland first discovered this phenomenon in 1969 (1). The prevalence of this phenomenon in ethylenediaminetetraacetic acid (EDTA) is estimated to be 0.03-0.27% of the general population (2-4); however, multiple anticoagulant pseudothrombocytopenias (PTCPs) with citrate, heparin, or sodium fluoride have also been described. Cation chelation by EDTA leads to a conformational change of the platelet membrane GPIIb-IIIa complex, unmasking a cryptic epitope, which becomes accessible for autoantibodies. Antibodies are predominantly of the IgG type but act as cold agglutinins that react with platelets *in vitro* (5). Although harmless, failure to make this important distinction leads to unnecessary diagnostic tests, delays in surgery and treatment, and unnecessary platelet transfusions. Therefore, it is crucial to take into account PTCP when a low platelet count occurs. In this study, we report a case of EDTA-dependent PTCP.

Case Report

A 53-year-old woman was admitted to the hospital for long-term weakness. The patient complained of progressive fatigue in the last year. A physical examination revealed that the patient was overweight and had a BMI of 28.7. Laboratory analysis including complete blood count, biochemistry, free T4, thyroid peroxidase antibody (anti-TPO), and thyroid stimulating hormone (TSH) were analyzed. TSH: 4.38 mU/L (normal range: 0.27 to 4.2 uIU/mL), free T4: 11.7 ng/L (8.9 to 17.4 ng/L), anti-TPO: 795.2 IU/mL (normal range: 0-9 IU/mL), and platelet count: 14.000/UL (normal range: 142.000 to 424.000 /mm^3).

Thrombocytopenia was detected in previous laboratory tests. Hypothyroidism was diagnosed, and treatment with levothyroxine was initiated. The patient was referred to the hematology department for an evaluation of thrombocytopenia. A peripheral blood smear was compatible with PTCP. The first suggestion was that a complete blood count should be performed in a citrate tube. The thrombocyte count performed in the EDTA tube was 14.000/UL, whereas that in the citrate tube was 175.000/UL. The patient was diagnosed with pseudotrombocytopenia on the basis of laboratory findings. The patient progressed well with levothyroxine treatment. All subjects provided informed consent, and patient anonymity was preserved.

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Discussion

Thrombocytopenia is defined as a platelet count of $<150\times10^9$/L, although patients with a platelet count $>50\times10^9$/L are usually asymptomatic. Patients with thrombocytopenia rarely experience severe spontaneous bleeding. It is more common when the platelet count is $<20\times10^9$/L, and particularly when $<10\times10^9$/L. The clinical history establishes the cause of thrombocytopenia. This includes asking about recent infections, drug/vaccination history, travel history, diet (B12/folate deficiencies), past medical history, pregnancy status, and alcohol intake, along with establishing any features associated with malignancy (6).

In a patient with new thrombocytopenia, a repeat full blood count along with a citrated or heparinized blood sample should be taken both to confirm thrombocytopenia and to exclude PTCP caused by artefactual clumping (7). An accurate assessment is essential to ensure the sustainable management of the patient. The initial approach should include a clinical history, examination, complete blood count, and blood film analysis.

Pseudothrombocytopenia, a relatively common finding in clinical laboratories, can lead to diagnostic errors, overtreatment, and further (even invasive) unnecessary testing. The condition is most often seen in blood samples anticoagulated with EDTA, although citrate, oxalate, or heparin have also been implicated (8-10). EDTA-induced PTCP, the most frequently seen form in clinical practice, occurs mainly due to the reaction of antiplatelet antibodies (11). This mechanism is based on the binding of an antiplatelet autoantibody to the glycoprotein (GP) IIb/IIIa receptor on the cell membrane of platelets. The combined effect of EDTA's chelating effect on calcium ions and low temperature affects platelet membrane GP complex IIb/IIIa and exposes the GP IIb epitope. When the autoantibody binds to the GP IIb epitope, platelet aggregation occurs, which is observed in peripheral blood smears (12).

To avoid anticoagulant-induced PTCP, mainly associated with EDTA, either citrate or magnesium sulfate should be used as an anticoagulant (13). What is striking, however, is that the patients did not have a history of bleeding or recurrent hematoma despite extremely low platelet counts. Another helpful possibility is to perform the platelet count as early as possible after blood sampling, but this is not suitable for routine use.

However, it is the laboratory's responsibility to detect, confirm, or exclude PTCP in daily routine analyses. The reliability of automated hematologic analyzers is unsatisfactory. New technologies, such as fluorescence or optical platelet counting, should be implemented in clinical laboratories because they will provide valuable and suitable support for correcting spuriously low platelet counts. In patients with known PTCP, a venipuncture system with an alternative anticoagulant should be used before measuring platelet counts. Based on the available information, *in vitro* platelet aggregates have been reported for all alternative anticoagulants except magnesium sulfate, although this is rare. Supplementation of anticoagulant samples with aminoglycosides before blood sampling is controversial and ultimately unsuitable for routine use (14).

No particular disease was strongly associated with the presence of PTCP or showed significant differences from a control population of healthy individuals. However, the incidence of EDTA-PTCP appears to increase with hospitalization or in patients with specific disorders, such as autoimmune diseases (13,15). In a study that included 49 patients with diagnosed PTCP and 69 healthy volunteers, 23.8% of the patients had diabetes mellitus, 32.5% were hypertensive, 26.3% had an atherosclerotic heart disease, 5% had a history of cerebrovascular accidents, and 10% had hypothyroidism. Hospitalization was required for thirty-eight of them (76.25%), and 8% of the patients had coexisting diseases (14).

As our study has shown, the combination of hypothyroidism and PTCP is unmistakable. According to this information, both clinicians and laboratory specialists must be careful when evaluating thrombocytopenia.

Ethics

Informed Consent: All subjects provided informed consent, and patient anonymity was preserved.

Authorship Contributions

Surgical and Medical Practices: E.O.A., Y.E.D., Concept: E.O.A., Y.E.D., M.K., Design: E.O.A., Y.E.D., M.K., I.Y., Data Collection or Processing: E.O.A., Y.E.D., Analysis or Interpretation: E.O.A., Y.E.D., M.K., Literature Search: E.O.A., Y.E.D., M.K., Writing: E.O.A.

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