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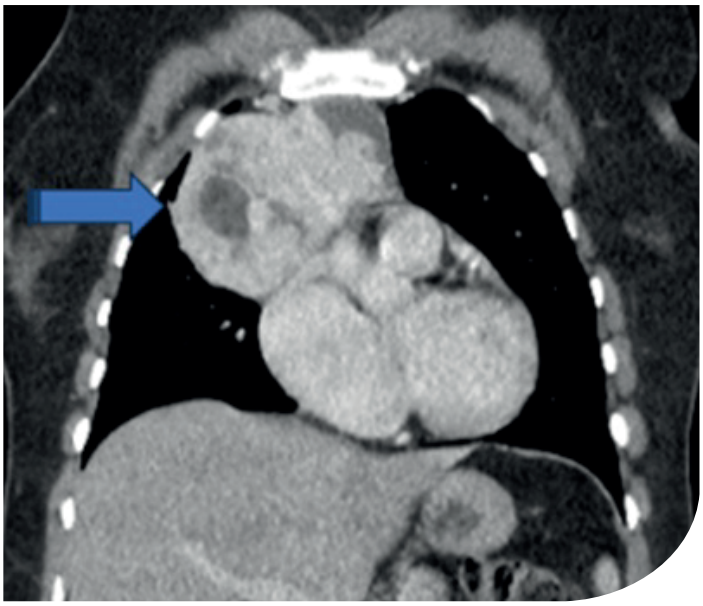

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Aims and Scope

Developments and Experiments in Health and Medicine (ISSN: 1300-6622; e ISSN: 2602-3148) is an international, double-blind peer-reviewed, open access publication of Dokuz Eylül University Faculty of Medicine which is published quarterly in January, April, July and October.

It publishes articles of original research conducted using scientific methods with appropriate hypotheses in all areas of medicine. In addition, it publishes reviews on current issues, rare medical cases, and letters to the editor containing the experiences and comments of specialist physicians in the field. Manuscripts are publishable in English. Developments and Experiments in Health and Medicine does not charge any fees to the author(s) for the evaluation and/or publication of submitted articles.

The aim of this journal is to provide scientists with the opportunity to publish their original scientific studies in the field of medicine and health, to share their discoveries, new original ideas and theories in this field.

The target audience of Developments and Experiments in Health and Medicine is physicians, specialists, researchers, specialists and doctoral students in all areas of medicine as well as medical faculty students. It aims to contribute to the spread of continuous professional development and research culture.

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Subject Areas

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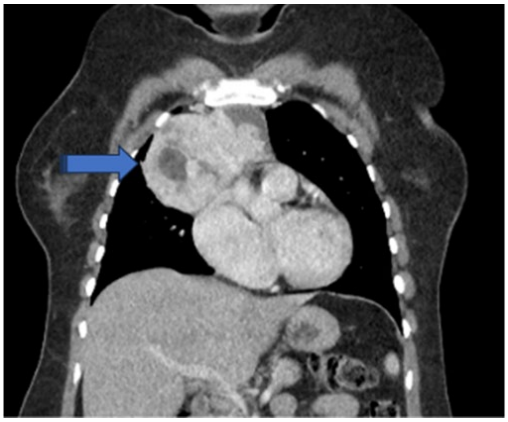

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Research Article

Low apelin levels are associated with a marked increase in risk of gestational diabetes mellitus development

Düşük apelin seviyeleri, gestasyonel diabetes mellitus gelişme riskinde artış ile ilişkilidir

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ABSTRACT

Background: Apelin is an adipokine which plays a role in the regulation of glucose homeostasis. Relationships between serum apelin concentrations and dysmetabolic conditions are still controversial. The aim of this study was to investigate the association of serum apelin levels with gestational diabetes mellitus (GDM).

Material and Methods: This study was designed as a cross-sectional research that consecutively recruited subjects with GDM (n=38), without GDM pregnant (n=41), and non-pregnant healthy women (n=39). Fasting blood glucose (FBG), serum apelin, insulin and lipids were measured. BMI and HOMA-IR were calculated for all subjects. Logistic regression analysis was performed to determine predictors of GDM development.

Results: Serum apelin levels (GDM = 1.99 ± 1.36 mg/ml, non-GDM pregnant = 2.95 ± 1.36 mg/ml, non-pregnant women = 2.62 ± 1.67 mg/ml) were significantly lower ($p = 0.017$), HOMA-IR ($p = 0.024$) and BMI ($p < 0.001$) were significantly higher in the GDM group compared with both the non-GDM and the nonpregnant women. Serum apelin levels were found to be negatively correlated with FBG ($r = -0.236$, $p = 0.010$), OGTT 1 h glucose ($r = -0.346$, $p = 0.002$) & 2 h glucose ($r = -0.248$, $p = 0.028$), HbA1c ($r = -0.209$, $p = 0.023$), HOMA-IR ($r= -0.360$, $p < 0.001$) and BMI ($r = -0.299$, $p = 0.001$).

Conclusions: Serum apelin levels were significantly lower in the GDM group as compared with both the non-GDM pregnant and the non-pregnant healthy women. Low apelin levels appear to be an independent predictor of GDM development.

Keywords: GDM, Apelin, HOMA-IR, HbA1c

ÖZ

Amaç: Apelin, glikoz homeostazının düzenlenmesinde rol oynayan bir adipokindir. Serum apelin konsantrasyonları ile dismetabolik durumlar arasındaki ilişkiler hala tartışmalıdır. Bu çalışmanın amacı serum apelin düzeylerinin gestasyonel diabetes mellitus (GDM) ile ilişkisini araştırmaktır.

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Gereç ve Yöntem: Bu çalışma, ardışık olarak GDM'li (n=38), GDM olmayan gebe (n=41) ve gebe olmayan sağlıklı kadınların (n=39) alındığı kesitsel bir araştırma olarak tasarlanmıştır. Açlık kan şekeri (AKŞ), serum apelin, insülin ve lipidler ölçüldü. Tüm denekler için BMI ve HOMA-IR hesaplandı. GDM gelişimini öngördürücüleri belirlemek için lojistik regresyon analizi yapıldı.

Bulgular: Serum apelin seviyeleri (GDM = 1,99 ± 1,36 mg/ml, GDM olmayan gebe = 2,95 ± 1,36 mg/ml, gebe olmayan kadın = 2,62 ± 1,67 mg/ml) anlamlı olarak daha düşüktü (p = 0,017), HOMA-IR (p = 0,024) ve BMI (p < 0,001) GDM grubunda anlamlı olarak yüksekti. -GDM ve gebe olmayan kadınlar. Serum apelin düzeylerinin AKŞ (r = -0,236, p = 0,010), OGTT 1 saatlik glukoz (r = -0,346, p = 0,002) ve 2 saatlik glukoz (r = -0,248, p = 0,028), HbA1c (r = -0,209, p = 0,023), HOMA-IR (r= -0,36) ile negatif korelasyon gösterdiği bulundu. 0, p < 0,001) ve BMI (r = -0,299, p = 0,001).

Sonuç: Serum apelin düzeyleri GDM grubunda hem GDM olmayan gebelere hem de gebe olmayan sağlıklı kadınlara göre anlamlı derecede düşüktü. Düşük apelin seviyeleri, GDM gelişiminin bağımsız bir göstergesi gibi görünmektedir.

Anahtar Kelimeler: Apelin, gestasyonel diabetes mellitus, HOMA-IR

INTRODUCTION

Glucose intolerance that develops during pregnancy is called as gestational diabetes mellitus (GDM) (1). Parallel to the increase in the prevalence of diabetes all over the world, the incidence of GDM is also increasing due to the increase in maternal age and obesity. Although there are different results between countries, the prevalence of GDM is estimated between 1.7-11.6% in developed economies; the frequency is increasing in young and obese women (2). More than 200,000 pregnancies per year are complicated by GDM (1).

Pregnancy progresses with the development of the fetoplacental unit and a decrease in insulin sensitivity physiologically. The main purpose of these changes is to meet the nutrition and energy needs of the fetus, which is in continuous development. In response to the development of insulin resistance in target organs during pregnancy, insufficient insulin secretion of the pancreas causes hyperglycemia (3). GDM is the cause of significant complications in both the mother and the baby. While macrosomia, hypoglycemia, hyperbilirubinemia, congenital anomalies and respiratory distress syndrome are adverse neonatal outcomes, hypertension and preeclampsia are some of the possible maternal complications. Women with GDM and their babies are at increased risk of developing diabetes and metabolic syndrome later in life (4).

Recent developments have made it possible to examine the pathophysiology of insulin resistance at the molecular level, aiming to reveal the factors that cause insulin resistance and to develop new treatment and diagnostic strategies targeting these factors. In cell culture studies conducted in recent years, it has been determined that apelin peptide, an adipocytokine, plays an important role in the development of insulin resistance. Apelin receptors (APJ) are expressed in muscle, liver and adipose tissue, which are insulin sensitive tissues (5). It was observed that glucose uptake was increased in adipocytes after apelin injection into adipocytes that had insulin resistance with TNF alpha in vitro. It has been determined that apelin provides this effect by promoting GLUT4 translocation from the cytoplasm

to the plasma membrane via AMP-activated protein kinase (AMPK) and endothelial nitric oxide synthase (eNOS). Again, apelin inhibits triglyceride hydrolysis by inhibiting hormone sensitive lipase in adipose tissue, thus preventing free fatty acid release into the systemic circulation (6). Apelin increases the expression of peroxisome proliferator activated receptor γ coactivator 1α (PGC1α) with AMPK activation in muscle tissue. In this way, glucose uptake, beta oxidation, nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM) levels increase in muscle tissue. NRF-1 and TFAM increase mitochondrial biogenesis and oxidative phosphorylation capacity (7-8).

It was determined that oxidative phosphorylation capacity and fat oxidation in muscle cell mitochondria increased, white adipose tissue mass decreased, thermogenesis increased in brown adipose tissue, and as a result, insulin sensitivity improved in obese and insulin resistant mice with apelin administration (8-9). The fact that apelin increases glucose utilization in insulin-sensitive tissues in mice suggests that it may be a promising target molecule in the management of insulin resistance (5).

As it is known, the development of insulin resistance during pregnancy is to ensure optimal fetal nutrition. Accordingly, the effect of apelin on pregnancy metabolism is not fully known. In the study by Telejko et al. apelin and APJ (endogenous ligand of the G protein-coupled receptor of Apelin) mRNA levels investigated in serum, subcutaneous adipose tissue, visceral adipose tissue and placenta tissue of pregnant women with normal glucose tolerance and pregnant women with GDM were examined, and the relationship of apelin with the GDM process was evaluated.

Although a statistically significant relationship between apelin and apelin receptor expression with metabolic parameters was not detected, it was found that apelin mRNA expression in placental tissue was ten times higher than in adipose tissue. This result indicates that apelin plays an important role in the regulation of placental vascularization and blood flow, which is necessary for a normal fetal development (10-11).

Recognition and targeting of the factors that cause insulin resistance not only enables early diagnosis and treatment of GDM, but also creates a new threshold in the treatment of diabetes, metabolic syndrome and its complications. The role of apelin in the development of insulin resistance, which plays a potential role in the pathophysiology of GDM, was investigated in this study.

MATERIALS AND METHODS

Our study was designed as a case-controlled cross-sectional study. Ethics committee approval was obtained for our study with the decision of Dokuz Eylül University Non-Interventional Research Ethics Committee dated 10.05.2012 and numbered 2012/17-23. Our research was carried out between 01st September 2012 and 31st March 2013.

Research group

The research group consisted of three different groups of 40 people, each being pregnant women diagnosed with GDM, healthy pregnant women with similar age and gestational weeks, and healthy non-pregnant women with similar demographic characteristics. The pregnant women included in the study were 24-30 weeks pregnant women who were followed up in the Dokuz Eylul University Hospital Gynecology and Obstetrics clinic and referred to the Hospital Endocrinology clinic for oral glucose tolerance test (OGTT) screening. Pregnant women who volunteered to participate in the study were divided into two groups as those with GDM and healthy pregnant women according to the OGTT result. The healthy non-pregnant women in the research group were also composed of healthy women in the similar age range who came to the Endocrinology clinic of University for routine control

and examination and were in compliance with the research protocol.

Written consent was obtained from the subjects who accepted to participate in the study, and the Declaration of Helsinki was complied with during the study.

Inclusion criteria;

- To be over 18 years old
- To be pregnant in 24-28. weeks
- Volunteer to participate in research

Exclusion criteria;

- Having a diagnosis of malignancy
- Having a systemic disease (liver or kidney failure, adrenal, thyroid and parathyroid disease, metabolic bone disease, type 1 and type 2 diabetes, malabsorption syndrome or connective tissue disease)
- Chronic alcohol abuse
- Having a twin pregnancy
- Diagnosed with pre-pregnancy diabetes
- Use of drugs that affect carbohydrate and lipid metabolism

Clinical Evaluation and Specimen Collection

Demographic data, anthropometric measurements, medical and reproductive histories of the research group were recorded. The blood pressure of the cases was measured manually with Erka brand blood pressure device after 15 minutes of rest. Body weights were measured with light clothing and shoes removed. Height measurements were made with a standard measuring tape. Body mass index was calculated as kg/m2. In the study, GDM screening and diagnosis were made according to International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria (12,13). OGTT was performed in the morning after fasting for at least eight hours with 75 grams of glucose. The patients were given water-sugar solution within 10 minutes. For blood glucose measurements, antecubital venous blood samples were taken at 0, 60, and 120 minutes during OGTT. The test was repeated the next day in patients who developed emesis after drinking water-sugar solution. For other parameters determined in the study (fasting insulin, fasting C-peptide, apelin, hs-CRP, TSH, PTH, creatinine) from the blood sample obtained from the study group for at least eight hours of fasting, for fasting blood glucose determination, GGT, triglyceride, HDL, LDL, total cholesterol sample was separated. Serum samples were separated from the blood samples taken from the subjects by cold centrifugation method (4000 rpm/min, 15 min). The serum samples obtained were stored at -80 degrees. Two cases in the GDM group and one case in the control female group were excluded from the study due to insufficient serum samples.

Laboratory measurements

Serum apelin (Raybiotech Inc., USA, intra-assay CV< 10%, inter-assay CV< 15%) levels of the research group were measured by enzyme-linked immunosorbent assay (ELISA) method. Fasting blood glucose, and serum levels of hs-CRP, creatinine, GGT, ALT, triglyceride, total cholesterol, HDL, sodium, potassium, calcium, phosphorus in our study were measured by spectrophotometric method using Abbott Diagnostics original kits and Abbott Architect C16000 autoanalyzer (Illinois, USA). Fasting insulin level, PTH, TSH, fT4, fT3 levels in Abbott Diagnostics original kits with Abbott Architect I2000 autoanalyzer (Illinois, USA), fasting C-peptide levels in Cobas e601 autoanalyzer with Roche Diagnostics original kits (Manheim, Germany) were measured by electrochemiluminescence immunoassay method. HbA1c was measured by high-performance liquid chromatography (HPLC) method on an Adams HA-8160 Arkray autoanalyzer (Longfield, England).

All parameters in our research were studied in the Central Laboratory of Dokuz Eylul University Medical Faculty Hospital, which has ISO15189 accreditation certificate. Our research was supported by the Dokuz Eylul University Scientific Research

Fund. (Project number: 2012.KB.SAG.094). Insulin sensitivity of the patients was calculated according to QUICKI (Quantitative insulin sensitivity check index), insulin resistance according to HOMA-IR (Homeostasis model assessment) and pancreatic insulin secretion according to HOMA-β (homeostasis model assessment-beta).

• QUICKI-IS: 1 / [(log (fasting insulin) (μU/mL) + log (fasting glukoz) (mg/dL) (14).

• HOMA-IR = fasting insulin (μU/ mL) x fasting glucose (mmol / L) / 22.5 (15).

• HOMA-β = fasting insulin (μU/ml)x20) / fasting glucose (mmol/l) (15).

Statistical analysis

Statistical analyzes were performed in the PASW Statistics 18 program. Descriptive statistics; percentage distributions, mean and standard deviation values are presented. One Way Anova test was performed to compare the independent variables of GDM, healthy pregnant and non-pregnant healthy women. If a difference was detected between the three groups, post-hoc analysis was performed with Bonferroni correction to understand which group caused the difference. Pearson's correlation analysis and the relationship between clinical and laboratory characteristics were examined.

In order to determine the relative risk, a logistic regression model was created between GDM and healthy pregnant women. Evaluation of the results: The $p< 0.05$ value was accepted as the significance value, taking into account the 5% margin of error in the 95% confidence interval.

RESULTS

The study included 38 pregnant women with GDM as the patient group, 41 healthy pregnant women and 39 healthy non-pregnant women as the control group. Demographic and anthropometric characteristics of the study cases are shown in Table 1.

Table 1. Demographic characteristics of the research group

Variables	GDM (n=38)	Control pregnant (n=41)	Control non-pregnant (n=39)	p
Age, years	32±5	28±4	26±3	<0.001
Education, years (>11 years)	%34.2	%34.1	%100	<0.001
DM in the mother	%39.5	%17.4	%10.3	<0.001
BMI (prior to gestation), kg/m^2	27±5.4	23±3.9	20.1±2.1	<0.001
Blood pressure, mm Hg				
Systolic	110±10	103±11	100±10	<0.001
Diastolic	68±8	62±9	66±6	0.011
Pregnancy-related issues				
Gestational week	27±2	26±2	N/A	0.281
Gravida	2.2	2.0	N/A	0.346
Parida	0.9	0.8	N/A	0.627
History of GDM	%13.2	%2.4	N/A	0.098
Weight of the baby, gr	3147±559	3254±420	N/A	0.35

BMI: body-mass index; **DM:** diabetes mellitus**; GDM:** gestational diabetes mellitus; **N/A:** not applicable

The levels of HbA1c ($p<0.001$), serum fasting blood glucose ($p<0.001$), serum fasting C-peptide ($p=0.002$), HOMA insulin resistance index ($p=0.024$), serum triglyceride ($p<0.001$), serum hs-CRP ($p<0.001$) and serum GGT ($p=0.007$) of pregnant women with gestational diabetes were found to be statistically significantly higher than the control group.

Although the serum fasting insulin level of pregnant women with gestational diabetes was found to be higher than the control group, this was not significant ($p=0.786$). Serum apelin levels ($p=0.017$),

QUICKI insulin sensitivity (p=0.002) and serum creatinine levels (p<0.001) of the pregnant women with gestational diabetes were found to be statistically significantly lower than the control group. The laboratory characteristics of the research groups are shown in Table 2.

Table 2. Laboratory results of the research group (Values are shown as X±Standard deviation)

Variables	GDM (n=38)	Control pregnant (n=41)	Control non-pregnant (n=39)	p
HbA1c, %	5.4±0.5	4.9±0.3	5.1±0.2	<0.001
Fasting glucose, g/dL	87±20	74±7	88±11	<0.001
Insulin, µIU/mL	9.7±5.3	8.4±8.8	8.2±14.4	0.786
Fasting C-peptide, ng/mL	2.6±1.1	2.2±1.4	1.7±0.5	0.002
HOMA-IR	2,1±1,62	1,56±1,75	1,3±0,54	0.024
HOMA-β	40.6±19.1	41.4±41.4	24.4±9.1	0.012
Quicki	0.350±0.033	0.370±0.035	0.370±0.027	0.002
Apelin, µg/mL	1.99±1.36	2.95±1.36	2.62±1.67	0.017
Triglyceride, mg/dL	219±81	179±103	66±20	<0.001
hs-CRP, mg/L	7±6	6±6	1±1	<0.001
GGT, U/L	13±9	9±4	11±5	0.007
Creatinine (serum), mg/dL	0.57±0.07	0.59±0.09	0.68±0.08	<0.001
PTH (pg/mL)	29.6±13.6	29.9±20.3	37.6±17.8	0.078
TSH, µIU/mL	1.45±0.89	1.49±0.77	1.50±0.78	0.965

BMI: body-mass index; **DM**: diabetes mellitus; **GDM**: gestational diabetes mellitus

The pearson correlation analysis of serum apelin levels with other clinical and laboratory data were examined. A significant and positive correlation was found between serum apelin levels and QUICKI insulin sensitivity (r = 0.42, p < 0.001). A negative correlation was found between serum apelin levels and HOMA insulin resistance index (r = -0.36, p < 0.001), body-mass index (r = -0.29, p < 0.001), fasting serum glucose levels (r = -0.23, p = 0.01), fasting C-peptide levels (r = -0.372, p = 0.001) and HbA1c levels (r = -0.20, p = 0.023). There was no negative or positive correlation between serum apelin levels and age, gestational week, fasting insulin levels and serum hs-CRP. There was a strong positive correlation between serum apelin levels and QUICKI insulin sensitivity (r=0.82, p<0.001) in the pregnant group with GDM compared to other case groups. A negative correlation was observed between serum apelin levels and HOMA insulin resistance index (r=-0.61, p<0.001) in the pregnant group with GDM.

Serum apelin levels, HOMA insulin resistance index, age, body-mass index, hs-CRP, and maternal diabetes were included in the logistic regression model. In the multivariate analysis (Table 3), older age (OR 1.20, 95% CI, p<0.001) was associated with a higher risk of GDM, while a higher serum apelin level was protective against GDM (OR 0.60, 95% CI, p = 0.040).

Table 3. Predictors of gestational diabetes mellitus in multivariate regression analysis

Variables	OR	95% CI	p
Age	**1.20**	**1.05-1.40**	**<0.001**
BMI	1.11	0.97 1.28	0.120
DM in the mother	2.87	0.81-10.19	0.101
Apelin	**0.60**	**0.30-1.00**	**0.040**
HOMA-IR	1.00	0.68-1.46	0.970
hs-CRP	1.02	0.94-1.11	0.580

BMI: Body-mass index; **DM:** diabetes mellitus; **HOMA-IR:** Homeostasis model assessment of insulin resistance; **hs-CRP:** High sensitive C-reactive protein.

Women in all three groups were compared in terms of serum apelin level (Figure. 1-a). A statistically significant difference was found between them (p=0.017). In the post-hoc analysis, it was understood that this difference was due to the difference in serum apelin levels between pregnant women with GDM and healthy pregnant women. The serum apelin level was found to be low in women with GDM. HOMA insulin resistance index was found to be statistically significantly higher in pregnant women with gestational diabetes compared to the control women group (p=0.025) (Figure. 1-b). Triglyceride / HDL ratio (p<0.001) and C-peptide / FPG ratio (p=0.02), which are indirect indicators of insulin resistance, were found to be significantly higher in the pregnant group with GDM compared to the control female group (Figure. 1-c/d).

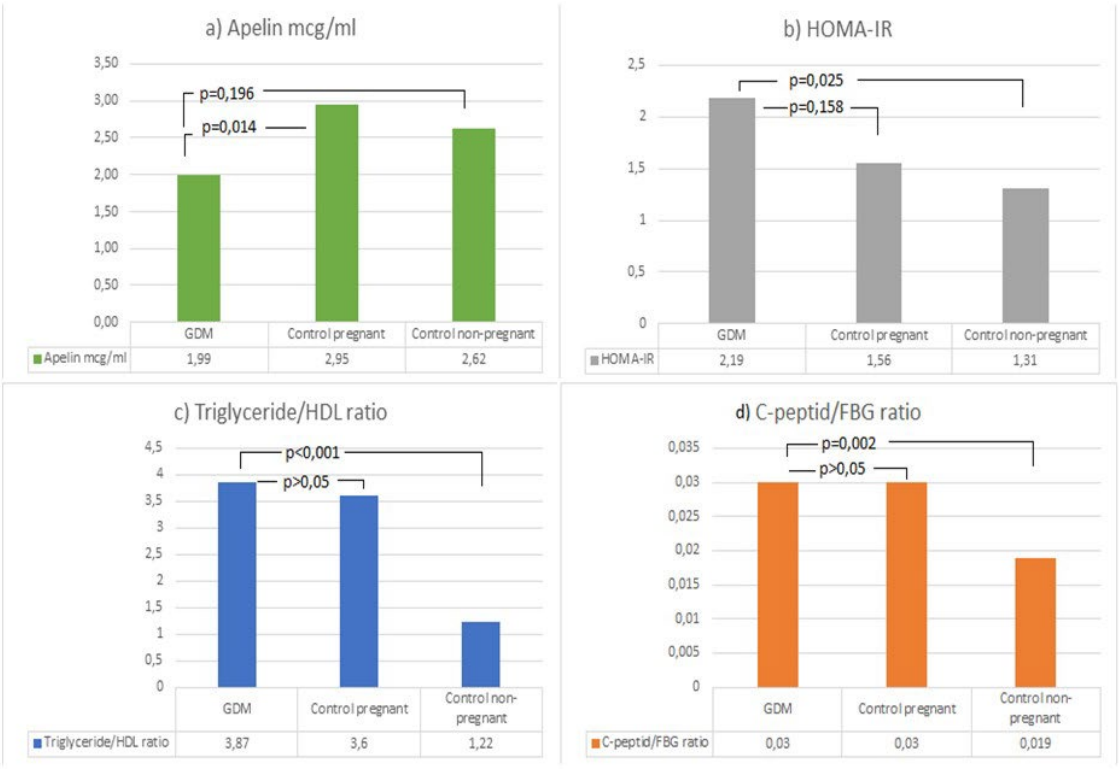

Figure 1. Serum apelin levels, HOMA-IR levels, triglyceride / HDL ratio, C-peptide/fasting blood glucose ratio in groups presented in figure 1.

DISCUSSION

The development of insulin resistance plays a fundamental role in the pathophysiology of GDM. Therefore, in our study, the effect of apelin, which is thought to be closely related to insulin resistance, on the development of GDM was examined. Apelin has been shown in cell culture and animal experiments to improve insulin sensitivity by increasing glucose utilization in insulin-sensitive tissues (5,8,9). In this study, serum apelin levels of pregnant women with GDM and control groups were determined and their relations with insulin resistance parameters were examined. Serum apelin level was found to be significantly lower in pregnant women with GDM compared to the control groups. While a significant and positive correlation was found between serum apelin level and QUICKI insulin sensitivity, a negative correlation was found between serum apelin level and HOMA insulin resistance index. In line with these analyzes, it was thought that there was a strong and important relationship between the development of GDM and serum apelin level.

Due to the role of apelin in placental blood flow and vasculogenesis, it is known that its production from the placenta increases in healthy pregnancy (12). According to the regression model in our study, it was determined that insufficient apelin production during pregnancy increased the risk of developing GDM (RR= 1.4, p=0.04). From this point of view, we think that insufficient apelin production or due to an increase in clereance during pregnancy is a factor that facilitates the development of GDM. There are few studies in the literature dealing with the effects of apelin on the physiology of pregnancy and the development of GDM (10- 11). In the research of Telejko et al., which includes the highest number of patients on this subject; 101 pregnant women with GDM and 101 pregnant women with normal glucose tolerance were included. There was no statistically significant difference between the two study groups in terms of serum apelin levels (10). In the same study, no correlation was observed between serum apelin level and insulin resistance indicators. In the study of Telejko et al., WHO criteria were used as the diagnostic criteria for GDM (75 g OGTT, serum fasting glucose was ≥ 100 mg/dl and ≥ 140mg/dl at 120. min) (11). This suggests that there may be pregnant women with GDM in the pregnant group, which was interpreted as normal glucose tolerance in the study. Therefore, in the study of Telejko et al., a difference in serum apelin levels and a relationship between apelin and GDM could not be found between the study groups. In the same study, subcutaneous-visceral adipose tissue and placental tissue sampling of 20 pregnant women with GDM and 16 pregnant women with normal glucose tolerance were performed in the peripartum period, and apelin mRNA levels in the tissues were measured. While there was no difference between pregnant women with GDM and healthy pregnant women in terms of apelin mRNA levels in tissues, it was determined that the level of apelin mRNA in placenta tissue was ten times higher than in adipose tissue. In our study, serum apelin level was found to be higher in healthy pregnant women compared to healthy non-pregnant women. From this point of view, it is thought that the production of apelin from the placenta increases during pregnancy and plays a role in the regulation of placental blood flow and vascularity.

In various studies conducted in obese and type 2 diabetics, the relationship between serum apelin level and insulin resistance parameters has been revealed (16-17). In a study by Erdem et al., which included 40 newly diagnosed type 2 diabetes patients and 40 healthy adults, serum apelin levels were found to be significantly lower in the diabetic group, and a negative correlation was found between serum apelin levels and HOMA insulin resistance index (17). Similarly, in our study, serum apelin levels were found to be significantly lower in the pregnant group with GDM compared to the healthy pregnant group, and a negative correlation was observed between serum apelin levels and HOMA insulin resistance index.

The limitations of our study are that it is a cross-sectional study and the determination of serum apelin level during pregnancy with a single measurement. Also we do not know whether the decrease in apelin in pregnant women with GDM is due to increased clearance or decreased production. Visceral adipose tissue and/or placental tissue mass range may affect on apelin level in patients with GDM. Visceral fat tissue determination with dual energy x-ray absorptiometry or post-partum placental mass was not measured. Also the low number of the study population should be considered in our limitations. On the other hand, the research group includes healthy pregnant women and healthy women and pregnant women with GDM expresses its methodological strength.

In conclusion, low serum apelin levels are associated with the development of GDM. More studies are needed to show if serum levels of apelin precede the development of GDM, and if it can predict GDM development early in pregnancy.

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Research Article

The effect of GNRI score on prognosis in patients with multivessel disease

GNRI skorunun çok damarlı hastalığı olan hastalarda prognoz üzerindeki etkisi

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ABSTRACT

Background: Geriatric Nutrition Risk Index (GNRI) is a simple and practical method used to evaluate the nutritional status of patients. Low GNRI scores are associated with poor outcomes and increased mortality. The aim of the study was to evaluate the association of the GNRI score with adverse outcomes in patients with multivessel disease.

Materials and Methods: Our study included 232 patients with multivessel disease from 2 centers between 01.01.2019-01.01.2021. Patients were divided into 2 groups according to GNRI score; GNRI > 98 normal nutrition and GNRI ≤98 malnutrition. All-cause mortality and major adverse cardiovascular events (MACE) rates were assessed at 36 months of follow**-up.**

Results: Approximately one third of the patients were in the low GNRI group (GNRI ≤ 98, n = 81, 34.9%). The low GNRI group had higher rates of MACE (45.7% vs. 21.9%, p < 0.001) and mortality (22.2% vs. 8.6%, p = 0.004). In multivariate Cox regression analysis, GNRI was identified as an independent predictor of both mortality and MACE (HR: 0.908, 95% CI: 0.864-0.954, p<0.001 and HR: 0.903, 95% CI: 0.873-0.934, p<0.001, respectively). In Kaplan-Meier analysis, both MACE and mortality were higher in the low GNRI group over time (Log-Rank Test=20.481, p<0.001 and Log-Rank Test=8.287, p=0.004, respectively).

Conclusion: In conclusion, this study demonstrated that GNRI is an independent predictor of MACE and all-cause mortality in patients with multivessel disease. Closer monitoring of patients with low GNRI and interventions to improve their nutritional status may contribute to improving their long-term prognosis.

Keywords: Multivessel disease, mortality, geriatric nutrition risk index, major adverse cardiovascular events.

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ÖZ

Geriatrik Beslenme Risk İndeksi (GNRI), hastaların beslenme durumunu değerlendirmek için kullanılan basit ve pratik bir yöntemdir. Düşük GNRI skorları kötü sonuçlar ve artmış mortalite ile ilişkilidir. Çalışmanın amacı, GNRI skorunun çok damarlı hastalığı olan hastalarda olumsuz sonuçlarla ilişkisini değerlendirmekti.

Gereç ve Yöntemler: Çalışmamıza 01.01.2019-01.01.2021 tarihleri arasında 2 merkezden çok damarlı hastalığı olan 232 hasta dahil edildi. Hastalar GNRI skoruna göre 2 gruba ayrıldı; GNRI >98 normal beslenme ve GNRI≤98 yetersiz beslenme. Her nedene bağlı mortalite ve majör olumsuz kardiyovasküler olay (MACE) oranları 36 aylık takipte değerlendirildi.

Bulgular: Hastaların yaklaşık üçte biri düşük GNRI grubundaydı (GNRI ≤ 98, n=81, %34,9). Düşük GNRI grubunda daha yüksek MACE oranları (%45,7'ye karşı %21,9, p<0,001) ve mortalite (%22,2'ye karşı %8,6, p=0,004) vardı. Çok değişkenli Cox regresyon analizinde, GNRI hem mortalite hem de MACE'nin bağımsız bir öngörücüsü olarak tanımlandı (sırasıyla HR: 0,908, %95 CI: 0,864-0,954, p<0,001 ve HR: 0,903, %95 CI: 0,873-0,934, p<0,001). Kaplan-Meier analizinde, hem MACE hem de mortalite düşük GNRI grubunda zaman içinde daha yüksekti (sırasıyla Log-Rank Test=20,481, p<0,001 ve Log-Rank Test=8,287, p=0,004).

Sonuç: Sonuç olarak, bu çalışma GNRI'nin çoklu damar hastalığı olan hastalarda MACE ve tüm nedenlere bağlı mortalitenin bağımsız bir öngörücüsü olduğunu göstermiştir. Düşük GNRI'li hastaların daha yakından izlenmesi ve beslenme durumlarını iyileştirmeye yönelik müdahaleler uzun vadeli prognozlarının iyileştirilmesine katkıda bulunabilir.

Anahtar Kelimeler: Çoklu damar hastalığı, mortalite, geriatrik beslenme risk indeksi, majör olumsuz kardiyovasküler olaylar.

INTRODUCTION

Coronary artery disease (CAD) remains one of the leading causes of morbidity and mortality worldwide (1). Multivessel disease represents a severe clinical picture in which there is more than 70% stenosis in more than one major coronary artery, and these patients are at higher risk of cardiovascular events(2-3). The frequency and severity of cardiovascular events in these patients may be exacerbated by both the nature of the disease and by comorbid conditions such as malnutrition. Individuals with multivessel disease often require more aggressive treatment approaches and their quality of life is severely affected.

Malnutrition is a widespread yet often overlooked issue among hospitalized patients, leading to significant and broad-ranging negative impacts on clinical outcomes (4). This situation results in high economic burden, longer hospital stays and higher mortality rates (5). Previous studies have reported that malnutrition leads to increased inflammatory response and progression of arterial calcification and atherosclerosis (6-7). This indicates that malnutrition may be a critical factor in the formation and progression of cardiovascular diseases, thus emphasizing the negative effects of nutritional deficiencies on cardiovascular risk factors. As a result, evaluating the nutritional status could play a crucial role in accurately determining risk stratification in patients with CAD.

The geriatric nutritional risk index (GNRI), developed by Bouillanne et al., is a simple and practical method that evaluates the nutritional status

of patients using parameters such as serum albumin level and body weight(8).GNRI is an index developed to comprehensively assess the nutritional status of elderly patients and to predict the morbidity and mortality risks that may develop due to nutritional deficiencies. According to this new nutritional tool, a low GNRI score is considered an indicator of malnutrition (9). Several studies have shown that GNRI predicts mortality in patients with chronic diseases such as chronic kidney disease, heart failure, and malignancies (10-12). In addition, there are some studies that have associated GNRI with adverse outcomes in patients with coronary artery disease (CAD) (13-15). However, the prognostic value of GNRI in CAD patients with multivessel disease has still not been fully elucidated.

The aim of this study is to comprehensively evaluate the prognostic value of GNRI in individuals with multivessel disease and also to determine the effect of GNRI on major adverse cardiovascular events (MACE) and all-cause mortality, thus revealing the contributions of this index to clinical practice.

MATERIALS AND METHODS

Study design

Patients who underwent coronary angiography in 2 centers between 01.01.2019 and 01.01.2021 and were detected to have multivessel disease were consecutively included in our study. A total of 255 patients were studied in a retrospective review, but 23 of these patients were excluded from the study because they did not meet the specified inclusion criteria, leaving the remaining 232 patients eligible for study analysis and included in the study (Figure. 1).

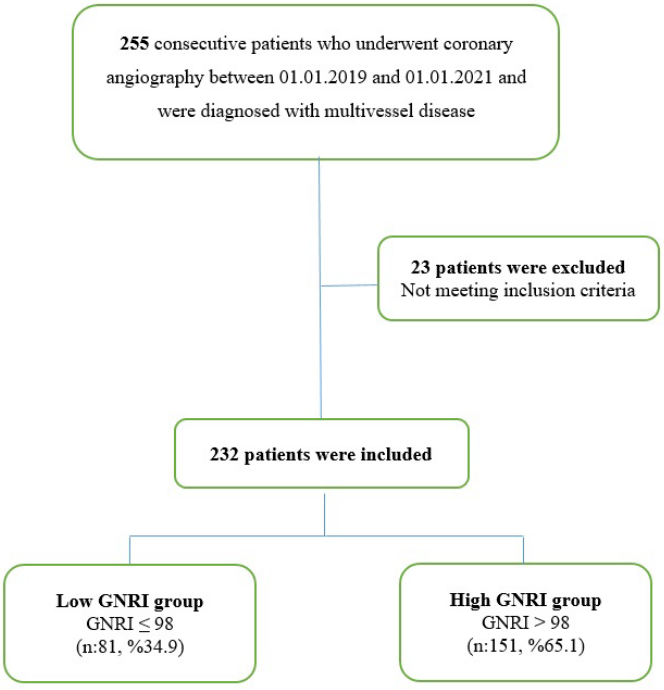

Figure 1. Study flowchart

The study group consisted of patients who underwent CAG for any reason and were diagnosed with multivessel disease. Multivessel disease was defined as the presence of two or more main coronary branches (vessel diameter ≥2.5 mm) with ≥70% stenosis degree(2). Patients with diagnoses of malignancy, severe liver and kidney disease, pregnancy, autoimmune diseases, severe valve disease, and severe pulmonary hypertension were excluded from the study. The study was conducted meticulously in accordance with the Declaration of Helsinki, which sets international ethical standards and research practice(2013). The local ethics committee approval required for the study was obtained (Diyarbakır Gazi Yaşargil Training and Research Hospital Ethics Committee, with date and number: 04/10/2024 and 173).

Assessment of patient characteristics

Data on demographic and clinical characteristics of patients were collected (e.g. gender, age, height, weight, BMI (body mass index), heart rate, blood pressure, medical history, family history and medical treatment received, etc.). Body mass index (BMI) was calculated by dividing individuals' body weight in kilograms by the square of their height in meters (kg/m2). Hypertension was defined as the use of antihypertensive medication to control blood pressure or the presence of blood pressure above 140/90 mm Hg. Patients with hemoglobin A1c >6.5% or those receiving antidiabetic therapy (treatment with diet, insulin, or oral agents) were considered to have Diabetes Mellitus. Patients with an estimated glomerular filtration rate (eGFR) of less than 60 ml/min/1.73 m^2 were defined as having chronic kidney disease. Peripheral blood samples were taken from the patients and basic metabolic parameters (glucose, urea, creatinine, etc.), complete blood count (white blood cell count, hemoglobin, hematocrit, etc.) and lipid profile were examined. Blood tests were taken at the time the patients were admitted to the hospital. All participants underwent echocardiography using the Vivid 7 (GE Vingmed Ultrasound, Horton, Norway) ultrasound system, and LVEF (left ventricular ejection fraction) was calculated with the modified Simpson method. Discharge medications [aspirin, clopidogrel, statins, β-blockers and angiotensin converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB)] were recorded.

Assessment of nutritional status

In our study, the nutritional status of the patients was analyzed with the GNRI score. The following formula was used to calculate the GNRI (8): GNRI = 41.7 × (weight/idealweight) (kg) + 1.489 × albumin (g/L).

Ideal body weight was calculated according to the participants' height, and was obtained by multiplying the square of the height in meters by 22, using the generally accepted method (16). In this score calculated, the weight/ideal body weight ratio is taken as 1 when the patient's' weight exceeds the ideal body weight (17). According to GNRI cut-off values, nutritional risk levels are as follows (9):

-GNRI >98: no risk
- GNRI 92–98: low risk
- GNRI 82–92: moderate risk
- GNRI<82: severe risk

According to the above values, GNRI >98 indicates that the patients have normal nutritional status, so we used 98 as the cut-off value in our study. Thus, patients were divided into two different groups in terms of nutritional status: GNRI >98 with normal nutrition and GNRI≤98 with malnutrition.

Follow-up and endpoints

The results of 36 months follow-up from the date of inclusion were reviewed. The endpoints of our study were all-cause death and MACE. All-cause death, nonfatal acute myocardial infarction, revascularization, and stroke were considered

MACE. Patient data were obtained from hospital digital databases and the national registry.

Statistical analysis

Statistical analysis of the data obtained in our study was performed using SPSS 22.0 (IBM Corp., Armonk, New York, USA) software. Kolmogorov-Smirnov test was performed to determine whether continuous variables showed normal distribution. Student t-test, a parametric test, was used to compare variables with normal distribution. On the other hand, Mann-Whitney U test, one of the non-parametric methods, was preferred for variables without normal distribution. Variables with normal distribution were described as mean ± standard deviation, and variables with abnormal distribution were described as median (interquartile range) values. In order to compare categorical variables, the Chi-square test was applied to determine the distribution differences between the groups. Kaplan-Meier analysis was performed to evaluate the 3-year survival probability of patients with low and high GNRI scores. In addition, the log-rank test was applied to determine the statistical significance of survival differences between the groups. To determine independent predictors of mortality and MACE, both univariate and multivariate Cox regression analysis models were applied to perform a comprehensive statistical analysis. In the statistical analyses, it was determined that the p value should be below 0.05 in order for the results to be considered statistically significant.

RESULTS

A total of 232 consecutive patients who underwent coronary angiography between January 1, 2019 and January 1, 2021 and were diagnosed with multivessel disease were included in the study. Patients were divided into two different groups based on GNRI values to determine their nutritional status: low GNRI group (GNRI ≤ 98, n=81, 34.9%) and high GNRI group (GNRI > 98, n=151, 65.1%). The basic characteristics, demographic data and results of these groups are summarized in Table 1.

Table 1. Basic clinical and laboratory characteristics of patients according to GNRI groups

	Low GNRI score (GNRI≤98, n= 81)	**High GNRI score (GNRI>98, n= 151)**	***p* value**
GNRI	91.1±4.3	106.4±5.6	**<0.001**
Gender (Female), n(%)	30(37.0)	49(32.5)	0.482
Age, (years)	70.8±7.1	65.4±8.0	**<0.001**
Body mass index, (kg/m2)	26.4±3.1	29.1±5.0	**<0.001**
Heart Rate (minute)	84.9±15.3	78.4±12.9	**0.001**
Systolic Blood Pressure (mmHg)	123.9±13.0	130.0±16.4	**0.005**
Diastolic Blood Pressure(mmHg)	76.7±9.7	80.3±10.4	**0.012**
Diagnosis -STEMI, n(%) -NSTEMI, n(%) -Unstable angina, n(%) -Stable CAD, n(%)	 9(11.1) 22(27.2) 30(37.0) 20(24.7)	 22(14.6) 35(23.2) 48(31.8) 46(30.5)	0.602
Angiography result, n(%) -Medical therapy -PCI	 17(21.0) 50(61.7)	 17(11.3) 101(66.9)	0.125

-CABG	14(17.3)	33(21.9)	
HT, n(%)	50(61.7)	113(74.8)	**0.037**
DM, n(%)	49(60.5)	83(55.0)	0.418
Dyslipidemia, n(%)	37(45.7)	97(64.2)	**0.006**
CKD, n(%)	21(25.9)	22(14.6)	**0.034**
Smoking, n (%)	44(64.3)	60(39.7)	**0.033**
LVEF, (%)	48.5±9.4	53.2±7.9	**<0.001**
WBC, ($x10^3$/uL)	10.5±2.1	9.8±1.8	**0.011**
Hgb, (gr/L)	11.7±2.2	12.6±2.0	**0.006**
Glucose, (mg/dl)	132(116-161)	128(115-148)	0.415
Creatinine, (mg/dL)	0.92(0.80-1.25)	0.88(0.75-1.04)	**0.042**
GFR, (mL/min)	72.8±21.8	82.4±20.6	**0.001**
Albumin, (gr/L)	33.2±2.9	43.5±3.7	**<0.001**
CRP, (mg/dL)	1.10(0.17-2.50)	0.71(0.10-2.50)	0.149
Total cholesterol, (mg/dl)	176.6±33.5	194.3±44.1	**0.002**
HDL cholesterol, (mg/dl)	36.5±6.5	37.2±6.3	0.448
LDL cholesterol, (mg/dl)	108.1±26.1	121.1±39.2	**0.003**
Triglyceride(mg/dl),	159.5±62.5	179.7±59.2	**0.016**
Uric acid, (mg/dl)	6.0±1.6	5.8±1.4	0.327
Medical treatment, , n(%)			
-Aspirin	80(98.8)	150(99.3)	0.653
-P2Y12 inhibitors	62(76.5)	118(78.1)	0.780
-Beta-blocker	50(61.7)	108(71.5)	0.127
-ACEI/ARB	35(43.2)	72(47.7)	0.515
-Statins	68(84.0)	132(87.4)	0.465
MACE, n(%)	37(45.7)	33(21.9)	**<0.001**
Mortality, n(%)	18(22.2)	13(8.6)	**0.004**

Abbrevations: ACEI: Angiotensin Converting Enzyme Inhibitor, **ARB**: Angiotensin Receptor Blocker, **BUN**: Blood Urea Nitrogen, **CABG**: Coronary Artery Bypass Grafting, **CAD**: Coronary Artery Disease, **CKD**: Chronic Kidney Disease, **CMP**: Cardiomyopathy, **CRP**: C-Reactive Protein, **DM**: Diabetes Mellitus, **GFR**: Glomerular Filtration Rate, **GNRI**: Geriatric Nutritional Risk Index, **HDL**: High-density Lipoprotein, **Hgb**: Hemoglobin, **HT**: Hypertension, **LDL:** Low-density lipoprotein, **LVEF**: Left Ventricular Ejection Fraction, **MACE**: Major Adverse Cardiovascular Events, **NSTEMI:** Non- ST-Segment Elevation Myocardial Infarction, **PCI**: Percutaneous Coronary Intervention, **STEMI:** ST-Segment Elevation Myocardial Infarction, **WBC:** White Blood Cell

Patients in the low GNRI group were significantly older (70.8±7.1 vs. 65.4±8.0, p<0.001) and had a lower body mass index (26.4±3.1 vs. 29.1±5.0, p<0.001) compared to those in the high GNRI group. In addition, the low GNRI group had higher heart rate, lower systolic and diastolic blood pressure, and higher prevalence of smoking. Hypertension and dyslipidemia were higher, while chronic kidney disease was lower, in the high GNRI group. In patients in the low GNRI group, LVEF, hemoglobin, glomerular filtration rate (GFR), albumin, total cholesterol, triglyceride and LDL cholesterol levels were significantly lower. In contrast, white blood cell count and creatinine levels were higher. Importantly, the low GNRI group exhibited worse outcomes, with a significantly higher rate of MACE (45.7% vs. 21.9%, p<0.001) and mortality (22.2% vs. 8.6%, p=0.004).

As a result of 3 years follow-up, a total of 70 patients had MACE and 31 patients had mortality.

Considering whether mortality and MACE developed, patients were compared in terms of GNRI scores. Significantly lower GNRI scores were found in patients who developed MACE and mortality [96.7(87.8-101.2) vs 104.2 (98.2-108.7), p<0.001 and 92.3 (86.3-101.2) vs 102.7(96.0-108.7), p<0.001, respectively] (Figure. 2)

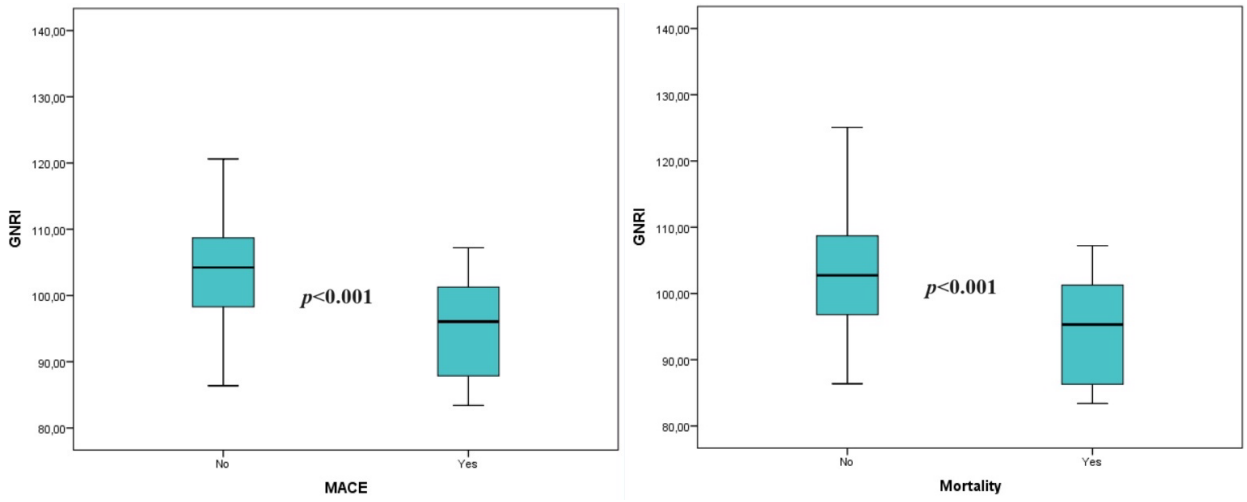

Figure 2. Comparison of patients with and without mortality and MACE in terms of GNRI scores in a box plot graph

Using univariate and multivariate Cox regression analysis models, independent predictors of 3-year mortality and MACE were determined in our study (Table 2).

Table 2. Independent predictors of mortality and MACE in Univariate and Multivariate Cox Regression analysis models

	Univariate analysis			**Multivariate analysis**		
Mortality	**HR**	**95%CI**	***p***	**HR**	**95%CI**	***p***
Gender	1.811	0.781-4.204	0.167			
Age	1.072	1.023-1.125	**0.004**	1.031	0.983-1.082	0.206
HT	0.903	0.425-1.917	0.790			
DM	2.200	0.984-4.918	0.055			
GNRI	0.898	0.857-0.940	**<0.001**	0.908	0.864-0.954	**<0.001**
Smoking	1.121	0.554-2.268	0.750			
Dyslipidemia	1.947	0.954-3.973	0.067			
CKD	0.818	0.314-2.129	0.680			
BMI	0.960	0.886-.1040	0.319			
	Univariate analysis			**Multivariate analysis**		
MACE	**HR**	**95%CI**	***P***	**HR**	**95%CI**	***p***
Gender	0.984	0.601-1.613	0.950			

Age	1.074	1.040-1.108	**<0.001**	1.032	1.000-1.065	0.052
HT	0.826	0.501-1.360	0.452			
DM	1.991	1.176-3.372	**0.010**	1.315	0.770-2.248	0.316
GNRI	0.888	0.860-0.916	**<0.001**	0.903	0.873-0.934	**<0.001**
Smoking	0.817	0.508-1.315	0.405			
Dyslipidemia	0.718	0.450-1.148	0.166			
CKD	1.015	0.555-1.853	0.962			
BMI	0.927	0.876-0.981	**0.009**	0.970	0.914-1.029	0.307

Abbrevations: BMI: Body Mass Index, **CI:** Confident Interval**, CKD:** Chronic Kidney Disease, **DM:** Diabetes Mellitus, **GNRI:** Geriatric Nutritional Risk Index, **HT:** Hypertension**, OR:** Odds Ratio

In the univariate analysis performed for mortality, age (HR: 1.072, 95% CI: 1.023-1.125, p=0.004) and GNRI value (HR: 0.898, 95% CI: 0.857-0.940, p<0.001) were determined as independent predictors. Multivariate analysis results showed that only GNRI (HR: 0.908, 95% CI: 0.864-0.954, p<0.001) was found to be significant as an independent predictor. For MACE, age (HR: 1.074, 95% CI: 1.040-1.108, p<0.001), DM (HR: 1.991, 95% CI: 1.176-3.372, p=0.010), GNRI (HR: 0.888, 95% CI: 0.860-0.916, p<0.001) and BMI (HR: 0.927, 95% CI: 0.876-0.981, p=0.009) were found to be independent predictors in univariate analysis. In multivariate analysis, GNRI (HR: 0.903, 95% CI: 0.873-0.934, p<0.001) was also found to be significant as an independent predictor.

Kaplan-Meier analysis was conducted to investigate the association between low and high GNRI groups and mortality and MACE during the 3-year follow-up period. According to this analysis, both MACE and mortality were higher in the low GNRI group over time (Log-Rank Test=20.481, p<0.001 and Log-Rank Test=8.287, p=0.004, respectively) (Figure. 3).

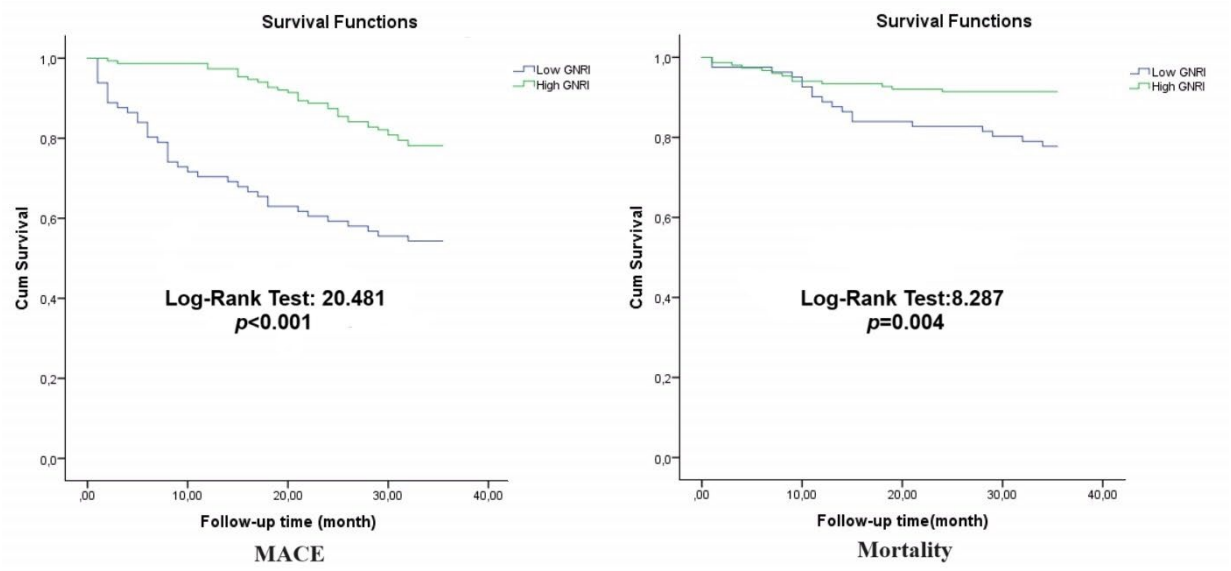

Figure 3. Kaplan-Meier analysis of the association between low and high GNRI groups and MACE and mortality during the 3-year follow-up period

DISCUSSION

In our study, we examined the prognostic value of GNRI in patients diagnosed with multivessel coronary artery disease and its effect on the clinical outcomes of patients. Our study showed that both MACE and mortality were significantly higher in patients with low GNRI values. These results suggest that GNRI may be a prognostic indicator in patients with multivessel disease and that malnutrition may have a significant impact on clinical outcomes in this group of patients.

GNRI is a score that indicates nutritional status and is calculated using routinely measured serum albumin, weight and height parameters in hospitalized patients (8). Serum albumin and BMI values used in GNRI measurement have also been evaluated as indicators of nutritional status in some studies (18-19). However, factors like inflammation, dehydration, and heart failure can influence these measurements (20). The GNRI covers more than just the overlap of these two parameters, hence serving as a more reliable indicator. The prognostic value of GNRI has been previously demonstrated in various studies in chronic diseases such as coronary artery disease, heart failure, and chronic kidney disease (15,17,21). However, the prognostic role of GNRI in CAD patients with multivessel disease has not been evaluated. Our study fills this gap, revealing that patients with low GNRI may experience higher MACE and mortality rates. In particular, the fact that patients in the low GNRI group are older, have lower body mass indexes, and have poor clinical features suggests that this patient group is more fragile.

The association of low GNRI with MACE and mortality suggests the adverse effects of malnutrition on cardiovascular events. Malnutrition may contribute to increased inflammation, progression of atherosclerosis, and acceleration of vascular calcification (22-23). Additionally, low albumin levels and loss of body weight can increase the risk of complications by weakening the body's defense mechanisms (24). The energy metabolism of cardiomyocytes plays a crucial role in the cardiac remodeling and heart failure processes that frequently occur following coronary artery disease (25). The occurrence of serious complications such as infection in individuals with multivessel disease may further reduce the already limited metabolic reserve. In addition, the inability of malnourished patients to perform recommended physical activities may increase the risk of hypercoagulation in this group, making them more vulnerable to coronary events (14). These findings emphasize that the nutritional status of patients with multivessel disease should be regularly assessed and patients with low GNRI should be closely followed.

Our study also revealed that patients with lower GNRI scores were older, had lower BMI, and presented with poorer clinical parameters such as low LVEF, low hemoglobin levels, and impaired renal function. These findings suggest that malnutrition may contribute to the poorer overall health status of these patients, further exacerbating existing cardiovascular burden. Interestingly, despite the well-known role of traditional risk factors such as hypertension, dyslipidemia, and chronic kidney disease, our findings suggest that nutritional status assessed by the GNRI provides additional prognostic value beyond these factors. Kaplan-Meier survival analysis further clarified the clear distinction between patients with low and high GNRI scores, with the low GNRI group showing significantly higher rates of MACE and death over time. These results suggest that routine assessment of nutritional status using simple tools such as the GNRI may aid in risk stratification of CAD patients, allowing for more

targeted interventions aimed at improving both nutritional status and cardiovascular outcomes.

Studies have shown that well-implemented nutritional interventions can lead to notable reductions in both hospital stay durations and mortality rates among malnourished patients (26). However, nutritional support is often neglected by physicians in patients with coronary artery disease (27). This article highlights the significance of evaluating nutritional status in individuals with coronary artery disease. The findings of our study also indicate that GNRI is not limited to elderly patients but may be a valuable prognostic tool in a wider patient population. The clinical use of GNRI can be expanded due to its simplicity and easy calculation, allowing early identification and intervention of high-risk patients. However, further studies in larger patient groups and different populations are required for the full integration of GNRI into clinical practice.

Limitations

Our study has some limitations that should be taken into consideration in terms of validity and reliability of the results obtained. First of all, the fact that this study was retrospective and conducted in two centers may restrict the generalizability of the results. Second, the use of the GNRI as a nutritional assessment tool, although practical, may not capture all aspects of malnutrition. Third, the GNRI score was measured only at admission, and changes over time could not be assessed. Finally, we did not assess other potential factors that may have influenced nutritional status, such as inflammatory markers or socioeconomic status, which may have influenced the results.

CONCLUSION

This study demonstrates the prognostic value of GNRI in patients with multivessel disease. According to our findings, patients with low GNRI values are at higher risk for both MACE and mortality. The negative effects of malnutrition on clinical outcomes in this patient group indicate that GNRI may be a valuable tool in cardiovascular risk assessment. Integrating nutritional status assessed by GNRI into routine clinical practice may be beneficial for early diagnosis of high-risk patients and improvement of clinical outcomes with nutritional interventions. However, further studies in larger and more diverse patient populations will contribute to our better understanding of the prognostic value and clinical utility of GNRI.

Conflict of interest disclosure

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Research Article

The role of stem cell markers in choriocarcinoma: Immunocytochemical analysis in JAR cell line

Koryokarsinomda kök hücre belirteçlerinin rolü: JAR hücre hattında immünositokimyasal analiz

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ABSTRACT

Objective: Choriocarcinoma is a type of cancer that originates from placental trophoblastic tissue and has a high malignant potential. The aim of this study was to investigate the distribution of stem cell and cancer stem cell markers in the JAR cell line. The study is to provide information about the biology of choriocarcinoma and the mechanisms of resistance to treatment.

Materials and Methods: JAR cells were cultured in RPMI 1640 culture medium. Colony formation assay was performed in JAR cells. The expression of OCT3/4, CD133, C-KIT, SALL4 and CD90 markers were analysed using indirect immunoperoxidase method. Healthy endometrial epithelial cell line RL95-2 was used for statistical analysis.

Results: JAR cells showed statistically high colony formation efficiency compared to the RL95-2 group. Immunocytochemical analyses showed strong immunoreactivity for CD133 and moderate immunoreactivity for SALL4. C-KIT, OCT3/4 and CD90 showed weak immunoreactivity.

Conclusion: The JAR cell line has emerged as a valuable model for trophoblastic cell invasion and placentation studies in choriocarcinoma. Markers such as CD133 and SALL4 provide important information about stem cell-like characteristics and choriocarcinoma malignancy. Lower expressions of C-KIT, OCT3/4 and CD90 reflect heterogeneous stem cell profiles in JAR cells. This diversity provides important insights into the complex nature of choriocarcinoma stem cells and sheds light on potential therapeutic approaches.

Keywords: Choriocarcinoma, JAR cell line, stem cell markers, cancer stem cells, trophoblastic malignancy.

ÖZ

Amaç: Koryokarsinom, plasental trofoblastik dokudan köken alan ve yüksek malignite potansiyeline sahip bir kanser türüdür. Bu çalışmanın amacı, JAR hücre hattında kök hücre ve kanser kök hücre belirteçlerinin dağılımını incelemek ve koryokarsinom biyolojisi ile tedaviye direnç mekanizmalarına dair bilgiler sağlamaktır.

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Gereç ve Yöntem: JAR hücreleri, RPMI 1640 kültür ortamında kültüre edildi. Indirekt immünoperoksidaz yöntemi kullanılarak OCT3/4, CD133, C-KIT, SALL4 ve CD90 belirteçlerinin ifadesi analiz edildi.

Bulgular: JAR hücre hattı, 2D kültür ortamında sferoid benzeri yapılar oluşturmuştur. İmmünositokimyasal analizler, CD133 için güçlü immünoreaktivite ve SALL4 için hafif immünoreaktivite göstermiştir. CD90 negatif gözlenmiştir, C-KIT ve OCT3/4 ise zayıf immünoreaktivite sergilemiştir.

Sonuç: JAR hücre hattı, koryokarsinomda trofoblastik hücre invazyonu ve plasentasyon araştırmalarında değerli bir model olarak öne çıkmaktadır. CD133 ve SALL4 gibi belirteçler, kök hücre benzeri özellikler ve koryokarsinom malignitesi ile ilgili önemli bilgiler sağlamaktadır. C-KIT ve OCT3/4'ün daha düşük ekspresyonları, JAR hücrelerinde heterojen kök hücre profillerini yansıtmaktadır. Bu çeşitlilik, koryokarsinom kök hücrelerinin karmaşık yapısına dair önemli bilgiler sağlayarak, potansiyel tedavi yaklaşımlarına ışık tutmaktadır.

Anahtar Kelimeler: Koryokarsinom, JAR hücre hattı, kök hücre belirteçleri, trofoblastik malignite, kanser kök hücreleri.

INTRODUCTION

Choriocarcinoma is a highly aggressive malignancy arising from placental trophoblastic tissue, characterized by rapid growth, early metastasis, and high mortality rates. Although choriocarcinoma is known to be sensitive to chemotherapy compared to other trophoblastic diseases, resistance to treatment can occur in advanced stages, emphasizing the importance of understanding the resistance mechanisms to develop more effective treatment strategies (1).

The human choriocarcinoma cell line (JAR) exhibits a sphere-like structure and is frequently used to understand the biology of choriocarcinoma in studies of cellular proliferation, differentiation, and trophoblastic malignancy. Despite several studies investigating the role of JAR cells in various biological processes, there is limited information on the distribution and functional roles of stem cell and cancer stem cell markers (2).

Analysis of stem cell markers plays an important role in cancer biology. OCT3/4, CD133, C-KIT (CD117), SALL4 and CD90 (THY1) are important markers associated with pluripotency, cancer stem cells and metastasis (3-7). OCT3/4 is a crucial transcription factor in maintaining pluripotency in embryonic stem cells and is involved in stem cell self-renewal and differentiation. It has also been shown to contribute to maintaining of cancer stem cell properties in various cancers. CD133 is a well-known marker for cancer stem cells in many tumor types, including brain and colon cancers. C-KIT, a proto-oncogene, is involved in tumor progression and especially metastasis of breast and prostate cancers, where they are mutated and associated with aggressive disease (8-9). SALL4, a transcriptional factor, is recognized for its role in promoting metastasis and enhancing the invasive ability of cancer cells through pathways such as TGF-β/SMAD (4, 10, 11). Furthermore, SALL4 expression is also linked to negative prognosis in various cancer contexts, including gastric and colorectal cancers associated with markers of epithelial-mesenchymal transition (EMT) (12-13). CD90 expression is a marker for the establishment of cancer stem cells and

promotes tumor initiation, progression and metastases by modulating cell adhesion and signalling pathways (14-16). Taken together, these markers provide strong evidence for the interdependent nature of tumor self-renewal, promoting cancer progression and malignancy. In light of this information, the expression and distribution of markers in JAR cells are critical to gainining important insights into trophoblastic malignancy and stem cell biology.

However, there is a lack of information in the literature regarding the expression of these markers in the JAR cell line. This is important for studies on choriocarcinoma biology and resistance mechanisms (17). This research aimed to analyze the distribution of key stem cell and cancer stem cell markers, including OCT3/4, CD133, C-KIT, SALL4, and CD90, in the JAR cell line. Investigating the expression of these markers in the JAR cell line is aimed at providing new information about trophoblastic malignancy and its potential to carry stem cell properties (18).

MATERIAL AND METHODS

Cell Culture

JAR (HTB-144) and endometrial epithelial cell line RL95-2 (CRL-1671) were obtained commercially from the American Type Culture Collection (ATCC). JAR cells were cultured in RPMI 1640 (11875093, Gibco) and RL95-2 cells were cultured in DMEM:F12 (Biosera, LM_D12221500) culture medium with 10% fetal bovine serum (FBS) (26140079, Gibco), 1% penicillin-streptomycin (15140148, Gibco) and 1% L-glutamine (25030081, Gibco). After the cells reached 80% confluence, the culture medium was removed and Trypsin-EDTA (T4049, Sigma-Aldrich) was added. After incubation at 37□C for 10 minutes, the cells were collected in 5 ml of culture medium and centrifuged at 1000 rpm for 5 minutes. The supernatant was removed, and the cells were suspended in new culture medium and seeded in a 24-well plate with a round coverslip for immunocytochemistry study.

Colony Forming Assay

A colony formation assay was performed to evaluate the clonogenic capacity of JAR cells. Cells were seeded in a 6-well plate at a density of 500 cells per well and cultured in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin-streptomycin and 1% L-glutamine. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 10 days (ESCO, CCL-170B-8).

Colonies were observed directly using a phase-contrast microscope (Olympus, IX71) to avoid any interference from fixation or staining. Images were taken at regular intervals, and colonies were defined as clusters containing >50 cells. Quantification was performed by manual counting of colonies in phase-contrast images, providing an accurate assessment of colony-forming potential (19- 20).

Immunocytochemistry

JAR and RL95-2 cells were fixed in 4% paraformaldehyde (158127, Sigma-Aldrich) prepared with phosphate buffered solution (PBS, P4417, SentezLab) for 30 min at 4 °C. Cells were treated with 0.01% Tween 20 (P1379, Sigma Aldrich) for 15 min for permeabilization. After washing with PBS, endogenous peroxidase activity was inhibited by 3% hydrogen peroxide (H2O2, 7722-84-1, Santa Cruz) for 5 min at room temperature. Cells were then washed with PBS and incubated with anti-OCT3/4 (A1759, R&D), anti-CD133 (MA5-44377, Thermo Fisher), anti-C-KIT (ab283653, Abcam), anti-SALL4 (#720030, Thermo Fisher), and anti-CD90 (ab307736, Abcam) at 4°C overnight at a dilution ratio of 1:250 for all primary antibodies.

Biotinylated secondary antibody in the Histostain-Plus IHC Kit (HRP, 859043, ThermoFisher) was dropped onto the slides and incubated for 10 minutes. Diaminobenzidine (DAB, 8059, Cell Signalling) was used as chromogen to show immunoreactivity and incubated for 5 minutes.

The samples were washed once with PBS and 3 times with distilled water and counterstained with Mayer haematoxylin (109249, Merck Millipore) for 1 minute. After washing 3 times with distilled water, the slides were covered with closing solution (109016, Merck Millipore). The slides were then examined under a light microscope (Olympus BX40, Tokyo, Japan).

Immunocytochemistry staining intensity for CD133, C-KIT, CD90, OCT3/4, and SALL4 markers was measured using the H-SCORE formula: H-SCORE = Σл(i + 1). Where i represents the staining intensity (1 = weak, 2 = moderate, 3 = strong) and л represents the percentage of cells stained at each intensity level (from 0 to 100%) (21). Intensity levels were categorised as follows:

Weak (1): Light brown staining visible under a microscope.

Moderate (2): Brown staining of medium intensity, clearly distinguishable from the background.

Strong (3): Dark brown staining indicating high marker expression.

H-SCORE provides a semi-quantitative assessment of markers by combining intensity and distribution.

Statistical Analysis

Normality tests of the experimental data were performed using the Shapiro-Wilk test. Parametric data were analysed using a one-way ANOVA test and then compared between the selected groups. Statistical comparisons were analysed using Sidak post hoc test. Non-parametric data were analysed using Kruskal-Wallis test followed by a post hoc Dunn's test. Data are presented as mean ± SEM. GraphPad Prism software version 9 was used for all statistical analyses, and statistical significance was set at p-value < 0.05.

RESULTS

JAR choriocarcinoma cells were epithelioid and demonstrated a colony-forming growth pattern in 2D culture medium. However, the endometrial epithelial cell line RL95-2 cells showed fibroblastic growth without colony formation (Figure. 1).

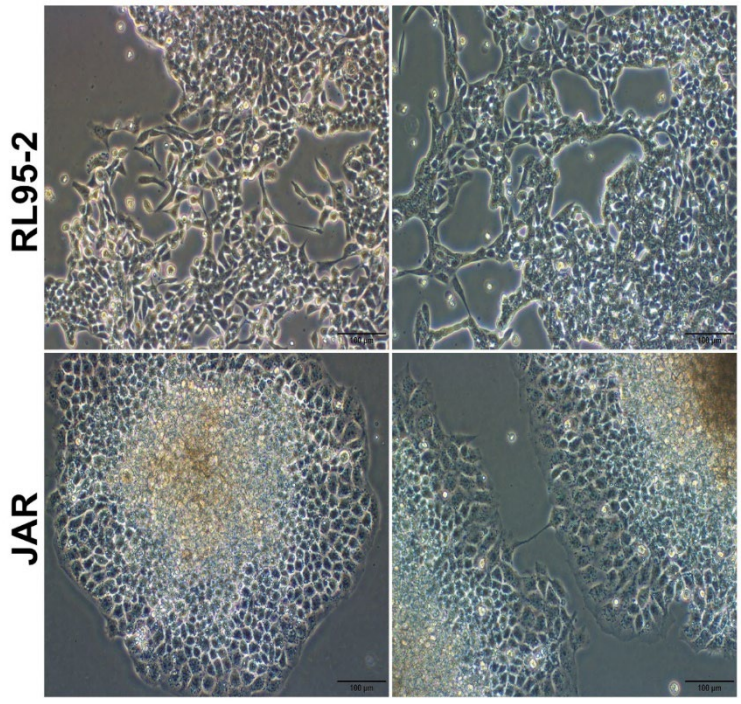

Figure 1. Cell culture photographs of RL95-2 and JAR cell lines. Scale bars: 100 µm.

The colonies formed in JAR cells were visualised by phase-contrast microscopy on days 1, 7 and 10 of culture and analysed by colony formation assay (Figure. 2).

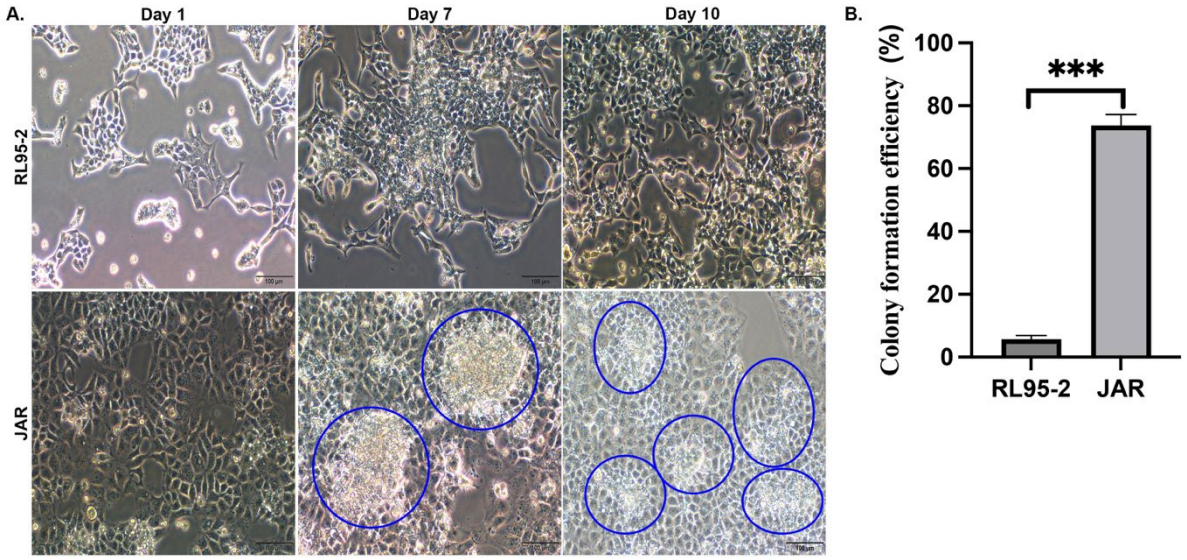

Figure 2. Phase-contrast image of colony formation on days 1, 7, and 10 of culture in RL95 and JAR cells. Scale bars: 100 µm (A). Statistical analysis of colony formation efficiency on day 10 of culture in RL95-2 and JAR cells (B).

The first colony formation in JAR cells was observed on the 7th day of culture, and the colony formation increased on the 10th day of culture (Figure 2A). However, no colony formation was observed in RL95-2 cells during the culture period (Figure 2A).

Colony formation efficiency was statistically higher in JAR cells compared to RL95-2 cells (Figure 2B).

Immunohistochemical analyses revealed that CD133, C-KIT, CD90, OCT3/4, and SALL4 immunoreactivities were negative in RL95-2 cells (Figure 3, Table 1).

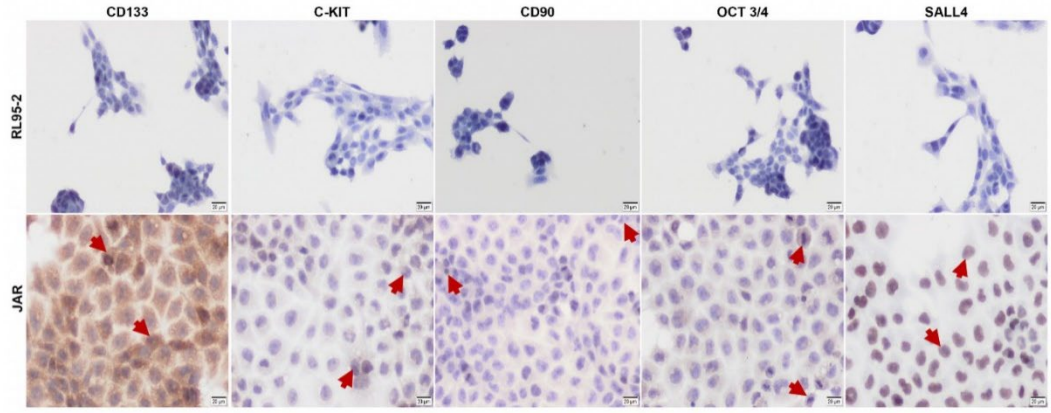

Figure 3. Immunohistochemical staining of CD133, C-KIT, CD90, OCT3/4 and SALL4 in RL95-2 and JAR cells. Red arrows indicate the staining areas of each antibody. Scale bars: 20 µm.

Table 1. H-SCORE results of CD133, C-KIT, CD90, OCT3/4 and SALL4 proteins in RL95-2 and JAR cells.

	CD133	**C-KIT**	**CD90**	**OCT3/4**	**SALL4**
RL95-2 cells	0	0	0	0	0
JAR cells	396.7 ± 2.887	230 ± 5.0	125 ± 8.05	275 ± 5.0	316.7 ± 5.774

In JAR cells, CD133 immunoreactivity was found to be strongly positive. Moreover, CD133 staining pattern was determined to be cytoplasmic (Figure 3, Table 1). In JAR cells, C-KIT immunoreactivity was weakly positive, and the staining pattern was cytoplasmic (Figure 3, Table 1). In JAR cells, CD90 immunoreactivity was weak, and the staining pattern was cytoplasmic and membranous (Figure 3, Table 1). OCT3/4 immunoreactivity in JAR cells was observed to be weakly intense, and the staining pattern was determined to be nuclear (Figure 3, Table 1). SALL4 immunoreactivity suggesting that it plays a role in the regulation of embryonic stem cells was found to be of moderate intensity in JAR cells, and the staining pattern was nuclear (Figure 3, Table 1).

DISCUSSION

Our study revealed that the colony-forming capacity of JAR cells, a choriocarcinoma cell line, is a critical indicator of biological properties such as proliferation ability, tumorigenic potential, cancer stem cell properties, and resistance to therapy. JAR cells have been characterized for their ability to form colonies, which is closely related to the properties of cancer stem cells. In particular, the capacity for self-renewal and differentiation is a hallmark of cancer stem cells, and JAR cells have been shown to exhibit these characteristics. These findings are consistent with the literature (22).

The markers OCT3/4, CD133, C-KIT, SALL4 and CD90 have been extensively studied in various cellular contexts, particularly concerning their role in cancer stem cells, tumor progression, and therapeutic resistance. OCT3/4 is a transcription factor critical for maintaining pluripotency in embryonic stem cells. Its expression is associated with stem cell self-renewal and differentiation and has been shown to play a role in several cancers, where it helps maintain cancer stem cell properties. For example, OCT3/4 expression in breast cancer is associated with aggressive tumor behaviour and poor prognosis (22). CD133 is a well-known marker for cancer stem cells in multiple tumor types, including brain and colon cancers. Since CD133+ cells show greater self-renewal capacity and resistance to conventional

therapies, their expression is associated with enhanced tumor formation and metastatic potential (12- 23). C-KIT expression is associated with poor prognosis and resistance to treatment in hematological malignancies, especially in acute myeloid leukemia. Its role in solid tumors such as gastrointestinal stromal tumors (GISTs) further emphasizes its importance in cancer biology (23). SALL4 is a transcription factor that has emerged as a critical player in various cancers, including gastric and ovarian cancers. It is associated with stem cell regulation and epithelial-mesenchymal transition (EMT) and promotes invasion and metastasis (4-12). SALL4 expression is often associated with aggressive tumor characteristics and poor patient outcomes, making it a potential therapeutic target (24-25). CD90 expression is associated with increased tumor aggressiveness in various cancers, including glioblastoma and nasopharyngeal carcinoma. CD90+ cells often exhibit increased migratory and invasive capabilities. This contributes to tumor progression (26).

The present study has revealed that JAR cells express high levels of CD133 and SALL4, which are associated with enhanced invasive behavior and proliferation. These findings are similar to those observed in other malignancies (4-24). OCT3/4 expression in JAR cells suggests a mechanism by which these cells avoid differentiation and retain their malignant phenotype. Furthermore, the presence of C-KIT indicates that there is a way in which JAR cells can support their growth and survival in the tumor microenvironment, which is in agreement with studies linking C-KIT expression to poor prognosis in various cancers (12-23). The role of SALL4 in JAR cells is particularly noteworthy because it has been shown to regulate cell proliferation and invasion, reinforcing its importance as a therapeutic target (24-25). In addition, CD90 expression in JAR cells suggests that these cells may use CD90-mediated pathways to facilitate tumor progression and metastasis. This finding is consistent with the previous study demonstrating that CD90+ cells exhibit increased levels of aggressiveness in a range of cancer types (26).

The expression of these markers in the JAR cell line used to study choriocarcinoma suggests their potential importance in understanding the biology of this malignancy. The presence of OCT3/4, CD133, C-KIT, SALL4, and CD90 in JAR cells suggests that these cells may exhibit characteristics similar to cancer stem cells and may contribute to their invasive ability and therapeutic resistance.

In summary, the expression of OCT3/4, CD133, C-KIT, SALL4 and CD90 in the JAR cell line highlights the complexity of choriocarcinoma biology and reveals the potential of these markers as therapeutic targets. The findings from our study not only contribute to the understanding of the molecular mechanisms driving choriocarcinoma but also pave the way for future research aimed at developing targeted therapies that may improve patient outcomes.

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Research Article

Knowledge and Awareness of Anesthesiologists about Di(2-EthylHexyl) Phthalate in Turkey: A Survey Study

Türkiyede anestezi hekimlerinin Dİ (2-ETİLHEKSİL) fitalat hakkında bilgi ve farkındalığı; Anket Çalışması

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ABSTRACT

Objective: This study aims to evaluate the knowledge and experiences of anesthesiologists in Turkey about the presence and hazards of DEHP and increase their awarenesses.

Materials and Methods: The questionnaire study consisting of web based 20 survey questions about DEHP is sent to anesthesiologists in Turkey via electronic mail. Participants were asked questions about whether they heard the name of the DEHP, whether they knew the harmful effects that the DEHP could cause, and whether it was the effect of DEHP in the selection of the operating room and intensive care medical supplies.

Results: We determined that 70% of anesthesiologists have never heard of 'the name or notion of DEHP. The study also demonstrates that 90-95% of them do not know whether the medical supplies that they use contain DEHP and they suffer from lack of knowledge about the purchase and selection of medical supplies.

Conclusion: According to the data of this research, the majority of the anesthesiologists in Turkey have insufficient information about DEHP. In order to prevent this threat, there is a need for multidisciplinary working from industrial organizations to health institutions.

Keywords: Anesthesiologist, Diethylhexyl Phthalate, Surveys and Questionnaires.

ÖZ

Amaç: Bu çalışmanın amacı Türkiye'deki anestezi uzmanlarının Di(2-EthylHexyl) Fitalatın (DEHP) varlığı ve zararları hakkındaki bilgi ve deneyimlerini değerlendirmek ve farkındalıklarını artırmaktır.

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Gereç ve Yöntem: DEHP hakkında web tabanlı 20 anket sorusundan oluşan anket çalışması Türkiye'deki anestezi uzmanlarına elektronik posta yoluyla gönderilmiştir. Katılımcılara DEHP'nin adını duyup duymadıkları, DEHP'nin neden olabileceği zararlı etkileri bilip bilmedikleri, ameliyathane ve yoğun bakım tıbbi malzeme seçiminde DEHP'nin etkisinin olup olmadığı ile ilgili sorular sorulmuştur.

Bulgular: Anestezi uzmanlarının %70'inin DEHP'nin adını veya kavramını hiç duymadığını belirledik. Çalışma ayrıca, %90-95'inin kullandıkları tıbbi malzemelerin DEHP içerip içermediğini bilmediğini ve tıbbi malzemelerin satın alınması ve seçimi konusunda bilgi eksikliği yaşadıklarını göstermektedir.

Sonuç: Bu araştırmanın verilerine göre, Türkiye'deki anestezi uzmanlarının çoğunluğu DEHP hakkında yeterli bilgiye sahip değildir. Bu tehdidin önlenebilmesi için sanayi kuruluşlarından sağlık kurumlarına kadar multidisipliner bir çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Anestezi uzmanı, Dietilheksil Ftalat, Anket ve soruları.

INTRODUCTION

While the rapid development of industry has brought many conveniences for life, it has caused organisms to be exposed to new synthetic chemical materials. A report published by the European Union Commission in 2002 found 60 materials with damage to “environmental and human health” clearly shown among hundreds of chemical materials. Phthalates are among these materials causing damage to human health (1). Phthalates have many industrial uses (2-3). Phthalates are commonly used to add softness and flexibility to naturally hard and brittle plastic items. Among phthalates, di-(2ethylhexyl) phthalate (DEHP) is the most commonly used and annual production is reported to be nearly two million tons (4-6).

Common use of phthalates has caused increased contact with humans and animals. Phthalates may be transmitted to humans via oral, dermal or inhalation routes. Phthalate esters and metabolites may be easily identified in items, care products, in urine, breastmilk and amniotic fluid. Additionally, phthalates may pass the placenta and may cause fetal effects in proportion to the mother’s exposure. At the end of DEHP metabolism, the more toxic MEHP (mono-ethyl hexyl phthalate) forms (7-12). Commonly used in practice in the health system and with high degree of lipophilia, DEHP may easily pass into body solutions (13). DEHP used in health applications may negatively affect human health. DEHP is assessed as a chemical material causing endocrine disorders in children (14). The FDA (Food and Drug Administration) and European Union Commission have made a range of recommendations to avoid this toxic material and to reduce its use. Especially in some countries like Canada, the use of plastic bags and medical material like teats containing phthalate, etc, have been banned.

The Republic of Turkey Ministry of Health recommended special precautions to protect children under the age of 3, especially, from phthalates. In 2011, a warning device regulation about the reduction or elimination of phthalates in medical material was published (15-17).

Surgeries and intensive care units are the health area where phthalates are most commonly used. The physician group working most in these areas and using most phthalate material are anesthesiologists. As a result, the physician group who should be most sensitive to the toxic effects of

DEHP and MEHP are anesthesiologists. In our study, we aimed to evaluate the knowledge of Anesthesia and Reanimation physicians about DEHP and to increase awareness on this issue.

MATERIAL AND METHODS

Our study was planned as a survey study after receiving permission from "Dokuz Eylül University Non-Interventional Ethics Committee". Ethical approval for this study (Ethical Committee No 855) was provided Ethical Committee Dokuz Eylül University Non-Interventional Ethics Committee, İzmir, Türkiye(Chairperson Prof B. Onvural) on 20th November 2015.

A web-based survey comprising 4 sections of 20 questions (App. 1) was sent to lecturers, specialists and specialist students (2343 people in total) employed in Anesthesiology and Reanimation Departments in Türkiye 3 times at 1-month intervals from 23.12.2015. It was stated that participation was not mandatory. Our study did not include those with invalid e-mail addresses and responses sent after 01.03.2016.

The participants answered a total of 20 questions in 4 groups (A-D). The A section of the survey comprised 6 questions collecting data related to demographics and place of employment.

B section included 6 questions about whether they had heard the name DEHP and about products containing DEHP.

C section included 2 questions about the effect of presence of DEHP on the selection of medical material used in surgery and intensive care. The D section included 6 questions collecting data about the possible harmful effects of DEHP and legal regulations.

Data Analysis

Data obtained in response to questions on the survey were analyzed with Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA) 15.0 version program for Windows. Data indicating frequency are shown as frequency (n) and percentage (%). Continuous variables are shown as mean ± standard deviation (mean±SD). Comparison of frequency data used the chi square statistical method.

After determining the normality of distribution patterns of data with continuous values, the Kruskall Wallis, Mann Whitney U, Student t test and ANOVA test were used in accordance with distribution pattern and number of groups. Significance was accepted as $p<0.05$.

RESULTS

Of the 2343 anesthesiologists that the survey questions were sent to, the data from 270 (11.52%) anesthesiologists who responded to the survey in time were assessed. Of the 270 participants answering the survey, 149 (55.2%) were female, 121 (44.8%) were male and the mean age was identified as 38.12±9.59 years. It was identified that research assistants (n=104) and specialist doctors (n=97) provided most responses to our survey. The mean professional experience duration of those answering the survey was 10.56±9.44 years.

The mean professional experience duration of research assistants was identified as 2.35±1.13 years, specialists 11.80±6.46 years of teaching assistants 21.33±9.27 years and lecturers 21.00±7.57 years of. Thus, it was identified that teaching assistants and lecturers were more experienced than others (p=0.0001).

Survey responses were mostly obtained identified to the Marmara (n=99), Aegean (n=86), and Central Anatolian (n=52) regions. The majority of anesthesiologists completed the survey lived in metropolitan areas (n=202). Of participants, 88.9% (n=240) worked in Ankara and western provinces, while 11.1% (n=30) worked in eastern provinces.

The majority of anesthesiologists were working in university hospitals. When responses to the question of whether they had ever heard the name of DEHP are assessed, 70.4% had not heard the name DEHP or had no idea about it (p=0.0001).When rates of hearing about DEHP according to academic career

were compared, the majority of research assistants and specialists had not heard the name of DEHP, while the majority of lecturers had heard of DEHP (p=0.0001) (Table 1).

Table 1. Awareness rate of the name DEHP according to academic career

	Research Assistant	Specialist Doctor	Teaching Assistant	Lecturer	Total
Heard of DEHP	17.3% (n=18)	26.8% (n=26)	43.6% (n=17)	63.3%*(n=19)	29.6% (n=80)
Not heard of DEHP	61.5% (n=64)	61.9% (n=60)	43.6% (n=17)	23.3% (n=7)	54.8% (n=148)
No idea about DEHP	21.2% (n=22)	11.3% (n=11)	12.8% (n=5)	13.3% (n=4)	15.6% (n=42)
Total	100% (n=104)	100% (n=97)	100% (n=39)	100% (n=30)	100% (n=207)

* p=0.0001

According to professional experience duration, 120 of those answering the survey had worked for >10 years, while 150 had worked for <10 years. As the working duration increased, the rate hearing about DEHP was identified to increase (p=0.0001).

The rate of those answering the survey who did not know where DEHP was used or had no idea about it was 68.5%. It was determined that participants with longer working duration knew where DEHP was used at higher rates (p=0.0001) (Table 2).

Table 2. Rates of knowing where DEHP is used according to employment duration

	>10 years working duration	<10 years working duration	Total
Know where to use DEHP	36.8%* (n=42)	17.5% (n=24)	26.3% (n=66)
Don't know where to use DEHP	37.7% (n=43)	36.5% (n=50)	37.1% (n=93)
No idea where to use DEHP	25.4% (n=29)	46.0% (n=63)	36.7% (n=92)
Total	100% (n=114)	100% (n=137)	100% (n=251)

*p=0.0001

The rate of knowing where DEHP was used according to academic career was lowest for research assistants, the rate of knowing where DEHP was used was increased as academic career progressed (p=0.0001) (Table 3).

Table 3. Rates of knowing where DEHP is used according to professional career

	Research Assistant	Specialist Doctor	Teaching Assistant	Lecturer	Total
Know	15.1% (n=14)	23.6% (n=21)	35.9% (n=14)	56.7%* (n=17)	26.3% (n=66)
Don't know	36.6% (n=34)	39.3% (n=35)	35.9% (n=14)	33.3% (n=10)	37.1% (n=93)
No idea	48.4% (n=45)	37.1% (n=33)	28.2% (n=11)	10.0% (n=3)	36.7% (n=92)
Total	100% (n=93)	100% (n=89)	100% (n=39)	100% (n=30)	100% (n=251)

*p=0.0001

In answer to the question "do you use material(s) containing DEHP?", 18.5% of participants responded yes (n=50), while 8.1% responded no (n=22) and 64.4% stated they had no idea (n=174). The responses to questions asked about awareness of DEHP content in products used are given in Table 4.

Table 4. Awareness of products used containing DEHP

Product	Yes - contains DEHP	No- does not contain DEHP	No idea	Did not answer	Awareness of brand	Most commonly used brand
Examination gloves	6.7% (n=18)	5.9% (n=16)	74.8% (n=202)	12.6% (n=34)	35.2% (n=95)	Beybi-28.5% (n=77)
Intravenous/ arterial cannula	6.3% (n=17)	4.4% (n=12)	78.5% (n=212)	10.7% (n=29)	37.4% (n=101)	Braun-17.8% (n=48)
Aspiration and nasogastric probe	6.3% (n=17)	4.8% (n=13)	77.4% (n=209)	11.5% (n=31)	27.8% (n=75)	Bıçakçılar-26.3% (n=71)
Peripheral and central venous catheter	4.8% (n=13)	5.2% (n=14)	78.5% (n=212)	11.5% (n=31)	37.8% (n=102)	Braun-18.5% (n=50)
Epidural set	5.2% (n=14)	4.4% (n=12)	79.6% (n=215)	10.7% (n=29)	45.6% (n=123)	Braun-29.3% (n=79)

Dropadjustment set/pain infusion set/venous extension line	6.7% (n=18)	3.0% (n=8)	79.6% (n=21)	10.7% (n=29)	21.1% (n=57)	Bıçakçılar-17.8% (n=48)
Injector	5.9% (n=16)	3.3% (n=9)	80% (n=216)	10.7% (n=29)	39.2% (n=106)	Hayat-12.2% (n=3)
Endotracheal tube (double lumen/single lumen)	6.3% (n=17)	4.8% (n=13)	77.4% (n=209)	11.5% (n=31)	32.2% (n=87)	Bıçakçılar-21.9% (n=59)
Laryngeal mask	5.9% (n=16)	4.4% (n=12)	78.5% (n=212)	11.1% (n=30)	27% (n=73)	Promed-8.9% (n=24)
Ventilator cycle/respiration mask	6.7% (n=18)	3.0% (n=8)	79.3% (n=214)	11.1% (n=30)	16.3% (n=44)	Covidien-3.7% (n=10)
Blood and serum set, blood bag, triple tap	6.3% (n=17)	4.8% (n=13)	77.8% (n=210)	11.1% (n=30)	19% (n=51)	Bıçakçılar-6.7% (n=18)
Dialysis material/ ECMO set/ hot chemotherapy device sets	5.6% (n=15)	1.1% (n=3)	81.9% (n=221)	11.5% (n=31)	1.8% (n=5)	Maquet-1.1% (n=3)

It is known that different amounts of DEHP are used in products from different plastic producers. In answer to the question of whether there were differences between DEHP content between plastic producing companies, 25.9% (n=70) answered yes, 2.6% (n=7) answered no, and 68.5% (n=185) answered they had no idea. It is thought that the majority of purchasing commissions in private and public hospitals have no information about DEHP content and toxicity in products.

When asked whether DEHP content is checked during selection or purchasing of medical material at your hospital, 6.3% (n=17) said yes, 21.5% (n=58) said no and 68.9% (n=186) said they had no idea. It is known that patients receiving anesthesia and monitored in intensive care are exposed to DEHP. When asked whether patients receiving anesthesia or in intensive care are exposed to DEHP, 33% (n=89) said yes, 1.5% (n=4) said no and 63% (n=170) had no idea.

DEHP is transmitted via perioral, IV, inhalation and dermal routes. We asked what are the routes of exposure to DEHP during anesthesia administration and the responses to the question with multiple answers allowed were 25.2% (n=68) perioral route, 28.9% (n=78) inhalation route, 33.3% (n=90) intravenous route, 33% (n=68) dermal route and 49.3% (n=133) had no idea.

Exposure to DEHP may cause harmful effects to the fetus, abnormalities in pubertal

development, undescended testis and hypospadias development, increased anogenital openings, endometriosis, liver adenoma and HCC development, and changes to the thyroid structure and activity. When asked what exposure to DEHP may cause, the participants responses were 23.7% (n=64) harmful effects to the fetus, 22.2% (n=60) abnormalities of pubertal development, 18.9% (n=51) undescended testis and hypospadias development, 14.1% (n=38) increased anogenital openings, 13% (n=35) endometriosis, 24.1% (n=65) liver adenoma and HCC development, 14.8% (n=40) changes to thyroid structure and activity, and 56.7% (n=153) had no idea.

Everyone in the anesthesia is exposed to DEHP. However, the group with the highest DEHP exposure are were the preterm.When asked which patient groups were most exposed to DEHP, the answers were 32.2% (n=87) pregnant cases, 37.4% (n=101) premature cases, 37.4% (n=101) children, 19.6% (n=53) adults and 50.7% (n=137) had no idea.

The release of DEHP from PVC is influenced by pH, temperature, fluid content within the material, and exposure duration. Participants' responses to the question about what affects release rates of DEHP from material used were 20% (n=54) pH, 30.7% (n=83) temperature, 18.1% (n=49) fluid content, 37% (n=100) exposure duration and 51.1% (n=138) had no idea.

DEHP exposure may cause pulmonary damage, bronchopulmonary dysplasia, and increased bronchial sensitivity.

When participants were asked what DEHP release causes in the lungs, 22.6% (n=61) said pulmonary damage, 18.1% (n=49) said bronchopulmonary dysplasia and, 24.1% (n=65) stated increased bronchial sensitivity. There were 56.7% (n=153) who said they had no idea.

The use of material containing DEHP may cause platelet aggregation and complement activation in the cardiovascular system. In response to the question "What complications may be expected due to the use of tools containing DEHP in surgeries related to the cardiovascular system?", 15.9% of participants (n=43) said platelet aggregation, 17.8% (n=48) said complement activation and 69.9% (n=188) said they had no idea.

The Ministry of Health has two announcements about DEHP (2005, 2011). When participants were asked whether the Ministry of Health had made any announcements about DEHP, 11.1% (n=30) said yes, 8.1% (n=22) said no, and 75.2% (n=203) had no idea.

The Food and Drug Administration (FDA) has made warnings about DEHP in different years (2001, 2008, 2010). When participants were asked whether the Food and Drug Administration (FDA) had made any announcements about DEHP, 24.4% (n=66) said yes, 1.9% (n=5) said no, and 69.9% (n=188) had no idea.

DISCUSSION

In our survey study aiming to assess the knowledge and experience about harmful effects of (DEHP), of Anesthesia and Reanimation physicians in our country, 70% of the 270 anesthesiologists who responded to the survey had not heard the name of DEHP. We determined that 75% did not know where DEHP was used. When we asked whic medical materials and devices were contain DEHP, 90-95% did not know whether the medical materials they used contained DEHP or not. Another interesting finding in the research is that 70-99% of anesthesiologists did not know the brand of the material they used.

When asked about differences in DEHP content between plastic producers, 75% of anesthesiologists did not know and 93.7% had no idea about the selection and purchasing of material. More than 50% of anesthesiologists had no idea about the topics of how DEHP was transmitted to humans, what affected release, which patient groups it was more

dangerous for and the effects on the human body. It was determined that more than 70% of anesthesiologists had no information about national and international warnings about DEHP. In addition to all of this, as the academic career and professional experience increased, the proportion who had heard of DEHP increased, which is an expected and predicted result.

During a literature search, we did not find any studies that questioned any knowledge on topics related to "anesthesiologist, DEHP, material and devices containing DEHP, DEHP effects on human health, and efforts of national and international organizations about DEHP". As a result, we sent all anesthesiologists in Türkiye a survey "questioning knowledge about DEHP and aiming to increase awareness of DEHP" via electronic mail.

According to our survey data, 70% of anesthesiologists had never heard the name of DEHP and had insufficient knowledge about the material they used, which exceeded our expectations. Material and devices containing DEHP are used by physicians in various branches in all fields of the health sector.

DEHP exposure is mostly experienced in intensive care units (18-19) and in surgeries (20). Apart from these, the DEHP exposure in areas like dialysis units (18-19), nutritional units (15, 19,21), blood banks (15,19,22,23),and oncology departments (15,21,24) is high..

Unsurprisingly, given the ubiquity of phthalates and bisphenols, biomonitoring studies reported detectable levels of DEHP and BPA in 75-90% of the general population in the study by Ramadan et al. (25). One study clearly demonstrated that ECMO patients receive a significantly higher internal exposure to DEHP, the most prominent plasticizer in medical devices to date (26).

Gaynor et al found large postoperative increases in urinary BPA (42%) and DEHP metabolite (1,500-2,100%) levels in pediatric patients undergoing cardiac surgery (27).

Many alternative plasticizers are now available and increasingly used in medical devices (28).

One of these plasticizers developed specifically for use in medical devices is tri-2-ethylhexyl trimellitate (TOTM or TEHTM). The different structure of TEHTM is hypothesized to lead to a higher degree of stability associated with a lower migration rate in blood or other fluids compared to DEHP, which has been shown in previous studies (29).

In our literature search, we did not find any study researching the knowledge of physicians and other health personnel working in these fields about DEHP. However, we did encounter a small number of animal and human research studies about DEHP exposure (3-30). As a result, we think the knowledge, experience and sensitivity of health personnel about DEHP are insufficient. Our survey results confirm this data.

The Republic of Türkiye Ministry of Health Turkish Medicines and Medical Devices Agency (TMMDA) states it has a duty to "serve human health by developing and applying regulatory, supervisory and directive policies about medicine, medical devices and cosmetic products" according to their main website (31). The target of this organization is defined as "being a pioneering and reference organization in the international field, based on health-oriented science and targeting perfection".

The 5th item of the duty statement declare that their duty is "to observe and supervise medicines, medical devices and products entering the market, if necessary to collect, destroy or have them destroyed, to determine reliable reporting methods for products in the market, to make the necessary declarations, to perform laboratory analyses or have them performed" (32). As understood from these statements, the licensing, regulation and control of material or medicines are under the auspices of the Ministry of Health.

The purchasing commissions of state and university hospitals work in conjunction with the

chief physician's office. Doctors, nurses and technical personnel serve on these commissions. The basic duty of these commissions under current circumstances is to obtain the cheapest and best quality material on time and to make it available for use.

No matter their branch, the duty of health personnel may be summarized by the statement 'Primum non nocere' attributed to Hippocrates; in other words, 'Do no harm'.

In this situation, the duty of health personnel includes having sufficient knowledge about treatment methods that do not harm the patient or themselves and in the selection of medicines, material and devices used. The prevention of production of toxic products containing DEHP may be obstructed by not licensing them at Ministry of Health level.

Toxic products passing this stage may be prevented from entering hospitals at the purchasing stage. The first route to achieve this for is national units like the Ministry of Health that provide import permissions, licenses for use and health certificates on the topics of medicine-material-devices,to become more aware of this topic.

The second route is to inform physicians serving on purchasing and medical material device technical specifications commissions at chief physician level. The third route is to inform and sensitize all health personnel and patients through professional organizations, patient safety organizations, etc.

Despite the Ministry of Health releasing 2 announcements related to DEHP in 2005 and 2011 (17-33), it is interesting that only 11% of anesthesiologists participating in our study had any information related to DEHP. A slightly higher amount (24.4%) of anesthesiologists were aware of repeated announcements by international organizations like the FDA(15). Considering that the physicians most informed about DEHP were experienced academics in universities, it is understood that physicians employed in state hospitals serving large public masses will not have sufficient information about this topic. The Ministry of Health announcement in the Official Gazette published on 7th June 2011 described phthalates in the 7th item of the Medical Device Regulation and included the statement "the label of the product must state that it contains phthalate".

The FDA recommends that instead of PVC containing DEHP, material not containing DEHP (containing alternative chemicals, e.g., trioctyl trimellitate, and diethyl hexyl adipate) should be used for special patient groups (premature cases, especially male newborns, pregnant, and breastfeeding mothers, etc.) (15-34).

In line with this research, defining PVC/DEHP containing material initially, it is recommended that alternatives should be used for these risk groups. It is reported that to reduce individuals' DEHP exposure to a minimum, PVC free material should be chosen (35). It is clear that these precautions will only be taken through the efforts of informed, sensitive physicians.

The "European Chemical Agency Socio-Economic Analysis Committee" in 2015 recommended limiting the use of the majority of chemicals included in the EU's "Registration, Evaluation, Authorisation and Restriction of Chemicals" (REACH) law.

The target was to ban 4 phthalates called butyl benzyl phthalate (BBzP), di (2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and di isobutyl phthalate (DIBP), especially. Plasticizers commonly used in a majority of products were banned above levels of 0.1% by weight (36).

The passing of laws and bans clearly did not solve the problem. It is necessary for international organizations like the FDA, national authorities like the Ministry of Health, environmental and professional organizations, and physicians themselves to expend effort on this topic. The duty of industrial organizations should be to produce

products that do not harm human and animal health and do not pollute the environment.

There are some limitations to our study. The low number of participants in our study may not sufficiently reflect the opinions and information of all anesthesiologists employed in Türkiye.

CONCLUSION

In conclusion, according to our study data, the majority of anesthesiologists working in Turkey were identified to have insufficient knowledge about DEHP. Linked to this lack of knowledge, physicians and patients are exposed to the toxic effects of chemicals.

To prevent this unwanted exposure, recommendations of international organizations should be followed more closely to increase the safety for patients and health personnel; national units like the Ministry of Health should provide training in line with international recommendations, and more closely regulate announcements; hospitals should become more sensitive and material and products considered harmful should be prevented from entering the hospital by trained purchasing commissions;

Manufacturing firms should avoid toxic products and be supported to develop non-toxic products. There is a need for multidisciplinary studies involving industrial organizations and health organizations to prevent the release and use of these toxic products.

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Appendix1: The survey used in this study

A1. Your age:

A2. Your gender:

☐ Female

☐ Male

A3. Your academic career:

☐ Research assistant

☐ Specialist

☐ Teaching assistant

☐ Lecturer

A4. Number of years working in the field of anesthesia (years)**:**

A5. Province of employment:

A6. Organization of employment:

☐ State Hospital

☐ University Hospital

☐ Education and Research Hospital

☐ Private Hospital

☐ Other ( )

B1**.** Have you ever heard the name DEHP?

☐ Yes

☐ No

☐ No idea

If your answer is "No" or "No idea", just write the brand names of material you use in answer to B3.1 to B3.12 and then complete the survey.

B2**.** Do you know where DEHP is used?

☐ Yes

☐ No

☐ No idea

B3**.** Do you use material(s) containing DEHP?

☐ Yes

☐ No

☐ No idea

B3.1. Do the "examination gloves" you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don't know : ☐

B3.2. Does the “brannula” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B3.3. Does the “nasogastric probe” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B3.4. Does the “central venous catheter” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B3.5. Does the “epidural set” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B3.6. Does the “dosiflow” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B3.7. Does the “injector” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B3.8. Does the “endotracheal tube” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B3.9. Does the “laryngeal mask” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐Evet

B3.10. Does the “ventilator cycle” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B3.11. Does the “blood and serum set” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B3.12. Do the “dialysis material” or “ECMO set” you use contain DEHP?

☐ Yes

☐ No

☐ No idea

*** Brand name:

I don’t know : ☐

B4. Do you think there are differences between companies producing plastic material in terms of DEHP?

☐ Yes

☐ No

☐ No idea

B5. Does your hospital check whether material contains DEHP or not while choosing or buying medical material?

☐ Yes

☐ No

☐ No idea

B6. Do you think patients receiving anesthesia or in intensive care are exposed to DEHP?

☐ Yes

☐ No

☐ No idea

C1. What are the exposure routes for DEHP in anesthesia administration? (You may mark more than one)

- ☐ Peroral
- ☐ Inhalation
- ☐ Intravenous
- ☐ Dermal
- ☐ No idea

C2. Which of the following do you think DEHP exposure can cause? (You may mark more than one)

- ☐ Harmful effects on the fetus
- ☐ Abnormalities in pubertal development
- ☐ Undescended testis, hypospadias development
- ☐ Increased anogenital openings
- ☐ Endometriosis
- ☐ Liver adenoma and hepatocellular cancer development
- ☐ Thyroid structure and activity changes
- ☐ No idea

D1. Which patient(s) are at risk of DEHP exposure in anesthesia administration? (You may mark more than one)

- ☐ Pregnant cases
- ☐ Premature case
- ☐ Children
- ☐ Adults
- ☐ No idea

D2. What do you think affects the DEHP release rate from material used? (you may mark more than one)

- ☐ pH
- ☐ Temperature
- ☐ Fluid content
- ☐ Exposure duration
- ☐ No idea

D3. What do you think DEHP release may cause in the lungs? (you may mark more than one)

- ☐ Pulmonary injury
- ☐ Bronchopulmonary dysplasia
- ☐ Increased bronchial sensitivity
- ☐ No idea

D4. What complications may be expected due to the use of tools containing DEHP in surgeries related to the cardiovascular system? (You may mark more than one)

- ☐ Platelet aggregation
- ☐ Complement activation
- ☐ No idea

D5. Do you think the Ministry of Health has made any announcements about DEHP?

- ☐ Yes
- ☐ No
- ☐ No idea

D6. Do you think the *Food and Drug Administration* (FDA) has made any announcements about DEHP?

- ☐ Yes
- ☐ No
- ☐ No idea

Research Article

The Relationship Between Depressive Symptoms and Treatment Adherence in Patients With Hypoparathyroidism

Kronik Hipoparatiroidizm hastalarında depresif belirtiler ve tedaviye uyum arasındaki ilişki

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ABSTRACT

Aims: Hypoparathyroidism (HPT) is a chronic disease characterized by low calcium levels and significant psychological and physical complications. The aim of this study was to investigate the relationship between depressive symptoms and treatment adherence in patients with HPT.

Materials and Methods: This cross-sectional, observational study included patients with chronic HPT from the endocrinology clinic of a tertiary care hospital. Adults receiving oral calcium and active vitamin D therapy, who volunteered to complete the Beck Depression Inventory (BDI), and who did not have any mental disability or concomitant chronic serious disease were included. Biochemical and complication data were collected, and treatment adherence was assessed on the basis of self-reported medication use.

Results: Of the 33 patients (mean age 53.7 ± 12.7 years; 78.8% female), 54.5% were non-adherent to treatment. Depressive symptoms ranged from minimal to severe, with 24.3% experiencing moderate to severe symptoms. A significant association was found between HPT etiology and depressive symptom severity ($p = 0.011$), with moderate symptoms more common in post-operative patients (34.8% vs. 0% in non-surgical cases). A weak negative correlation was observed between depressive symptom scores and highest calcium levels ($r = -0.359$, $p = 0.04$). Conclusions: This study emphasizes the importance of treatment goals by demonstrating the negative correlation between Ca levels and BDI scores. The high prevalence of depressive symptoms and non-adherence in patients with HPT underlines the need for tailored mental health and adherence interventions.

Keywords: Hypoparathyroidism, depression, hypocalcemia.

ÖZ

Amaç: Hipoparatiroidizm (HPT), düşük kalsiyum seviyeleri ve önemli psikolojik ve fiziksel komplikasyonlarla karakterize kronik bir durumdur. Bu çalışma, HPT hastalarında depresif belirtiler ile tedaviye uyum arasındaki ilişkiyi araştırmayı amaçlamıştır.

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Gereç ve Yöntemler: Bu kesitsel, gözlemsel çalışmaya, Haydarpaşa Numune Eğitim ve Araştırma Hastanesi Endokrinoloji Kliniği'nden kronik HPT'li hastalar dahil edilmiştir. Oral kalsiyum ve aktif D vitamini tedavisi alan, Beck Depresyon Ölçeği'ni (BDÖ) doldurmayı gönüllü olarak kabul eden, zihinsel engeli veya ciddi eşlik eden kronik hastalığı olmayan yetişkinler çalışmaya alınmıştır. Biyokimyasal ve komplikasyon verileri toplanmış, tedaviye uyum hastaların ilaç kullanımını kendi bildirimlerine dayalı olarak değerlendirilmiştir.

Bulgular: Otuz üç hastanın (ortalama yaş 53,7 ± 12,7 yıl; %78,8 kadın) %54,5'inde tedaviye uyumsuzluk tespit edilmiştir. Depresif belirtiler minimalden şiddetliye kadar değişiklik göstermiş, %24,3'ü orta ila şiddetli belirtiler yaşamıştır. Depresif belirti skorları ile en yüksek kalsiyum seviyeleri arasında zayıf bir negatif korelasyon bulunmuştur (r = -0,359, p = 0,04).

Sonuç: Bu çalışma, kalsiyum seviyeleri ile BDÖ skorları arasındaki negatif korelasyonu ortaya koyarak tedavi hedeflerinin önemini vurgulamaktadır. HPT hastalarında depresif belirtilerin ve tedaviye uyumsuzluğun yüksek prevalansı, ruh sağlığı ve tedavi uyumuna yönelik özel müdahalelere ihtiyaç olduğunu ortaya koymaktadır.

Anahtar Kelimeler: Hipoparatiroidizm, depresyon, tedavi uyumu.

INTRODUCTION

Hypoarathyroidism (HPT) is a rare disease characterized by low serum calcium (Ca) levels and relatively high phosphorus (P) levels due to parathormone (PTH) deficiency(1). Chronic HPT has findings that persist for more than 6 months (2). The most common cause is post-surgical HPT caused by damage to parathyroid tissue following to neck surgery, usually after thyroidectomy. Genetic mutations and autoimmune diseases are also less common causes. Chronic HPT is mostly observed in adults aged 55 years and older and is three times more common in women than in men (3).

The clinical manifestations of HPT are mostly due to hypocalcemia and most commonly include increased neuromuscular excitability (tetany) and calcification of the kidneys, brain and vascular structures. In addition to physical symptoms such as fatigue, pain, muscle spasms, and paresthesia, there may be cognitive symptoms such as "brain fog" and emotional symptoms that affect quality of life such as depression and anxiety disorders. The relationship between depression and HPT is complex and likely involving the chronic stress of disease management, neurochemical changes due to hypocalcemia, and the psychological distress of persistent physical symptoms, all of which can lead to depression (4–6).

Chronic HPT treatment is a long-term therapy. Even active vitamin D and calcium therapies included in standard replacement therapy may not be sufficient to achieve ideal serum calcium and phosphorus levels. In these patient groups, recombinant PTH therapies have emerged as an as an alternative solution (7-8). Effective management of HPT requires lifelong adherence to therapy. However, adherence is particularly challenging in these patients due to the high medication burden, frequent gastrointestinal side effects, and the long-term nature of the treatment. These factors can lead to fatigue with daily regimens and reduced motivation to maintain adherence over time. Non-adherence to

treatment increases complications, may worsen psychological outcomes (9). This study examines the relationship between depressive symptoms and treatment adherence in patients with HPT, with a focus on achieving optimal biochemical control.

MATERIALS AND METHODS

Study Design and Participants

This cross-sectional, observational study was conducted at the endocrinology clinic of a tertiary care hospital in Istanbul between March 2018 and March 2021. Adults (≥18 years) diagnosed with chronic HPT and receiving conventional treatment (oral calcium carbonate supplementation at dose of 1 to 2 g/day and calcitriol at dose of 0.25 to 1.0 mcg/day) were eligible. All patients received standardized medication counselling from the same physician throughout the study period to ensure consistency in treatment explanations and adherence guidance. Patients with significant comorbidities or those receiving recombinant PTH therapy were excluded. Patients with a previously diagnosed psychiatric disorder or those using psychiatric medications or supplements (such as antidepressants, anxiolytics, mood stabilizers, or herbal supplements with psychoactive effects) were also excluded from the study to minimize potential confounding effects on depression scores. Ethical approval (decision number: 2018-KAEK-4/37) and written informed consent were obtained from all participants.

Data Collection

Demographic characteristics, level of education,body mass index (BMI), and duration of disease were recorded. The etiology of HPT was categorized into two main groups: post-surgical HPT and other causes. Post-surgical HPT included patients who developed the condition following thyroidectomy, parathyroidectomy, or other neck surgeries. The other causes group included patients with autoimmune, genetic, idiopathic, or other non-surgical forms of HPT. Classification was based on patient history and medical records. Biochemical parameters: Ca, corrected Ca, P, magnesium (Mg), 25-OH vitamin D3, creatinine and alkaline phosphatase (ALP), collected during patients' clinical visits approximately every 3 to 6 months. To assess treatment response and variability, the lowest serum Ca and highest serum P levels observed during the entire treatment period were recorded and included in the analysis. These values were considered potential indicators of treatment failure. Blood samples were taken by venipuncture in the morning after fasting. Measurements were performed using standard autoanalyzer devices in our laboratory.

Urine was collected for 24 hours to assess urinary calcium excretion. 24-hour urine collection was performed. The first-morning urine was discarded. All subsequent urine collected over the next 24 hours in a designated container. Urinary calcium levels was measured by using atomic absorption spectrophotometry. Creatinine clearance was calculated, and the results were corrected for urine volume. Hypercalciuria was defined as urinary calcium excretion >300 mg/day (or >4 mg/kg/day for weight-based assessment). The reference ranges are as follows: Serum Ca: 8.4-10.2 mg/dL, serum P: 2.5-4.5 mg/dL, serum Mg: 1.6-2.6 mg/dL, PTH: 15-65 pg/mL, 25-OH vitamin D: 20-50 ng/mL, and creatinine: 0.6-1.2 mg/dL. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation.

HPT complications (e.g., nephrolithiasis, cataracts, fractures, etc.) were obtained from medical records. Various imaging modalities were used to assess complications associated with HPT, based on clinical indications and patient history. Renal complications, including nephrolithiasis and nephrocalcinosis, were evaluated using renal ultrasound (USG) and, if necessary, non-contrast computed tomography (CT). Skeletal complications, such as osteopenia, osteoporosis, and pathological fractures, were assessed by using dual-energy X-ray

absorptiometry (DEXA) and plain radiographs of relevant skeletal regions. Intracranial calcifications, in particular basal ganglia calcifications (BGC), were assessed by computed tomography of the brain. Extrapyramidal signs, parkinsonism or seizures were evaluated by a neurologist and considered to be cerebrovascular complications of HPT unless an alternative cause was identified. Cardiovascular complications, including vascular calcifications, were evaluated using echocardiography and vascular Doppler ultrasound when clinically indicated. Arrythmia or prolonged QT interval was noted if seen on electrocardiography. Cataract was assessed by ophthalmic examination performed by an ophthalmologist.

Assessment Tools

Treatment adherence was defined as consistent use of prescribed medication, with "non-adherence" defined as more than one missed dose per week. This definition is based on standard adherence criteria commonly used in chronic disease management, where multiple missed doses have been associated with suboptimal outcomes. This definition consistent with adherence criteria in chronic disease management (10). Depressive symptoms were assessed using the Beck Depression Inventory (BDI), a validated 21-item self-report tool (11). For patients who were illiterate or suspected of having limited intellectual capacity, the survey was administered by a physician to ensure comprehension and accurate responses. This approach aimed to minimize response bias and improve the reliability of the depression assessment. The version developed by Hisli for Turkish population was used (12). BDI scores were categorized as minimal (0-9), mild (10-16), moderate (17-29), and severe (30-63).

Statistical Analysis

A post-hoc power analysis was performed using GPower version 3.1.9.7 to assess whether the study's sample size was sufficient to detect significant differences. Power calculations were performed for parametric (Student's t-test) and non-parametric (Mann-Whitney U, Kruskal-Wallis) tests based on an expected moderate effect size (Cohen's d = 0.5 for t-tests, r = 0.3 for Mann-Whitney U, and η^2 = 0.06 for Kruskal-Wallis), a significance level of α = 0.05, and a target power of 80%.

All statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Continuous variables were tested for normality using the Kolmogorov-Smirnov test. Variables with normal distribution were presented as mean ± standard deviation (SD), while non-normally distributed variables were presented as median (minimum-maximum). Categorical variables were expressed as numbers (percentages).

Comparison of treatment adherence groups (adherent vs. non-adherent) was performed using Student's t-test for normally distributed continuous variables, Mann-Whitney U test for non-normally distributed continuous variables, Fisher's exact test for categorical variables. Comparison of depressive symptom severity groups (minimal, mild, moderate, severe) based on the BDI scores was performed using: Kruskal-Wallis test for continuous variables, chi-square or linear-by-linear association test was dused to assess trends across ordered categories.

Correlations between biochemical parameters and depressive symptom scores were assessed using Spearman's rank correlation coefficient.A p-value <0.05 was considered statistically significant.

RESULTS

Power Analysis

The post-hoc power analysis showed that the study had sufficient power to detect medium-to-large effect sizes, especially for comparisons using t-tests. However, for non-parametric analyses (Mann-Whitney U, Kruskal-Wallis), the sample size may have limited the ability to detect small effect sizes.

Patient Characteristics

The study included 33 patients (mean age 53.7 ± 12.7 years; 78.8% female). The median disease duration was 9 years (IQR: 1-41), and the mean BMI was 29.3 kg/m² (range: 19.8-48.8). Treatment non-adherence was observed in 54.5% of patients, and 39.4% had at least one complication. The majority of participants were female (78.8%), and most had a primary school education level (48.5%). The proportion of illiterate patients was 21.2% (Table 1).

Table 1. Patient characteristics and biochemical profiles

Total number of patients, n		33
Age, mean (±SD)		53.70±12.72
Sex, n (%)	Female	26 (78.8)
	Male	7 (21.2)
Education, n (%)	Illiterate	7 (21.2)
	Primary	16 (48.5)
	High School	6 (18.2)
	Higher Education	4 (12.1)
BMI (kg/m^2), median (min-max)		29.3 (19.8-48.8)
Disease duration (years), median (min-max)		9 (1-41)
Etiology, n(%)	post-surgical	23 (69.7)
	other	10 (30.3)
Presence of complications, n (%)	yes	13 (39.4)
	no	20 (60.6)
Treatment adherence, n (%)	yes	15 (45.5)
	no	18 (54.5)
Serum calcium (mg/dl), mean (±SD)		8.15±0.82
	Lowest Ca	6.38±1.03
	Highest Ca**	9.4 (7.9-11.9)
Serum phosphorus (mg/dl), mean (±SD)		4.94±0.68
	Lowest P	4.09±0.67
	Highest P**	5.8 (4.3-9.6)
CaxP (mg^2/ml^2), mean (±SD)		39.17±6.08
Creatinine (mg/dl), median (min-max)		0.9 (0.6-2)
eGFR (mL/min/1.73m²), mean (±SD)		74.6 (±18.9)
Mg (mg/dl), mean (±SD)		1.74±0.17
PTH (pg/ml), median (min-max)		4.30 (0-33)
Serum 25-OH vitamin D (ng/ml), mean (±SD)		27.18±11.71
24-hour urinary Ca (mg/day), median (min-max)		159.6 (25-478)
ALP (U/l), mean (±SD)		66.27±17.73

ALP; alkalin phosphatase, **BMI**; body mass index, **eGFR**; estimated glomerular filtration rate, **PTH**; parathormone

Biochemical Data

Mean serum Ca levels were 8.15 ± 0.82 mg/dL, with a median peak of 9.4 mg/dL (range: 7.9-11.9 mg/dL). P levels showed a median peak of 5.8 mg/dL (range: 4.3-9.6 mg/dL). Patients had a mean serum Mg level of 1.74 ± 0.17 mg/dL, and a median serum PTH levels of 4.3 pg/mL (range: 0-33 pg/mL). Median 24-hour urinary calcium excretion was 159.6 mg/day (range: 25–478 mg/day). Hypercalciuria was observed in two patients (6.1%) (Table 1).

Complications

Of the patients, 60.6% reported no complications. However, 39.4% experienced one or more complications, including nephrolithiasis (18.2%), cataracts (18.2%), and infections (12.1%) (Figure 1). Cardiovascular and cerebrovascular complications were less common (9.1% and 3%, respectively).

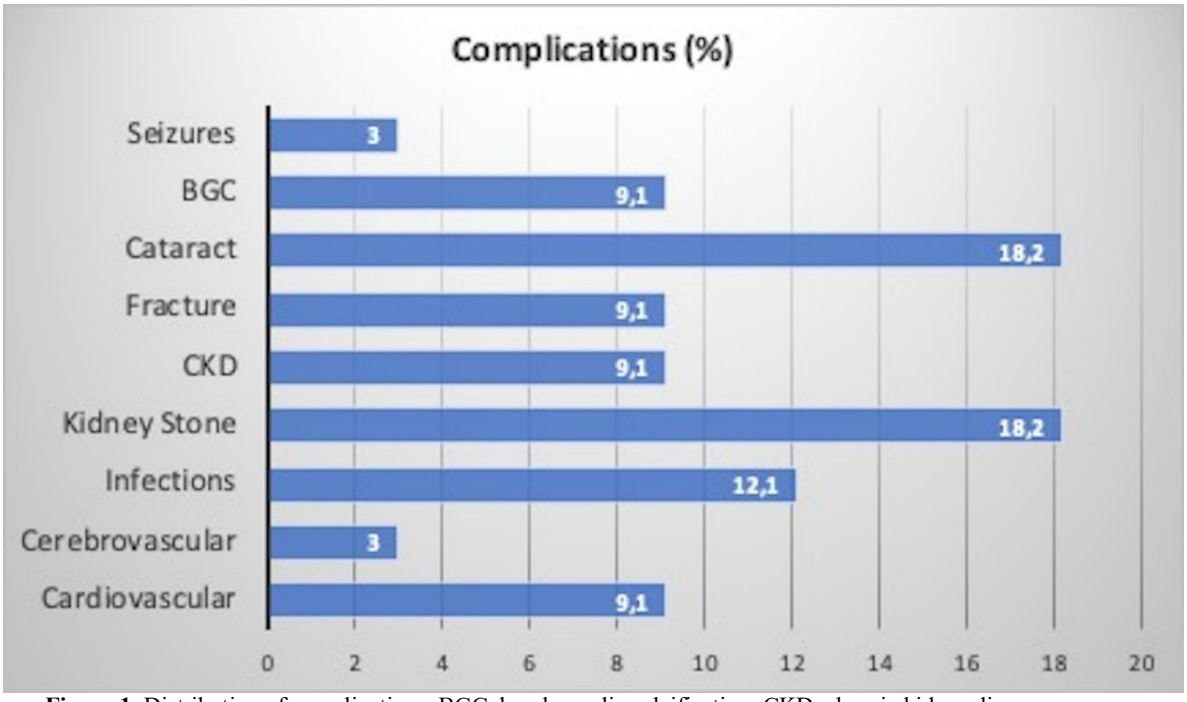

Figure 1. Distribution of complications. BGC, basal ganglia calcification; CKD, chronic kidney disease

Depressive Symptoms

The BDI scores indicated that 39.4% of patients had minimal depressive symptoms, 36.4% had mild symptoms, and 24.2% had experienced moderate to severe symptoms. Severe depressive symptoms were combined with the moderate group for statistical analysis due to the small sample size in the severe category (Figure 2).

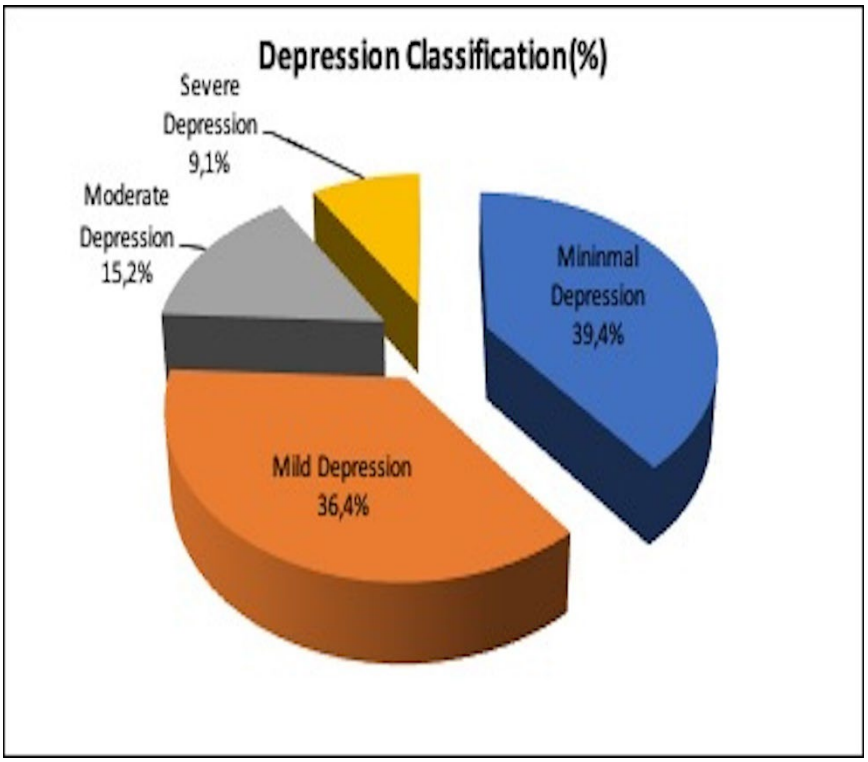

Figure 2. Depressive symptom severity classification of patients according to Beck Depression Inventory (BDI) scores

Patients with moderate-to-severe depressive symptoms (as indicated by BDI scores) were referred to a psychiatrist for further assessment. However, the effect of psychiatric interventions was not assessed in this study. No significant differences were observed between the minimal, mild, and moderate depressive symptoms groups, except for HPT etiology (Table 2).

Table 2. Comparison of patient characteristics, biochemical parameters, and presence of complications between treatment-adherent and non-adherent groups, as well as among different depressive symptom severity groups based on Beck Depression Inventory (BDI) scores [mean (standard deviation) or number (percentage)]

	Treatment Adherence		p	**BDI Score Severity**			p
	Yes (n=15)	**No (n=18)**		**Minimal (n=13)**	**Mild (n=12)**	**Moderate (n=8)**	
Age (years), mean (±SD)	56.4±7.6	51.3±15.6	[a]0.328	56.4±10.2	53.08±14.1	50.13±14.6	[c]0.385
Female, n (%)	12(80.0)	15 (83.3)	[b]1.000	9 (69.2)	10 (83.3)	8 (100)	[c]0.109
Male, n (%)	3(20.0)	3(16.7)		4 (30.8)	2 (16.7)	0 (0)	
BMI, mean (±SD)	28.98±5.7	30.63±8.6	[a]0.731	30.82±8.3	28.69±4.4	30.13±9.74	[c]0.959
Post-surgical, n (%)	11 (73.3)	12 (66.7)	[b]0.722	6 (46.1)	9 (75.0)	8 (100)	[c]0.009
Other etiology, n (%)	4 (26.6)	6 (33.3)		7 (53.9)	3 (25.0)	0 (0)	
Disease duration (years), mean (±SD)	13.40±13.8	10.11±8.3	[a]0.841	13.69±11.8	9.92±11.8	10.75±9.3	[c]0.554
Ca (mg/dl), mean (±SD)	8.14±0.70	8.17±0.93	[a]0.928	8.21±0.85	7.95±0.76	8.38±0.90	[c]0.473
P (mg/dl), mean (±SD)	4.83±0.66	5.03±0.70	[a]0.393	5.05±0.63	4.66±0.64	5.19±0.75	[c]0.133
Corrected Ca (mg/dl), mean (±SD)	8.10±0.96	7.82±0.74	[a]0.346	8.13±0.67	7.78±1.05	7.89±0.80	[c]0.471
CaxP (mg^2/dl^2), mean (±SD)	389.93±60.26	393.17±63.07	[a]0.882	407.69±39.83	362.75±68.12	409.13±68.28	[c]0.167
Creatinine (mg/dL), mean (±SD)	0.94±0.21	0.87±0.301	[a]0.480	0.99±0.36	0.86±0.08	0.81±0.26	[c]0.150
eGFR (mL/min/1.73m^2), mean (±SD)	73.4±15.9	75.8±20.7	[d]0.083	69.8±24.0	72.4±9.4	95.2±11.5	[c]0.190
Albumin (g/l), mean (±SD)	42.60±2.41	43.62±3.75	[a]0.370	42.17±3.47	44.08±2.64	43.38±3.46	[c]0.404
Mg (mg/dl), mean (±SD)	1.73±0.20	1.75±0.15	[a]0.686	1.78±0.13	1.69±0.22	1.76±0.15	[c]0.509
PTH (pg/ml), mean (±SD)	2.13±4.02	6.11±10.02	[a]0.137	4.23±9.05	4.17±5.92	4.63±9.96	[c]0.836
25-OH Vitamin D (ng/ml), mean (±SD)	26.80±8.46	27.50±14.10	[a]0.862	30.38±12.67	25.68±9.97	24.24±12.74	[c]0.475
ALP (U/l), mean (±SD)	67.93±21.6	64.89±14.24	[a]0.644	65.15±13.5	71.33±21.77	60.50±17.17	[c]0.529
Lowest Ca (mg/dl), mean (±SD)	6.35±1.28	6.40±0.81	[a]0.885	6.34±1.23	6.55±0.84	6.18±1.04	[c]0.785
Highest Ca (mg/dl), mean (±SD)	9.65±1.01	9.58±0.84	[a]0.832	9.95±0.96	9.58±0.83	9.10±0.76	[c]0.187
Lowest P (mg/dl), mean (±SD)	5.93±1.26	5.86±0.84	[a]0.058	4.38±0.58	3.73±0.76	4.19±0.39	[c]0.060
Highest P (mg/dl), mean (±SD)	3.85±0.70	4.29±0.59	[a]0.847	6.18±0.64	5.40±0.87	6.15±1.53	[c]0.072
24-h urinary Ca (mg/day), mean (±SD)	174±128	146±82	[d]0.725	162±68	174±136	102±68	[c]0.568
Presence of complications, n (%)	9 (60.0)	11 (61.1)	[b]1.000	10 (50.0)	7 (35.0)	3 (15.0)	[c]0.075
BDI score, medium (min-max)	11 (1-31)	8 (0-35)	[d]0.717	4 (0-6)	11 (10-14)	21 (18-35)	[c] <0.001
Treatment adherent, n (%)				4 (30.7)	8 (66.7)	3 (60.0)	[c]0.576
Treatment non-adherent, n(%)				9 (69.2)	3 (33.3)	5 (40.0)	

[a]Student T Test [b] Fischer's Exact Test [c] Kruskal Wallis Test, [d] Mann-Whitney U test [e] Linear-by-Linear Association

ALP; alkalin phosphatase, **BDI;** Beck Depression Inventory, **BMI**; body mass index, **eGFR**: estimated GFR**; PTH**; parathormone

A significant association was found between the severity of depression and the etiology of HPT ($p = 0.011$). Patients with post-surgical HPT had a higher proportion of moderate depressive symptoms category (34.8%), whereas none of the patients in the non-surgical group had moderate depressive symptoms. In contrast, minimal depressive symptoms were more evenly distributed between the two groups (26.1% vs. 70%). The distribution of depressive symptom levels was not significantly different between the adherent and non-adherent groups, suggesting that treatment adherence was not a major determinant of depressive symptom severity in this study population.

Adherence vs. Non-Adherence

Patients in the adherent group had a slightly longer median disease duration than those in the non-adherent group (13.4 vs. 10.1 years). There were no statistically significant differences in serum calcium, phosphorus, PTH levels or BDI scores between the adherent and non-adherent groups. Similarly, the prevalence of complications was comparable between the two groups (Table 2).

Correlations

Although no biochemical differences were observed between the groups according to BDI scores, correlation analysis between BDI scores and biochemical parameters, revealed a weak negative correlation between BDI scores and the highest recorded Ca levels ($r = -0.359$, $p = 0.04$), suggesting that lower calcium levels may be associated with higher depressive symptom scores (Figure 3).

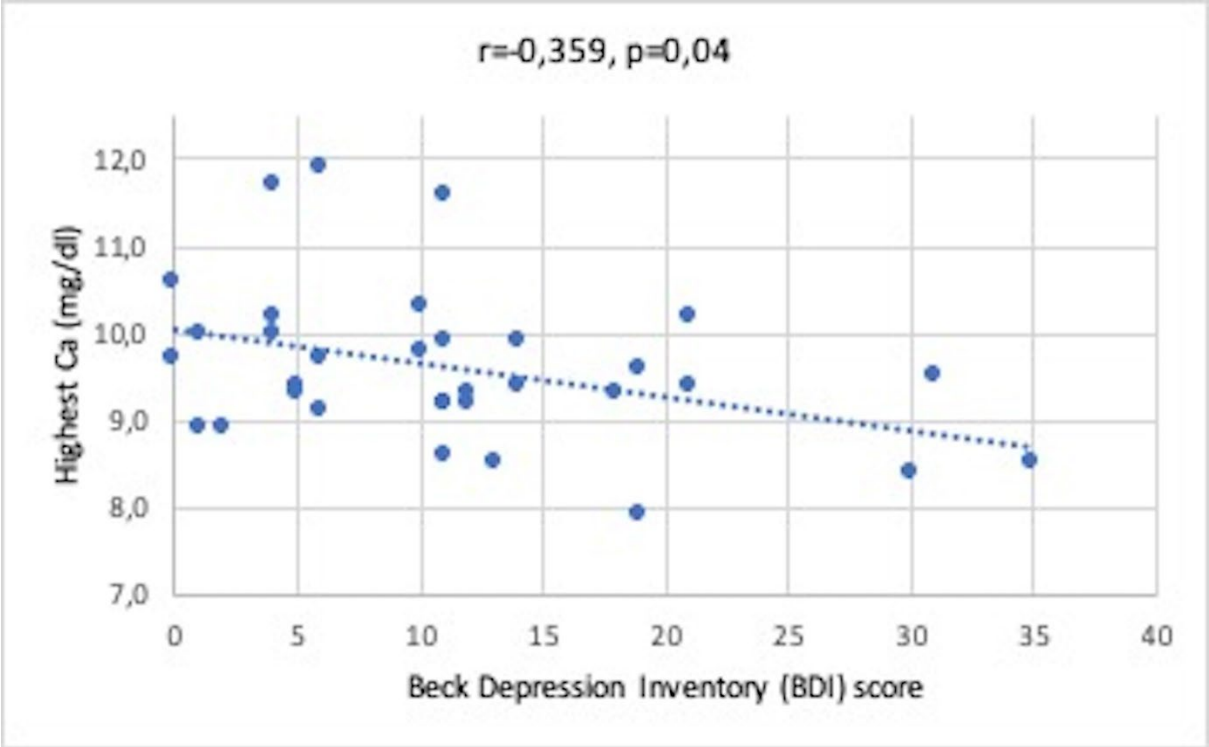

Figure 3. Negative Spearman correlation graph between Beck Depression Inventory (BDI) score and highest measured Ca value (rho=-0.359, p=0.04)

DISCUSSION

(HPT) is a chronic disease that requires long-term treatment, and its management is complicated by various factors, including patient adherence and psychological well-being. Although treatment adherence is important in achieving calcium and phosphorus target levels, Hadker et al. reported that 72% of patients had more than 10 a symptoms of hypocalcemia, despite one year of treatment (13). Anaforoğlu et al. found no difference in biochemical data and complications between HPT patient groups with and without treatment adherence (9). In our study, the complication rate was 32.4% and there was no difference in complications between the adherent and non-adherent groups.

Despite the expectations that treatment adherence would influence biochemical parameters and complication rates, no statistically significant differences were observed between the adherent and non-adherent groups. This finding may be due to the relatively small sample size of the study, the short follow-up period, and lpossible compensatory physiological mechanisms. Also, patients classified as non-adherent may have had partial adherence rather than complete treatment discontinuation. In addition, patients with severe non-adherence may have also missed follow-up visits, resulting in under-representation of true non-adherence underrepresentation of true non-adherence-related complications. This could lead to selection bias, with only partially adherent patients remaining in the study, potentially underestimating the true impact of poor adherence.

Treatment non-adherence was found to be 39.3% in the study by Anaforoglu et al. and 54.5% in our study. However, in our study, treatment non-adherence was defined as missing more than one medication per week and interview tools such as the Brief Medication Questionnaire (BMQ) and the Medication Adherence Rating Scale (MARS) which are used to assess non-adherence, were not used in this study (14-15). However, as discussed by Lieber et al. rather than using the same questionnaires for all chronic diseases to assess adherence, we suggest that the development of specific questionnaires tailored to HPT may better reveal behavioural tendencies and improve adherence assessment in future studies (10).

Patients with HPT are known to haveIt e difficulties in adhering to treatment compliance due to multiple drug use and gastrointestinal side effects (16). In addition, Büttner et al. have shown that patients diagnosed with HPT have an unmet information gap on many issues, particularly the long-term effects of the disease and treatment side effects (17). Comprehensive patient education at diagnosis and follow-up may improve adherence, although this requires further validation in prospective randomized trials. In our study all patients with HPT were treated with conventional therapies (calcium, vitamin D and active vitamin D metabolites). Recombinant PTH (1-84) therapy was introduced for the treatment of HPT after the REPLACE phase 3 trial in 2017 (18). When used in patients who do not respond to standard treatments and cannot use these treatments due to side effects, it is known to reduce the required doses of calcium and active vitamin D and improve quality of life (16).

In a multicentre study designed to evaluate the long-term effects of recombinant PTH (1-84) given as an adjunct to standard treatment for 24 weeks, Vokes et al compared quality of life measures using the Short Form-36 with a control group receiving placebo in addition to standard treatment. Although no statistically significant difference was found between the groups, the group receiving recombinant PTH showed statistically significant improvements compared to their baseline. In addition, in August 2024, the U.S Food and Drug Administration (FDA) approved the use of

palopegteriparatide, an extended-release PTH (1-34) prodrug, for chronic HPT (8). In a phase 3 trial, palopegteriparatide demonstrated maintenance of normocalcemia without conventional therapy, reduced urinary calcium, improved renal function, and quality of life in patients with chronic HPT (19). Based on these considerations, we believe that new trials on the use of PTH treatment alone should focus on its efficacy on neuropsychiatric outcome in addition to quality of life.

The exact mechanisms underlying the association between HPT and depressive symptoms remain unclear, but there are several hypotheses. One possible pathway involves the effect of hypocalcemia on neurotransmission. Calcium plays a crucial role in neuronal excitability and synaptic transmission (6). Severe hypocalcemia can disrupt these processes, potentially leading to alterations in brain function and mood regulation and contributing to depressive symptoms (20). The observed improvements in cognitive function and potentially depressive symptoms with calcium restoration in several studies support this hypothesis (5-21). Calcifications in brain regions involved in mood regulation could disrupt neuronal activity and lead to depressive symptoms (22).

Several studies have directly linked HPT to an increased prevalence of depression and other mood disorders. In a large cohort study including 688 non-malignant HPT patients and 2064 controls, Underbjerg et al. showed an increased risk of depression and bipolar disorder in HPT patients with an HR (hazard ratio) of 1.99 (23). A nationwide cohort study in Korea showed a significantly higher incidence of depression, especially bipolar depression, HPT patients compared to controls (24). Specifically, 21.0% of patients with nonsurgical HPT had depression and bipolar disorder, compared to 12.4% in the control group. This study also found that patients with nonsurgical HPT had a significantly increased risk of depression and bipolar disease, with a hazard ratio of 1.82 (95% CI, 1.30–2.56) compared to controls. Hillary et al. also, found that patients with post-surgical HPT reported more fatigue and loss of energy compared to controls, but overall quality of life was not significantly different (25).

In our study, the observed association between hypoparathyroidism etiology and depression severity suggests that post-surgical patients may be at higher risk for moderate depressive symptoms. This may be due to differences in disease perception, post-operative complications, or psychological adjustment after surgery. Interestingly, none of the patients with non-surgical hypoparathyroidism had exhibited moderate depression. Overall, 24.2% of the patients in our study were found to have moderate to severe depressive symptoms based on BDI scores. This highlights the significant psychiatric burden associated with HPT, as reported in many previous studies (26–28). The clinical presentation of depression in the context of HPT can vary. Some studies report cases where depression is a prominent symptom (29-30). In addition, some studies suggest that patients with HPT have higher scores on validated questionnaires measuring anxiety and depression, suggesting a clinically relevant association (9-26). The study by Anaforoglu et al. showed a statistically significant difference in BDI scores between the groups with and without treatment adherence and significant negative correlations were also observed between anxiety/depression scores and serum Ca and Mg levels. Our study found a weak negative correlation between depression severity and Ca levels (rho=-0.359, p=0.04), suggesting that as Ca levels decrease, depression severity may increase. While this finding suggests a possible association, the weak correlation suggests caution in interpreting these results. In their 2013 study, Aggarwal et al. found neuropsychological dysfunction in approximately one third of idiopathic HPT patients and showed that this was correlated with female

gender, Ca levels, and CaxP products (31). The fact that 78.8% of the HPT patients included in our study were female may have influenced the results. The increase in BDI scores with decreasing patient Ca levels is consistent with the study by Aggarwal et al. However, as self-reporte scales likesuch as the BDI were used, future studies incorporating structured psychiatric evaluations are needed to establish causality and assess changes over time. For example, the Hypoparathyroid Patient Questionnaire (HPQ) specifically identified depression and anxiety as significant scales in assessing the impact of the disease on patients' lives (32). This highlights the need for comprehensive assessment tools that include mental health measures.

This study has several limitations. First, all patients with HPT were treated with conventional therapies (oral Ca and active vitamin D metabolites), and no patients received recombinant PTH (1-84) or palopegteriparatide therapy, limiting comparisons between treatment modalities. Second, treatment non-adherence was defined as missing more than one medication per week, but no standardized adherence assessment tools were used, which may have limited the comprehensiveness of the adherence assessment. In addition, clinical findings related to HPT were not systematically recorded, preventing a detailed correlation between treatment adherence and clinical outcomes. Another limitation is that patients underwent multiple dose adjustments during follow-up, leading to variability in treatment regimens. As a result, dosing data were not included in the final analysis, which may have influenced the interpretation of adherence-related biochemical and clinical outcomes. In addition, depressive symptoms were assessed using self-report scales (BDI) without structured psychiatric assessment or longitudinal follow-up, which limits causal inference. Finally, the study population had a high proportion of female participants (78.8%), which may have influenced the results, as gender differences may play a role in both treatment adherence and depression severity. Overall, despite the small sample size, observed effect sizes suggest clinically meaningful associations, highlighting the need for further research with larger cohorts and longer follow-up period.

CONCLUSIONS

HPT is a rare chronic disease that leads to alterations in the metabolism of minerals such as Ca, P, and Mg, resulting in various long-term cardiovascular, neurological, and psychiatric complications. In addition to demonstrating an increased frequency of depressive symptoms in patients diagnosed with HPT, this study highlights the importance of treatment goals by revealing the negative correlation between Ca levels and BDI scores. Furthermore, our findings suggest that disease etiology may influence neuropsychiatric symptoms, emphasizing the need for tailored management strategies for different patient subgroups. Given the complex interplay between biochemical stability and psychological health in HPT, a more disease-specific approach to neuropsychiatric assessment is warranted, particularly in light of emerging treatment options. The development and validation of targeted assessment tools could provide a better understanding of the psychological burden of HPT and help to refine individualized therapeutic strategies for these patients.

Informed Consent:

Written informed consent was obtained from each participant.

Authorship Contributions

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

Conflict of Interest:

No conflict of interest was declared by the authors.

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Case Report

Adrenal Lymphangiomatous CYST with papillary and pseudopapillary endothelial proliferation and sinaptophysin positivity: A Case Report

Papiller ve psödopapiller endotelyal proliferasyon ve sinaptofizin pozitifliği gösteren adrenal lenfanjiyomatöz kist: Olgu Sunumu

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ABSTRACT

Benign adrenal cysts are rare lesions. Although they can cause some clinical symptoms such as abdominal distension and pain, they are mostly encountered incidentally. The most common are pseudocysts, endothelial, epithelial, and parasitic cysts. Endothelial cysts may be of vascular or lymphatic origin. In this case report, a unilocular lymphangiamotous adrenal cyst showing marked papillary and micropapillary proliferation and synaptophysin positivity is discussed in the light of current literature.

Keywords: Adrenal cyst, endothelial proliferation, lymphangiomatous cyst, synaptophysin

ÖZ

Benign adrenal kistler nadir görülen lezyonlardır. Karında şişkinlik, ağrı gibi klinik belirtilere neden olabilse de çoğunlukla insidental olarak karşılaşılmaktadır. Psödokistler, endotelyal kistler, epitelyal kistler ve parazitik kistler en sık görülenlerdir. Endotelyal kistler vasküler veya lenfatik kökenli olabilir. Bu olgu sunumunda belirgin papiller ve mikropapiller proliferasyon ve sinaptofizin pozitifliği gösteren uniloküler lenfanjiamotöz adrenal kist güncel literatür ışığında tartışılmıştır.

Anahtar Kelimeler: Adrenal kist, endotel proliferasyonu, lenfanjiomatöz kist, sinaptofizin

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A 20-year-old female patient was admitted to the hospital due to abdominal pain. Abdominal computed tomography revealed a unilocular cyst with a diameter of 10.5 cm in the right adrenal gland. The patient was operated by open excision.

In the macroscopic examination of the mass, a unilocular cystic lesion with a diameter of 10.5 cm was observed adjacent to normal adrenal and fatty tissue (Figure 1).

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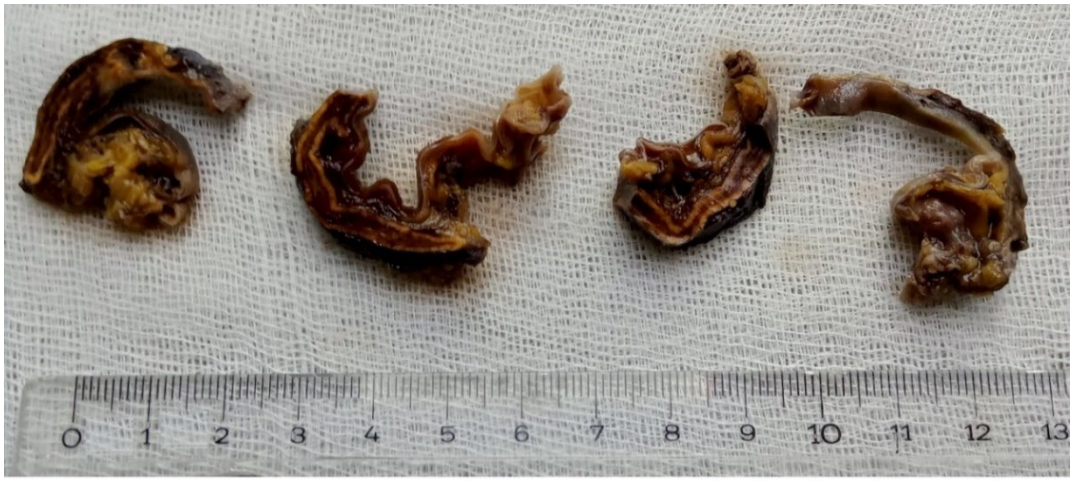

Figure 1. Macroscopic apperence of the cyst and adrenal tissue

Histological examination revealed a cystic lesion lined with endothelial cells next to the adrenal gland. Papillary and pseudopapillary proliferation was observed throughout the cystic lesion. The cells of the lining epithelium were generally columnar in shape, with an increased nucleus-cytoplasm ratio, and some consisted of hobnail-like cells. No mitotic activity, necrosis, or hyperchromasia was observed. Hyalinized connective tissue containing many dilated vessels was observed at the base of the papillary structures (Figure 2-3).

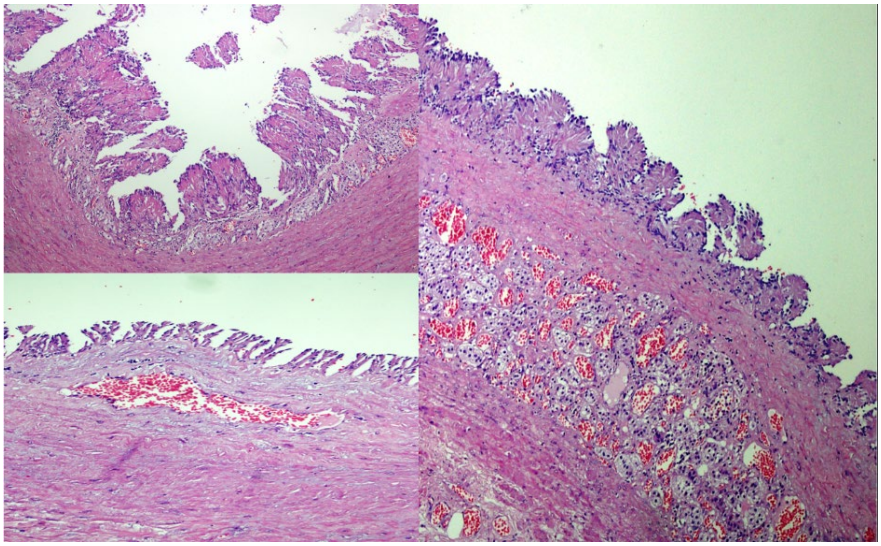

Figure 2. Papillary and micropapillary structure of the cysts H&E X100 and H&E X200.

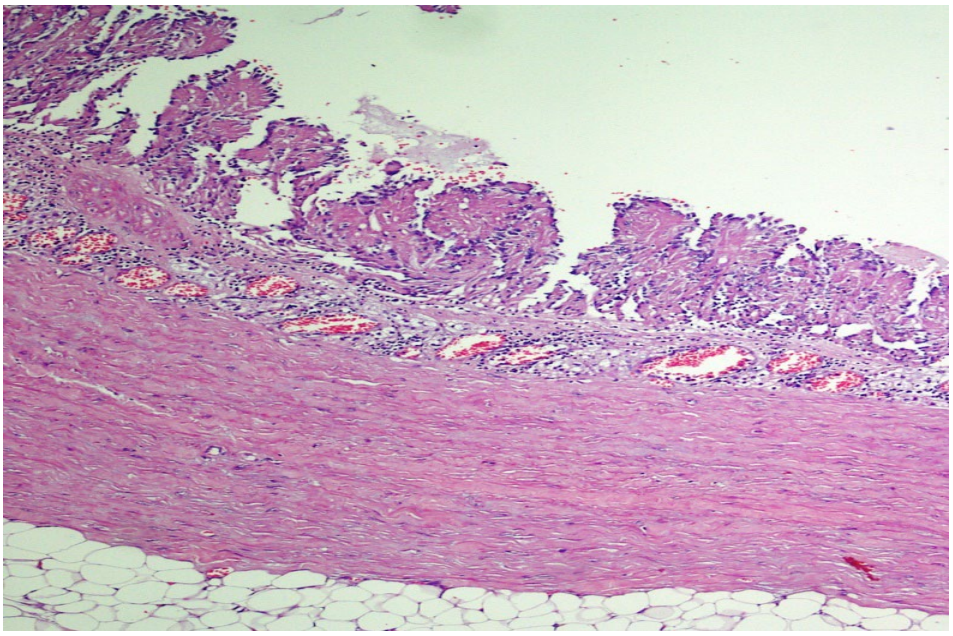

Figure 3. Papillary structure H&E X 400.

Immunohistochemical examinations were performed for the differential diagnosis of adrenal tumors and renal tumors. Negative staining was obtained with CAIX, CK7, PAX8, Melan-A, inhibin, CD10, RCC, HMB45, and chromogranin. CD 31, FLI-1, factor VIII, podoplanin, synaptophysin, and ERG were shown to have immunopositivity (Figure 4).

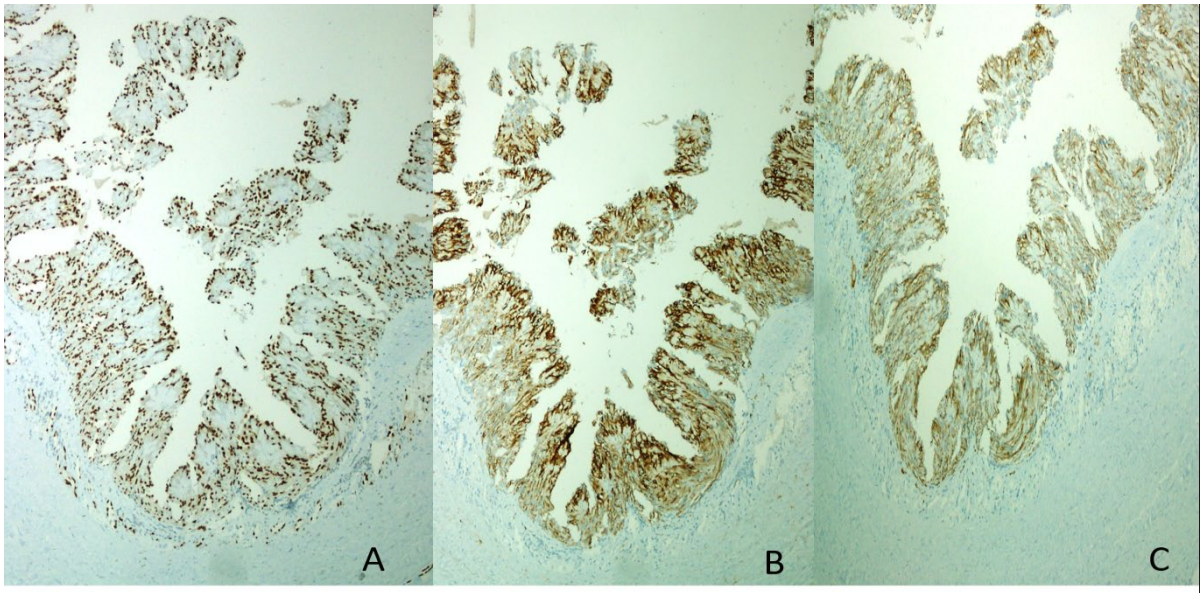

Figure 4. Immunohistochemistrial staining A: Podoplanin, B: ERG C: Synaptophysin.

Based on histological and immunohistochemical findings, the case was diagnosed with a lymphangiomatous cyst showing papillary proliferation.

DISCUSSION

Benign adrenal cysts are rare lesions. These cysts can vary in size and may be multiloculated or unilocular. Although there are differences in autopsy series and surgical studies, pseudocysts and endothelial cysts constitute the majority of these cysts (1). Pseudocysts are not lined by epithelium. Epithelial cysts are subdivided into glandular or retention cysts, cystic adenomas, and embryonal cysts. Hamartomatous, angiomatous, and lymphangiomatous cysts are subtypes of endothelial adrenal cysts.

Sometimes, benign adrenal cysts need to be surgically removed so that a definitive diagnosis can be made through pathological examination. Surgical indications for adrenal cysts are generally large size, complications, and suspicion of malignancy (2-5). Cystic metastases and cystic pheochromocytoma may be confused with benign adrenal cysts and pathological examination may be required (6).

The differential diagnosis of these lesions is based on histological and immunohistochemical features (7-10). Lymphatic cysts are usually lined with a flattened epithelium and contain serous fluid. Koperski et al. classified lymphatic cysts histologically as ‘typical multicystic lymphatic malformation’, ‘unilocular lymphangiomatous cyst’, and ‘lymphangiomatous cyst with papillary endothelial proliferation’. In this review, 4 cases showed diffuse papillary or pseudopapillary proliferation (9). All of these cases were unilocular cysts, similar to our case, and in this study, no radiological and clinical differences were observed, except that the cysts showing papillary proliferation were larger in size than other lymphatic cysts. Immunohistochemistry can be used to distinguish tumours with this histological appearance from tumours presenting with papillary structures. While lymphatic cysts are expected to stain positively with D2-40, CD31, and ERG, they stain negatively with pankeratin and CD34. However, although its importance is unknown, in our case, synaptophysin positivity was accompanied by the markers indicating lymphatic origin. Although synaptophysin positivity has been reported in vascular lesions, no satisfactory information has been found in the literature regarding synaptophysin positivity in lymphatic cysts (11).

CONCLUSIONS

Benign adrenal cysts can be operated if the lesions are large, cause complications, or to rule out malignancy. Papillary and pseudopapillary structures can be seen in cysts of lymphatic origin, and the differential diagnosis of these lesions can be made by pathological examination accompanied by macroscopic, microscopic, and immunohistochemical findings.

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Case Report

Ectopic thyroid tissue presenting as a mediastinal mass: A Case Report

Mediastinal kitle olarak prezente olan ektopik tiroid dokusu: Olgu Sunumu

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ABSTRACT

The presence of ectopic thyroid tissue in the mediastinum is a rare entity, accounting for approximately 1% of cases of ectopic thyroid tissue in adults. When detected, it typically presents as a mediastinal mass. Following the differential diagnosis, the primary treatment is surgery. In this case report, a female patient with complaints of back pain and shortness of breath was evaluated, and following examinations, mediastinal ectopic thyroid tissue was identified as the preliminary diagnosis. The patient underwent thyroid lobectomy and mediastinal mass excision. The aim of this case report is to discuss the diagnosis and treatment of mediastinal ectopic thyroid tissue.

Keywords: Ectopic thyroid, mediasten, mass.

ÖZ

Mediastende ektopik tiroid dokusunun varlığı nadir bir durumdur ve yetişkinlerde ektopik tiroid dokusu vakalarının yaklaşık %1'ini oluşturur. Tespit edildiğinde, tipik olarak mediastinal bir kitle olarak ortaya çıkar. Ayırıcı tanıyı takiben, birincil tedavi cerrahidir. Sunulacak vakada sırt ağrısı ve nefes darlığı şikayetleri olan bir kadın hasta değerlendirilmiş ve yapılan tetkikler sonrasında ön tanı olarak mediastinal ektopik tiroid dokusu tespit edilmiştir. Hastaya tiroid lobektomi ve mediastinal kitle eksizyonu uygulandı. Bu olgu sunumunun amacı mediastinal ektopik tiroid dokusunun tanı ve tedavisini tartışmaktır.

Anahtar Kelimeler: Ektopik tiroid, mediasten, kitle

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INTRODUCTION

Ectopic thyroid tissue is defined as thyroid tissue that is not located anterolateral to the second to fourth tracheal cartilages, which is its normal anatomical position. Anatomically, ectopic thyroid tissue can be located in areas such as the tongue, sublingual area, and anterior larynx. (1) Mediastinal ectopic thyroid tissue is a rare pathology with an incidence of 1%. (2) Surgical intervention is the treatment of choice; however, it is essential to first differentiate mediastinal ectopic thyroid tissue from other mediastinal masses during the diagnostic process. In the case presented in the article, a mediastinal mass was initially detected and, through

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differential diagnosis, identified as ectopic thyroid tissue. Subsequently, a treatment plan was made.

CASE REPORT

A 40-year-old female patient was admitted to the outpatient clinic with complaints of shortness of breath, and back and neck pain in May 2024. The patient's past medical history, family history and laboratory tests were unremarkable .Based on the PA chest X-ray (Figure 1), neck ultrasonography, and thorax CT (Figure 2 and 3) performed at a different medical institution before the patient presented to our hospital, a mediastinal malignancy, approximately 8x7 cm in size, was the primary consideration.

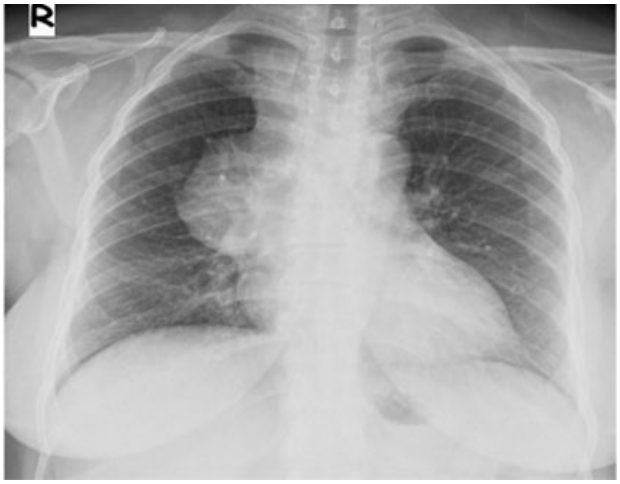

Figure 1. Mediastinal mass on preoperative chest X-ray

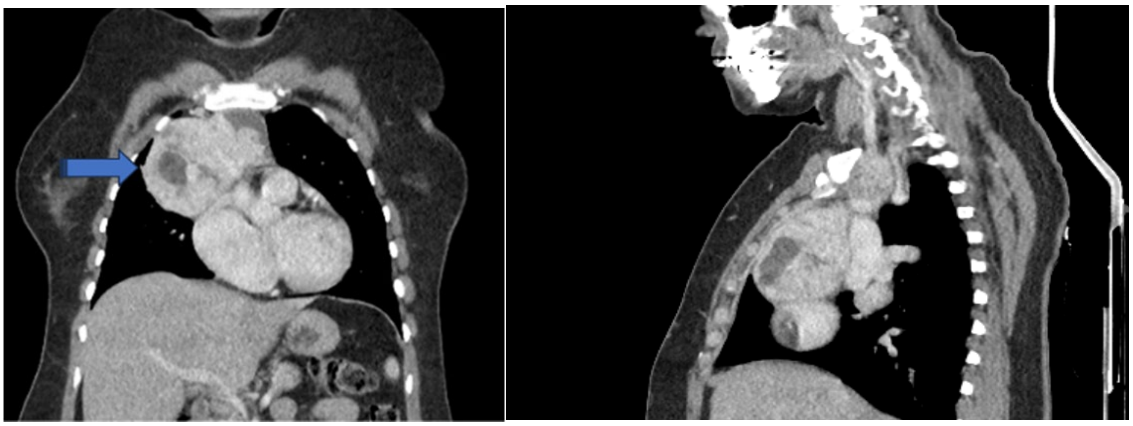

Figure 2-3.CT image of mediastinal ectopic thyroid tissue not associated with thyroid tissue.

Additionally, a 3 cm nodule was detected in the right thyroid lobe on ultrasonography. USG guided biopsy and PET-CT were performed. The biopsy confirmed benign ectopic thyroid tissue in the mediastinum. However, due to persistent suspicion of malignancy on the PET-CT, the patient was referred

to another center for further evaluation. PET-CT results described the mass as a heterogeneous, necrotic,hypermetabolic lesion originating from the mediastinum. The mass, was approximately 9x7 cm. Surgical intervention was planned in collaboration with the thoracic surgery department. Due to a 3 cm nodule in the right thyroid lobe, the patient underwent a right thyroid lobectomy. Additionally, a sternotomy was performed for the excision of the mediastinal mass. It was observed during the operation that the mediastinal mass was not connected to the thyroid tissue (Figure 4 and 5).

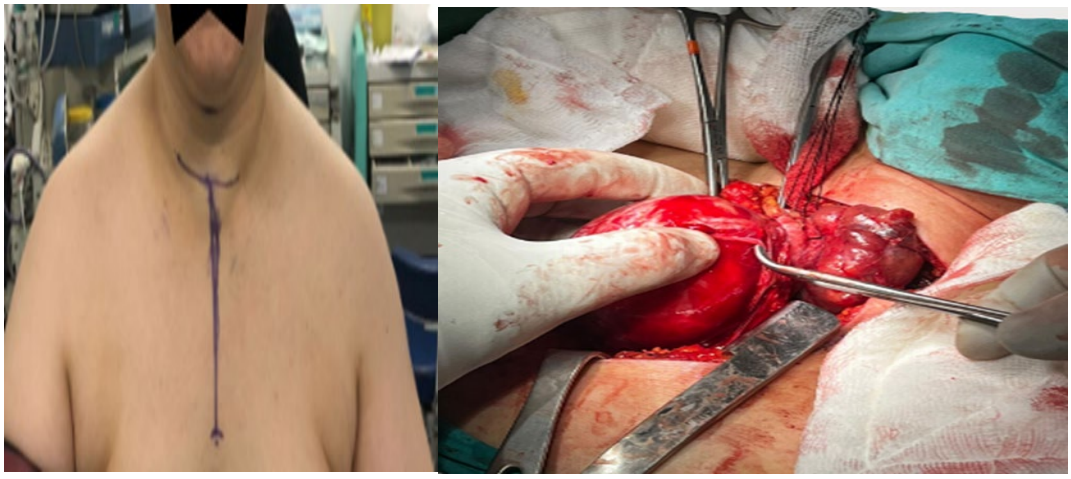

Figure 4-5. Thyroid right lobectomy and mediastinal mass excision performed through Kocher incision and sternotomy.

A frozen section of the mass removed during surgery confirmed it as thyroid tissue. On the postoperative day 1, the chest X-ray showed the complete disappearance of the mass (Figure 6) .

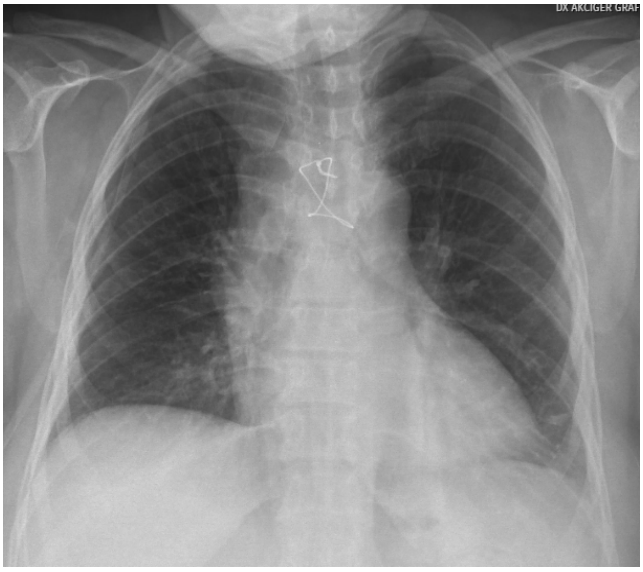

Figure 6. Postoperative chest X-ray

The patient was discharged on the postoperative day 4. Pathologic evaluation of the mediastinal mass was consistent with multinodular ectopic thyroid hyperplasia.

DISCUSSION

Ectopic thyroid tissue (ETT) refers to thyroid tissue that is not situated in its normal anatomical location at the front of the neck, between the 2nd and 4th tracheal cartilages, at the level of the C5-T1 vertebrae, but is found in an alternate location. (1) It is observed in 1 in 100,000 to 300,000 adults. (2) Approximately 90% of ectopic thyroid tissues are located in the lingual area. However, it has also been reported to be seen in the submandibular region, adrenal glands, gallbladder, esophagus and mediastinum (3).

Mediastinal thyroid tissue constitutes approximately 1% of ectopic thyroid tissues (4-5). Less than 15 cases have been reported in the last 40 years. Mediastinal ectopic thyroid tissue has no connection with normal thyroid tissue and receives its blood supply from intrathoracic vascular structures (2-6). In the presented patient, it was also observed during surgery that there was no such connection with the normal thyroid tissue.

Mediastinal ectopic thyroid tissue is usually detected incidentally on imaging or during surgery (7). However, when the ectopic thyroid tissue becomes large enough to cause symptoms, it can lead to compression symptoms such as shortness of breath, cough, and back pain (8). In the patient presenting to our clinic, symptoms of cough and back pain were present, which were attributed to the size of the mass.

In diagnosing mediastinal ectopic thyroid tissue, the first step is to differentiate it from other mediastinal lesions. The most common mediastinal tumors are thymomas, germ cell tumors, lymphomas, neurogenic tumors, and benign cysts. If ectopic thyroid tissue is suspected, a neck ultrasound to assess the normal localization of the thyroid gland, and thyroid scintigraphy to identify ectopic tissue; should be conducted. Additionally, if clinically feasible, a biopsy of the mass is recommended (1-9). In the presented case, a neck ultrasound and CT scan had been performed. Additionally, due to the prominence of necrotic tissues and the suspicion of malignancy, a PET-CT scan has been performed at a different medical institution. Malignancy could not be excluded by the PET result, and PET-CT was thought to have no contribution to the diagnosis.

In cases of mediastinal ectopic thyroid tissue, the patient may present as either euthyroid or hypothyroid. Yoon JS et al., noted that two-thirds of patients with ectopic thyroid tissues had hypothyroidism (10). In the presented patient, thyroid function test results were normal.

The treatment for ectopic thyroid tissue is surgical. The location of the mass and its relationship with surrounding structures on the CT scan are crucial for planning the surgical approach. While the thyroid tissue in the upper mediastinum can be excised only through a cervical approach, additional sternotomy is necessary for safer excision of the mass for thyroid tissues located lower. This approach not only alleviates the patient's symptoms in the early postoperative period but also reduces mortality(11). For the patient with mediastinal ectopic thyroid tissue, a cervicosternotomy was planned in collaboration with the thoracic surgery department due to the location of the mass and its proximity to neighboring organs. Due to the presence of a nodule in the right thyroid lobe, a right lobectomy was performed in addition to the excision of mediastinal ectopic thyroid tissue. The patient's complaints in the preoperative period had also dramatically regressed postoperatively.

CONCLUSION

Mediastinal ectopic thyroid tissue is a very rare pathology. In case of detection of a mediastinal mass, differential diagnosis is crucial as it significantly impacts the treatment plan and the surgical approach. When evaluating mediastinal masses for diagnostic purposes, ectopic thyroid tissue should also be considered.

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Review

Androgen receptor signalling pathway and possible therapeutic targets ın prostate cancer

Androjen reseptör sinyal yolağı ve prostat kanserinde muhtemel terapötik hedefler

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ABSTRACT

A kind of cancer affecting millions of men globally is prostate cancer (PCa). The way that PCa is treated is always changing because of new insights into human genetics and biological processes. A number of novel therapeutic classes have emerged in recent times, such as bone-targeted medications, poly(ADP-ribose) polymerase (PARP) inhibitors, and next-generation androgen receptor (AR) signaling inhibitors. Additionally, PSMA-targeting drugs show promise as theranostics that might enhance the precision of diagnosis and effectiveness of treatment. The outcome of prostate cancer is significantly influenced by the AR signaling pathway. This study examines novel molecules that are either now targeted or may be targeted in the AR signaling pathway, which is highly successful in the carcinogenesis of prostate cancer.

Keywords: Prostate cancer, androgen receptor signaling pathway, therapeutic targets.

ÖZ

Prostat kanseri (PCa) dünya çapında milyonlarca erkeği etkileyen bir kanser türüdür. İnsan genomunun ve biyolojik işlevlerinin anlaşılmasındaki ilerlemeler sayesinde, PCa tedavisi sürekli olarak gelişmektedir. Son zamanlarda, yeni nesil androjen reseptör (AR) sinyal inhibitörleri, kemik hedefli ajanlar ve poli(ADP-riboz) polimeraz (PARP) inhibitörleri dahil olmak üzere birkaç yeni ilaç sınıfı geliştirilmiştir. Ayrıca,prostat spesifik membran antijenini (PSMA) hedefleyen ajanlar, tanısal doğruluğu ve terapötik etkinliği artırabilecek umut verici teranostiklerdir. AR sinyal yolağı prostat kanserinin prognozunda kritik bir role sahiptir. Bu derlemede, özellikle PCa'nın karsinogenezinde etkili olan AR sinyal yolağı ve bu yolakta halihazırda hedeflenen veya hedeflenmesi muhtemel yeni moleküller gözden geçirilmiştir.

Anahtar Kelimeler: Prostat kanseri, androjen reseptör sinyal yolağı, terapötik hedefler.

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INTRODUCTION

In Türkiye and across the world, prostate cancer is the cancer that occurs most frequently in males (1-2). Adenocarcinomas of prostate cancer, share many features with other common epithelial cancers such as breast and colon cancer. Family history of prostate cancer, age, race, vitamin E, hormones, calcium, and folic acid are known to be important causes for prostate cancer. In addition, finasteride and dutasteride, which inhibit the conversion of folate and testosterone to dehydrotestosterone (DHT), are reported to reduce the risk of developing prostate cancer (3).

The androgen receptor (AR) signalling pathway has a critical role in the function, development and homeostasis of the prostate (4). The initiation and prognosis of prostate cancer are also dependent on AR. The usual course of treatment for advanced prostate cancer is androgen deprivation therapy. Even though androgen restriction treatment produces a favorable response initially, nearly all patients eventually develop an extra aggressive, castration-resistant phenotype(4). Castrate-resistant prostate cancer (CRPC) development seems to be directly linked to AR. It is imperative to comprehend the pivotal moments and intricacies of AR signaling in the advancement of CRPC in order to devise efficacious therapeutics in the future (5). Therefore, in this review, the AR signalling pathway, an important signalling pathway in prostate carcinogenesis, will be examined and new molecules currently targeted or likely to be targeted in this pathway will be reviewed.

AR STRUCTURE

The AR gene synthesizes a 110 kD protein with 919 amino acids. It has a conserved DNA-binding domain (DBD) and androgen-binding domains (ABD), moderately conserved ligand-binding domains (LBD), and a less conserved N-terminal transactivation domain (NTD). The transcriptional activity of AR is affected by polymorphic trinucleotide repeat segments, that can activate transcription in LBD-deletion mutants regardless of androgenic stimulus (6) (Figure 1).

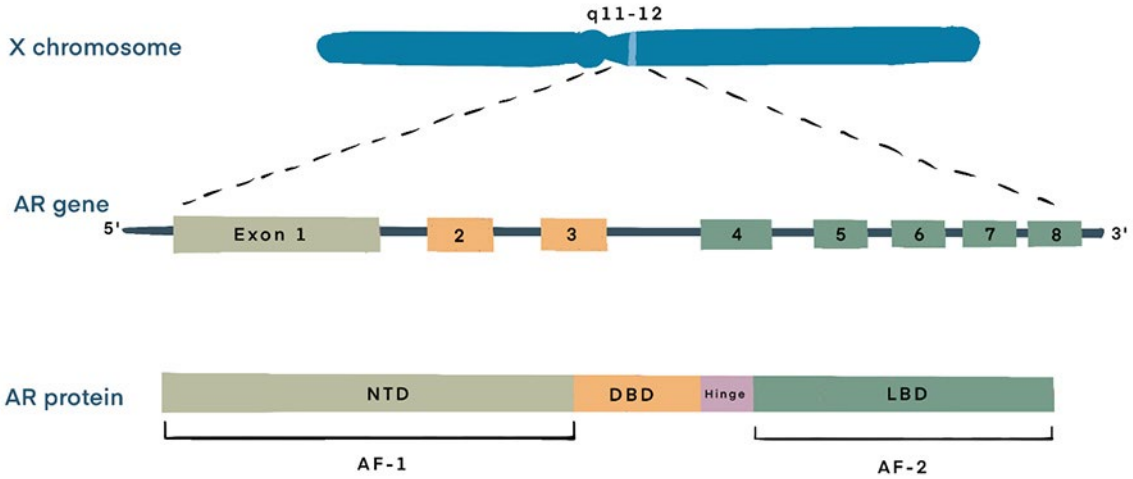

Figure 1. Schematic representation of the androgen receptor gene and protein with indication of its specific motifs and domains. (Lonergan, P. & Tindall, D. Androgen receptor signalling in prostate cancer development and progression. Adapted from J Carcinog 10, (2011) article 5).

AR SIGNALING PATHWAYS

Classic AR Signaling Pathway

AR, a conserved protein in androgen-independent prostate cancers, promotes cell survival and growth. It forms complexes with chaperone proteins such as HSP90, in the absence of ligands like DHT and testosterone. When AR binds to androgens, it is released, translocates to the nucleus, and promotes gene transcription, accelerating normal prostate cell maturation and tumor progression (7).

Following a sequence of steps known as dimerization, the AR attaches to certain recognition sequences in promoter and enhancer domains of target genes, which are called androgen response elements (AREs), once it has entered the nucleus. Co-regulators are brought into the AR transcriptional group to complete it,eventually modifying gene expression (5) (Figure 2).

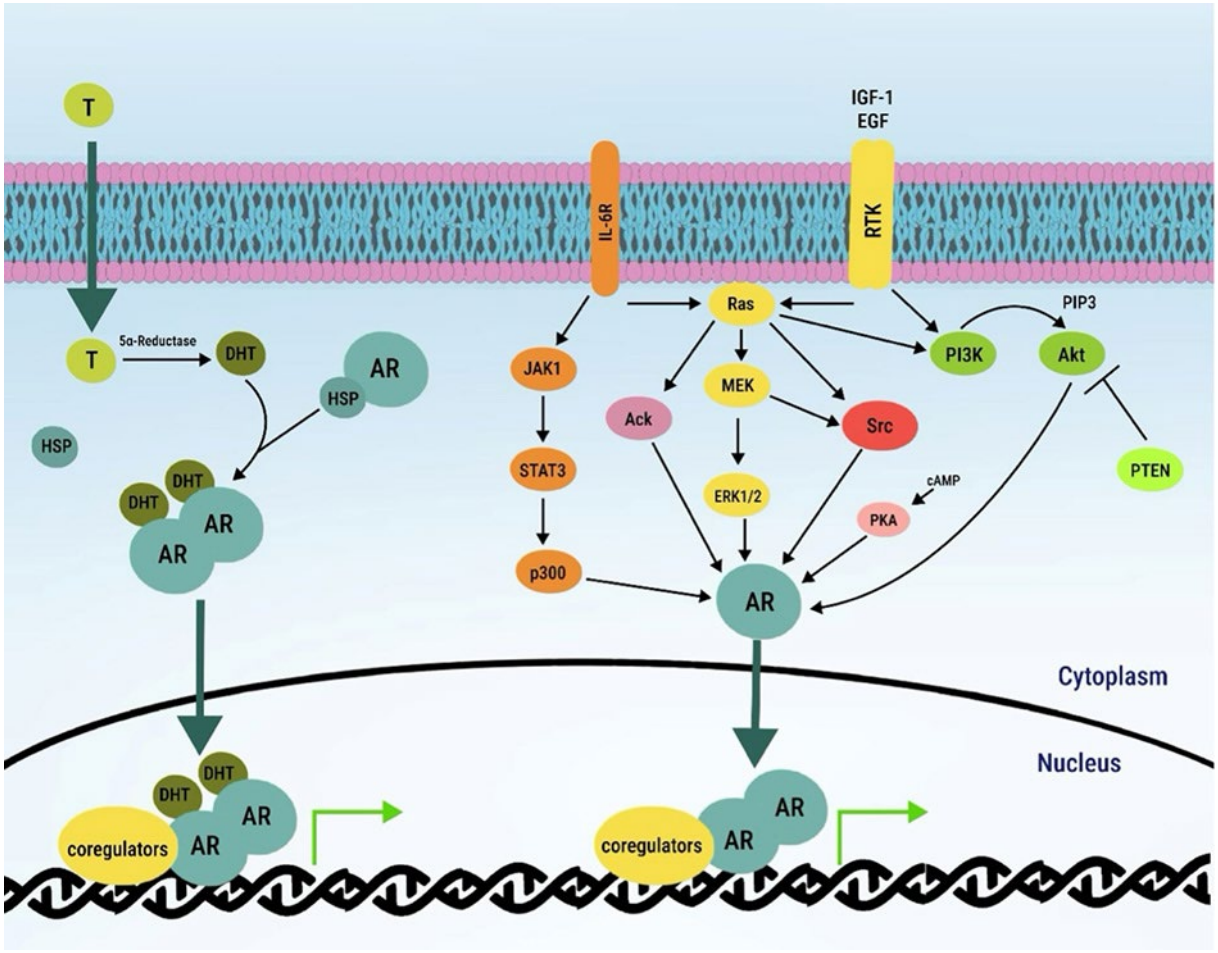

Figure 2. Summary of the major androgen receptor signalling pathways in prostate cancer. (Lonergan, P. & Tindall, D. Androgen receptor signalling in prostate cancer development and progression. Adapted from J Carcinog 10, (2011), article 6).

AR Co-regulator Proteins

AR co-activators do not significantly change basal transcription rates and are capable of binding DNA. They function as either enhancers or repressors based on the target gene. Rather, they attract general transcription factors linked to RNA polymerase activity by acting on the promoter of the AR target gene. Molecular chaperons, transcription coordinators, histone modifiers, and DNA structure modifiers are the four primary categories of these proteins (5).

Co-regulators are recruited onto ligand-bound nuclear AR to form the functional AR-direct transcription pre-initiation complex (8). The most well-known group of co-activators is the p160 family, which includes SRC1, TIF2, and SRC3. In 50% of samples of androgen-dependent prostate cancer, there was a rise in SRC1 expression; in 63% of samples of CRPC, SRC1 and TIF2 expression were noted. In clinically localized illness, elevated SRC3 levels are associated with the grade and stage of prostate tumors. By interacting with AR NTD and LBD, these p160 co-activators improve target gene transcription mediated by AR. The ability of AR to process information is directly impacted by their recruitment, whereas chromatin remodeling and the acquisition of secondary co-activators possessing protein acetyltransferase capacities are indirectly encouraged (9-10).

AR Target Genes

The study reveals chromosomal rearrangements in the promoter of the androgen-regulated TMPRSS2 gene, forming novel fusions to the 3' ends of oncogenic ETS transcription factor family members, ETV1 or ERG, significantly contributing to prostate cancer progression and development (10). Utilizing cDNA microarrays from human prostate tissues, TMPRSS2 was found to investigate the transcript-expressing patterns of androgen-sensitive LNCaP cells. It was discovered to be limited to prostate luminal epithelial cells and unique to prostatic tissue (11).

The inhibition of c-Myc, which has an antagonist transcriptional network with AR, occurs with the activation of AR (12). It has also been revealed that AR regulates c-Myc via histone methyltransferase, DOT1L (disruptor of telomeric silencing 1-like), and an enhancer. Other transcription factors, such as c-Myc, enhance the proliferation of AR-target genes in response to decreased AR expression caused by inhibition of DOT1L, which controls the AR and MYC pathways (13).

The "pioneer factor" FOXA1, which is present in more than 60% of AR binding regions in prostate cancer cells, is essential for the transcriptional control of prostate cancer. Throughout the genome-wide description of the AR cistrome in prostate cancer cell lines, a 70% overlap between FOXA1 and AR binding sites was found, suggesting a significant role for FOXA1 in the transcriptional regulation of prostate cancer. Both a rise and a fall in the number of ARBs can be caused by FOXA1 depletion. Moreover, FOXA1 has been demonstrated to inhibit AR transcriptional activity in prostate cancer cells, perhaps outcompeting AR co-activators (14).

A transcription factor called OCT1 combines with AR to strengthen signaling pathways that promote PC. In a research, immunoprecipitation of chromatin sequencing (ChIP-seq) was used to discover genes controlled by OCT1 in a set of genes linked to neural precursor cell proliferation in patient-derived CRPC cells. PFN2 and STNB1 were both shown to be substantially expressed in human CRPC cells by immunohistochemistry. Notably, it has been demonstrated that PFN2 knockdown significantly inhibits tumor development in vivo (15).

TBLR1, a key component of the nuclear receptor repressor (NCoR) complex, shows both co-repressor and co-activator activities on nuclear receptors (16). In a research it was demonstrated that TBLR1 physically interacts with AR and specifically occupies the androgen responses of the impacted AR gene targets in a way that is reliant on androgen (17).

In another study, ChIP-seq analysis showed that knockdown of SMAD3 reduced AR binding to chromatin, while knockdown of SMAD2 or SMAD4 had little or no effect. Furthermore, SMAD3 has been shown to bind to intron 3 of the AR gene to promote AR expression. When comparing primary prostate cancer to metastatic prostate cancer and CRPC data, it was shown that the mRNAs for AR and SMAD3 were up-regulated (18).

ALTERNATIVE AR SIGNALING PATHWAY

There is considerable evidence that AR remains transcriptionally active in CRPC. Numerous immunohistochemical studies have shown that AR protein is expressed at high levels (compared to levels in untreated tumours) in most cases of CRPC (4).

Interleukins

A significant body of research suggests that growth factors and cytokines, which are examples of extracellular peptide signaling, have a role in maintaining AR's transcriptional activity in CRPC. Numerous studies have been conducted on the function of interleukins (IL), particularly IL-6 and IL-8, in controlling cellular processes in various cancer types (19).

IL-6 is one of the greatest indicators of persistent inflammation in prostate cancer. Patients with untreated metastatic or CRPC have elevated serum IL-6 levels, that negatively correlate with tumor survival and response to therapy. In-vitro and in-vivo, IL-6 promotes prostate cancer cell proliferation and inhibits apoptosis via multiple signal pathways such as the JAK-STAT, extracellular ERK1/2-MAPK, and PI3K pathways. Prostate cancers with an aggressive character are linked to IL-6, which may play a role in the metastatic process by controlling the epithelial-mesenchymal transition (EMT) and driving cancer cells to the bone (20).

Another cytokine implicated in the development of prostate cancer is IL-8. Serum IL-8 levels aid in distinguishing among benign and malignant illness and are linked to the clinical phase of prostate cancer. IL-8 induces metalloproteinase-9, which aids in angiogenesis and metastasis. Like IL-6, IL-8 increases the transcriptional activity of AR, which promotes the development of tumors both in vitro and in vivo. The overexpression of IL-8 induces tumor development that is androgen-independent while decreasing the effectiveness of bicalutamide and anti-androgens (21).

Growth Factors

Prostate cancer development is associated with epidermal growth factor (EGF) and its receptor, which is the epidermal growth factor-1 receptor (EGFR). The AR antagonist bicalutamide blocks the MAPK pathway, which is activated in prostate cancer cells by EGF and EGFR activation. AR activation by Src and Ackl kinases is induced by EGF treatment in the absence of androgen. The MAPK pathway is triggered by androgen-activated AR, but the androgen response is modulated by EGF-activated MAPK signaling, which disrupts AR function. The down-regulation of AR in differentiated cells caused by EGF is reversed by MAPK extracellular kinase (MEK) inhibition, indicating that in non-tumor epithelial cells, there is an inverse relationship between EGF and androgen signaling (22).

Intracellular Kinase Signals

Prostate cancer is among the several forms of cancer for which the MAPK signaling pathway is implicated. The MAPK signaling pathway can be activated by a variety of external inputs. Numerous possible regulators of AR activity are offered by the

several targets downstream of the MAPK pathway. p42/44 extracellular signal-regulated kinases (ERK) and sarcoma-associated kinase (Src) are two examples of characterized regulators that have been found (9).

A powerful oncogene that is triggered in a variety of tumors is Src. Src phosphorylates AR at tyrosine-534 in LNCaP and LAPC-4 cells, which, in the absence of androgen, promotes nuclear transfer and DNA binding to activate transcriptional activity. EGF is one of the many stimuli that might cause Src to activate. Activated protein kinase C-1, the associated scaffolding protein's binding partner and receptor, helps regulate Src's influence on AR trans activation (RACK1) (23).

The PI3K pathway, which integrates growth signals with cellular activities such proliferation, protein synthesis, metabolism, survival and differentiation, is essential in the development of CRPC prostate carcinogenesis. Preclinical research has demonstrated a dynamic interplay between the androgen receptor signaling pathways and the PI3K-AKT-mTOR pathways, indicating a connection among these pathways throughout the progression of androgen deprivation treatment (ADT) resistance, which permits cancer cells to live and spread (24).

Members of the transcription factor nuclear factor-κB (NF-κB) are significant oncogenesis mediators in a variety of malignancies, including prostate cancer. In comparison to androgen-dependent grafts, NF-κB activity is elevated in androgen-independent cell lines, androgen-independent xenografts, as well as in metastatic prostate cancer as opposed to localized disease (25). In initial prostate cancer, high NF-κB activity is linked to poor prognosis and indicates a biochemical recurrence (26).

In the absence of androgens, protein kinase A, which is controlled by intracellular cyclic adenosine monophosphate (cAMP) levels, can modify AR activity. When androgens are not present, an alternative signaling route including forskolin can activate the AR. This mechanism stimulates adenylyl cyclase, which raises intracellular cAMP levels and, in turn, protein kinase A (PKA) (27).

AR MUTATIONS, AMPLIFICATIONS, SPLICE VARIANTS AND THEIR RELATIONSHIP WITH RESISTANCE

AR antagonists have been found to increase the incidence of mutations in metastatic prostate cancer patients compared to androgen deprivation therapy alone. Single amino acid substitutions in AR mutations allow anti-androgens to function as AR agonists, providing an advantage in cancer progression. The T877A mutation is a common example, enabling AR activation by other adrenal androgens. Specific AR germline polymorphisms have been associated with a six-fold higher risk of prostate cancer development. Other mutations increase AR-transcriptional activity by enhancing AR binding to co-regulators (28-29)

ADT inhibits the synthesis of androgens and/or works in opposition to antiandrogens that bind to the C-terminal ABD of AR and effectively prevent androgens from activating AR. To address the NTD and DBD, other medications have been created. EPI-001 was the first inhibitor of the AR's NTD. It works by covalently attaching to the NTD and obstructing protein-protein interactions that are necessary for the AR's transcriptional activity. The activation function-1 (AF-1) domain in the NTD of AR, which is in charge of AR's transcriptional activity, is bound by a combination of four stereoisomers called EPI-001 (30-31).

Exon skipping can result in AR variants like ARv567es during mRNA splicing. Even in the absence of a particular antibody, ARv567es is continuously active and capable of being translated into its protein form. It is suggested that the AR variation is the cause of this additional resistance mechanism. In contrast to full-length AR, AR-V7 and ARv567es exhibit distinct transcriptional activity. AR-V7 stimulates the production of genes, such as

UBE2C, that are involved in the advancement of the cell cycle. Samples of benign prostate tissue, hormone-depleted PCa, and CRPCa have all been shown to have both AR variant mRNAs; the CRPCa subgroup had greater amounts of both mRNAs than the hormone-depleted PCa subgroup (32).Androgen receptor signaling inhibitors (ARSIs) are the primary drugs used in endocrine treatment to block androgen receptors as much as possible by blocking the activity of ARs or preventing androgen production by cancerous cells (33).

Bicalutamide, flutamide, and nilutamide are a few examples of first-generation anti-androgens. These drugs work by binding competitively to the androgen receptor, or as an antagonist, to block the effects of androgen. Unfortunately, in LNCaP cells, which overexpress the AR and resemble castration-resistant prostate cancer, these first-generation drugs showed agonist properties in this cell line (6).

Among the several ways to generate drug resistance that have been identified include the production of constitutively active versions of AR, including AR-V7; intracrine androgens; upregulation of androgen synthesis enzymes, like AKR1C3; and enhanced drug efflux via ABCB1. Targeting these pathways and regaining medication sensitivity are the goals of the therapeutic approaches being investigated. A deeper comprehension of the processes through which medication resistance arises would enable the development of more effective treatment plans (34).

Precursor factor FOXA1 mutation and overexpression have a significant role in PCa. Gain-of-function mutations in FOXA1 are commonly seen in patients of mCRPC and primary PCa (35). Crucially, FOXA1 overexpression also inhibits the immune system, which makes ICIs less effective as a treatment for PCa. Proteolysis-targeting chimeras (PROTACs), such oligonucleotide-based PROTACs (O'PROTACs), may provide a new avenue for the development of novel anti-cancer medicines that target FOXA1 and its mutations, despite the fact that FOXA1 is known to be extremely difficult to target (36).

Drug resistance in metastatic colorectal cancer (mCRPC) is frequently caused by both prevalent resistance mechanisms similar by other malignancies and biological processes exclusive to mCRPC. General drug resistance pathways can be connected to decreased drug levels DNA damage repair, suppression of cell death, epigenetic regulation, immune system adaptations in tumors, changes in the tumor microenvironment (TME), and other variables. Furthermore, ARSIs and PARPi are the main components of the intrinsic biological mechanism of mCRPC (Figure. 3) (33).

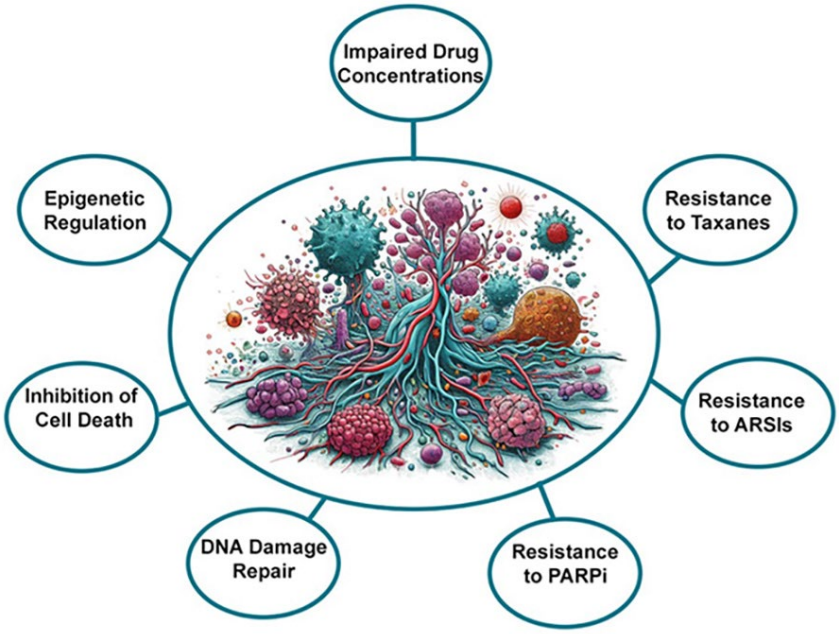

Figure 3. Drug resistance mechanisms in metastatic castration-resistant prostate cancer.
Symbols: PARPi stands for poly (ADP-ribose) polymerase (PARP) inhibitors, and ARSIs for androgen receptor signaling inhibitors. (Cai, M. et. al. Current therapy and drug resistance in metastatic castration-resistant prostate cancer. Adapted from Drug Resistance Updates, Volume 68, (2023), article 58).

Immune cells are now the primary therapy target in immuno-oncology, displacing cancer cells through immune checkpoint blockade (37). PCa is unique as a form of tumor with a worse prognosis due to increased CD8+ T cell infiltration. The limited efficacy of T cell targeted therapy in PCa may be due to the poor prognostic significance of TILs in PCa compared to other solid tumors. Prostate cancer tissues contain a larger percentage of B cells, which are more aggressive in this cancer. They produce lymphotoxin, that accelerates castration resistance and PCa metastatic spread by activating the IKKA-STAT3 and BMI1 signaling pathways in cancer clones. IgA class-directed IL10+PD-L1+ plasma cells are associated with higher levels of disease aggressiveness in prostate TME and have the ability to inhibit anti-tumor efficacy by rducing CTL activation. Macrophages are a significant immune cell population associated with PCa malignancy, and they are generally classified into two phenotypes: classical (M1) and alternative (M2). The presence of excess M2 macrophages in in prostate tumors has been linked to early biochemical recurrence and extracapsular spread. M2 macrophages and cancer-associated fibroblasts (CAFs) interact to cause neovascularization and the epithelial-to-mesenchymal transition in prostate cancer cells, which in turn affects prostate carcinogenesis. ADT has the ability to induce autocrine TGFB signaling, induce hypoxia, and transdifferentiate CAFs into myofibroblasts secreting CXCL13. This percentage of CAFs attracts IgA+ plasmacytes, that block CTL activity. Further studies are required to fully understand the thightly coupled resistance to immune-targeting therapy in PCa, (37).

In short, all molecules in the systems discussed above are targets for PCa treatment and further research is needed.

DISCUSSION

Metastatic CRPC resistance to chemotherapy is a form of drug resistance spesific to prostate cancer that involves ongoing signaling, oncogenic survival pathways, crosstalk, resistance mechanisms, and interactions between cancer cells and their microenvironment (38). Novel therapeutics targeting these mechanisms are being evaluated in clinical trials. Studies show that tumor-associated macrophages (TAM), CAF, and myeloid-derived suppressor cells (MDSCs), as well as adaptive immune cells such as CD4 + T cells and CD8 + T cells, are significant regulators of tumor growth and potential treatment targets in the prostate cancer tumor microenvironment (39). The practical translation and efficiency of these medicines are hampered by the lack of valid patient selection indicators and our poor understanding of the interactions among biological components and PCa cells.

MCP-1, also known as CC-motif chemokine ligand 2 (CCL2), is a crucial cytokine that regulates macrophage migration and invasion, promoting growth and metastasis in prostate cancer (39). CCR2 inhibitors have the ability to inhibit the CCL2/CCR2 signaling pathway and reduce TAM recruitment. The inflammatory protein S100A9 was directly bound by the oral active quinoline-3-carboxamide tasquinimod, which changed TAM accumulation and MDSC activity. Patients who received Tasquinimod had a longer disease progression-free survival, according to a phase III clinical research. By activating the HER3 receptor, CAFs create NRG1, which can increase PCa cell growth and resistance to anti-AR therapy. Tumor-promoting cytokines and chemokines are produced by CAFs. In organoids and xenograft models, U3-1402 and anti-HER3 antibody have demonstrated anti-cancer properties. By blocking VEGFR, MET, and AXL, the multi-targeted tyrosine kinase inhibitor cabotinib is being demonstrated to slow the growth of prostate cancer and bone metastases. It has demonstrated strong anti-tumor action in primary and metastatic PCa mice models. A phase III clinical trial using the anti-PD-L1 drug atezolizumab revealed potential anti-tumor effects, suggesting immunotherapy in conjunction with cabozantinib may have a promising future in the therapy of prostate cancer (39).

Histone deacetylases (HDACs) play a critical role in the advancement of prostate cancer and influence tumour suppressor genes as a component of a transcriptional co-repressor complex. Given that they have been demonstrated to slow down prostate cancer cell proliferation, promote differentiation, and/or trigger apoptosis, HDAC inhibitors are becoming more and more popular as chemotherapeutic treatments. Prostate cancer treatment with HDAC inhibitors in combination with radiation therapy or other chemotherapeutic drugs has demonstrated encouraging outcomes (40).

Since they have been around for a while, non-steroidal anti-inflammatory medications, or NSAIDs, have been used extensively all over the world. It has also been demonstrated that NSAIDs inhibit AR transcription by altering the transcription factors that control AR expression. Numerous preclinical investigations have demonstrated that NSAIDs both in vitro and in vivo cause cellular death and reduce PCa growth (41). Compounds containing selenium have also been thoroughly investigated and shown to inhibit the production of AR, perhaps at the level of transcription. Studies conducted in vitro on androgen-dependent LNCaP and androgen-independent PC-3 cells have demonstrated that selenium may both cause apoptosis and limit cell growth (42).

Taxane-based chemotherapy is commonly used to treat mCRPC, with docetaxel (DOC) being the most widely used. However, mitoxantrone (MIT) has a lower tumor response rate. Cabotazitaxel (CAB) is suggested as a second-line therapy after DOC failure. Pemetrexed (PEM) has been evaluated in patients with DOC treatment, but its effectiveness is less significant than MIT. As a result, new therapies like platinum-based chemotherapy, including

satraplatin, carboplatin, and cisplatin, are being studied in clinical trials (33). The PROSPECT research focused on immunotherapeutic medications like immune checkpoint inhibitors (ICIs) and PROSTVAC, but it was found that the main goal was not achieved. ICIs like atezolizumab, pembrolizumab, nivolumab, and ipilimumab have not proven effective in prostate cancer, despite their varied clinical outcomes in other solid tumors (33).

Cancer vaccines boost the immune response against tumor cells by increasing the amount of T lymphocytes spesific for tumor-associated antigens. Despite their limitations, they are frequently used due to their potential usefulness, low side effects, affordable cost and ease of synthesis (33). Sipuleucel-T is a customized vaccination containing ex-vivo dendritic cells from patients produce prostatic acid phosphatase, an important tumor antigen. In comparison to the control group, it demonstrated a prolonged survival of a minimum 4.1 months and an overall survival of nearly 20 months, with a 22.5% decreased chance of mortality (43). To date, the FDA has approved Sipuleucel-T as the only vaccine for the theraphy of cancer, specifically for metastatic castration-resistant prostate cancer (44).

Recent advances in drugs for the treatment of advanced PCa have led to numerous limitations that require the development of personalized therapies and targeted agents. Unfortunately, treatments aimed at AR are not always successful, even if AR is still the main cause of CRPC patients (Patel et al., n.d.). As AR inhibitors, beclomethasone, nilutamide, and flutamide are often employed; however, second-generation ADT drugs, such as apalutamide (APA), have a greater affinity for AR and shown superior efficacy in mouse xenograft models (45). Darolutamide (DAR) is a more powerful AR antagonist than ENZ or APA. One full response and another partial replies were seen in the Phase I and II clinical studies (ARADES), which had a favorable safety profile and suggested that DAR is helpful in people with mCRPC (46). Hormonal therapeutic drugs like corticosteroids, diethylstilbestrol, and ketoconazole have been abandoned due to their poor effeicacy as third-line treatment for patients (33). Two molecule Proteolysis-targeting chimera (PROTAC), one of the AR degraders, has become a viable alternative therapy for metastatic prostate cancer. Compared to conventional small-molecule inhibitors, this small-molecule disrupter is expected to result in greater target inhibition by lowering target protein levels in prostate tissues, resulting in greater therapeutic efficacy (47).

The increasing number of patients with distant metastases for prostate cancer is linked to treatment-induced neuroendocrine PCa (NEPC), which often leads to poor clinical outcomes. NEPCs often exhibit AR-indifferentiation, drug resistance, and genomic alterations due to mutations of TP53, PTEN, and RB1. Other phenotypes, such as double-negative PCa or PCa due to AR non-canonical function are also seen in patients. Key molecules involved in these pathways have been identified as therapeutic targets for PCa, including GR, IGF2, ONECUT2, GATA2, POM121, N-Myc, AURKA, HP1α, SRRM4, PEG10, BRN2, PRMT5, and SOX2 (48).

Immunosuppressive medications do not affect PCa, an immunological "cold" tumor (ICIs). In order to enhance the effectiveness of immunotherapy, novel drugs or combinations that target cancer-associated fibroblasts, hypoxia, myeloid-derived suppressor cells, PTPN2, SOCS1, ADAR1, MYC, KDM5B, and integrin β6 should be created (7-49).

Globally, PCa is a severe and intricate kind of cancer. However, scientific developments like genome sequencing and prediction algorithms are constantly expanding our understanding of the biology behind this illness, which makes it easier to find novel medications with the best possible combination of low toxicity and excellent performance. Future PCa clinical results are anticipated to be enhanced by these developments, which are anticipated to boost precision and personalized medicine.

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LETTER TO THE EDITOR

Dear Editor,

I read with great interest the article titled "The Relationship Between Clinical and Ultrasonographic Findings and Treatment Modification in Patients Diagnosed with Lateral Epicondylitis: 6-Month Results," published in Volume 38, Issue 3 of your journal on December 27, 2024. This study makes a significant contribution to the literature by highlighting the role of clinical and ultrasonographic evaluations in the treatment of lateral epicondylitis (1). I would like to congratulate the authors for their valuable contribution to this field. However, I believe that clarifying certain points could further strengthen the study.

In the Materials and Methods section, it is stated that the study was conducted at the Physical Therapy Clinic of Hatay University. However, none of the authors appear to be affiliated with Hatay University. It seems likely that this is a typographical error and that the intended institution is Dokuz Eylul University. Correcting this discrepancy is crucial for ensuring the study's credibility.

Providing more detailed information about the 12 patients who underwent treatment modification could make the study more comprehensive. Extracorporeal Shock Wave Therapy (ESWT) is a frequently used method in the treatment of musculoskeletal disorders, including lateral epicondylitis. It is known to create mechanical stress by applying focused or radial shock waves, thereby increasing local blood flow, modulating inflammatory processes, and promoting tissue regeneration. Its mechanism of action involves biological processes such as enhancing collagen synthesis, supporting neovascularization, and reducing the sensitivity of nerve endings that transmit pain signals (2–5).

ESWT has been successfully used to treat conditions like tendinopathies, plantar fasciitis, calcific tendonitis, and greater trochanteric pain syndrome. In lateral epicondylitis, it stands out as an effective method for reducing pain and improving functional outcomes. However, the application parameters (e.g., energy intensity, frequency, and number of shocks, number of sessions) vary across clinical protocols, which can directly impact treatment efficacy (2–5).

The study does not detail the protocol used for ESWT (e.g., energy intensity [joules], frequency [hertz], number of shocks, interval between sessions, and total number of sessions). This information is critical for evaluating treatment outcomes. Furthermore, it should be clarified whether the same dose and protocol were applied to all patients. Considering the heterogeneity in lateral epicondylitis treatment, it is important to address how differences in protocols may have influenced the results.

I believe that addressing these points would make the study more comprehensive and scientifically robust.

Sincerely

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Monograph in electronic form: CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reeves JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego: CMEA; 1995.

Complete Book: Ravel R. Clinical Laboratory Medicine. Fourth Edition. Chicago: Yearbook Medical Publishers Inc, 1984; 265-281.

Turkish Book: Yazıcı O. İki uçlu duygudurum bozuklukları ve diğer duygudurum bozuklukları.Psikiyatri Temel Kitabı (ı) içinde Ed: C Güleç, E Köroğlu, Hekimler Yayın Birliği, Ankara 1997; 429-448.

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Chapter in Book: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis and management. 2nd ed. New York: Raven Press; 1995. p. 465-478.

If the author is an organization: The Cardiac Society of Australia and New Zealand. Clinical

exercise testing. Safety and performance guidelines. Medl Aust 1996; 164-228.

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