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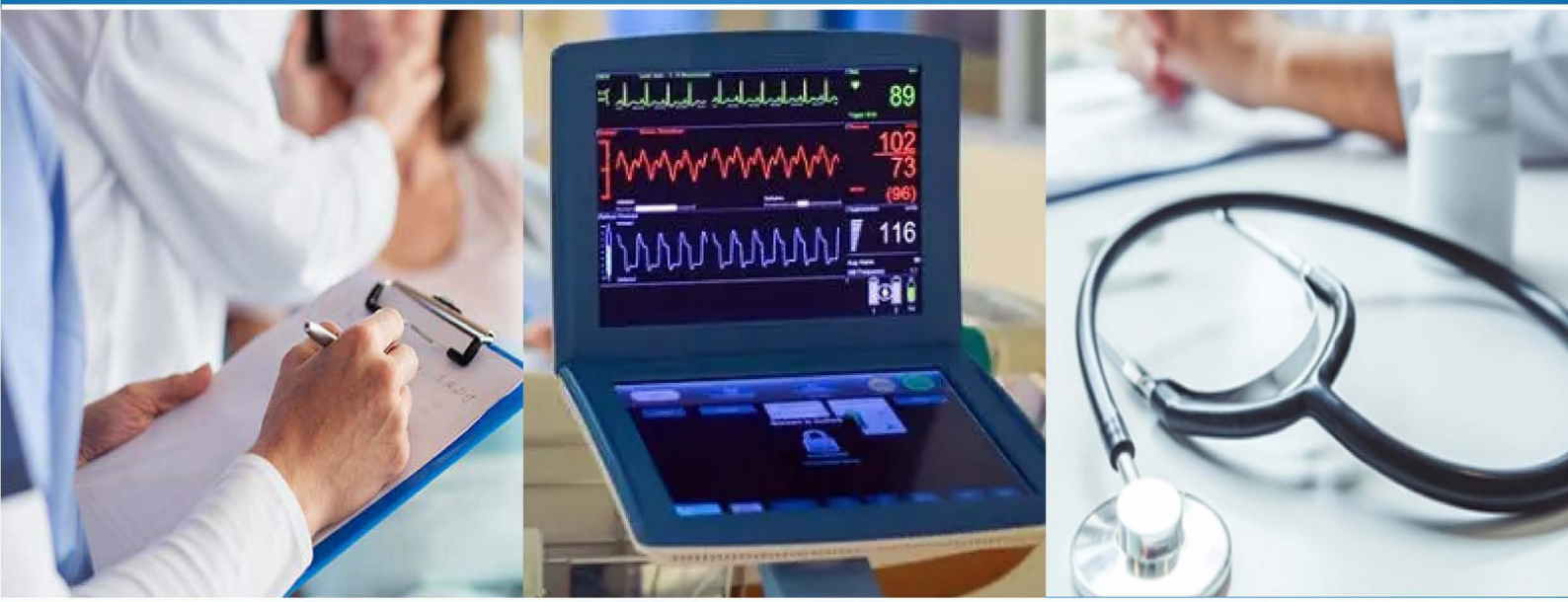

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Dear Colleagues,

We shared with you the happiness that our journal Intercontinental Journal of Internal Medicine contributed to the literature with up-to-date studies in 2024. We are happy and proud to continue our work in 2025 in the light of up-to-date information. Our goal is to become a respected journal in the international community on behalf of our country and to ensure that valuable studies from all over the world reach you.

In the 3rd year of our journal, we will contribute to the literature with original articles and case reports in this first issue. I would like to thank our readers for their interest throughout the year, our authors for submitting manuscripts to the journal, our reviewers for their impartial review of the manuscripts, all our editors, and everyone who contributed to ensure that ICJIM continues its regular publication life.

Sincerely yours,

Asst. Prof. Duygu FELEK
Editor-in-Chief

The Intercontinental Journal of
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ORIGINAL ARTICLES

CASE REPORT

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Original Article

Evaluation of pulmonary artery pressure variations in end stage renal disease patients before and after renal transplantation

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ABSTRACT

Aims: This study aims to evaluate changes in systolic pulmonary artery pressure (sPAP) in end-stage renal disease (ESRD) patients before and after kidney transplantation, as well as the prevalence and impact of pulmonary hypertension (pHT) on post-transplant outcomes.

Methods: A total of 87 ESRD patients undergoing kidney transplantation at the Nephrology Department of Ankara University Faculty of Medicine were prospectively followed. Demographic data, pretransplant, and posttransplant (3rd and 12th months) serum tests, as well as echocardiographic measurements, were analyzed. Patients with pHT due to non-renal causes were excluded. Mean systolic pulmonary artery pressure was measured echocardiographically and a level of above 30 mmHg was accepted as pHT. Statistical analyses were performed using SPSS, with a p-value <0.05 considered statistically significant.

Results: Among the 87 patients, 40 (45.9%) were male and 47 (54.1%) were female, with a mean age of 42.6±11.7 years. The mean pretransplant sPAP was 36.6±7.97 mmHg, which decreased significantly to 31.7±5.5 mmHg at 3 months and 30.1±6.2 mmHg at 12 months post-transplant (p<0.05). Pretransplant pHT was present in 72.4% of patients, which reduced to 36.7% at 3 months and 22.9% at 12 months post-transplant. Preoperative triglyceride and parathormone levels were significantly correlated with sPAP (p<0.05), while patients with pretransplant sPAP ≥40 mmHg had a higher incidence of early graft dysfunction (p<0.05).

Conclusion: Pulmonary hypertension is prevalent in patients with ESRD but significantly decreases after kidney transplantation. Patients with preoperative sPAP ≥40 mmHg are at higher risk for early graft dysfunction, highlighting the importance of screening for pHT in transplant candidates. Addressing pHT in ESRD patients may improve post-transplant outcomes, reduce morbidity, and enhance overall patient management.

Keywords: Pulmonary hypertension, chronic kidney disease, end stage renal disease, hemodialysis, peritoneal dialysis

INTRODUCTION

End-stage renal disease (ESRD) is associated with high morbidity and mortality, exacerbated by comorbidities such as atherosclerotic heart disease (ASHD), diabetes mellitus, congestive heart failure, and hyperlipidemia. These conditions not only increase the disease burden but also lead to significant healthcare costs, particularly due to dialysis and kidney transplantation.

Pulmonary hypertension (PH), specifically classified as type 5 in ESRD patients, is a notable complication. The global rise in chronic diseases, including chronic obstructive pulmonary disease (COPD), ASHD, and heart failure, has led to an increase in PH prevalence, particularly in older adults. Recent data suggest that hospitalization and mortality rates related to PH have nearly doubled in this population. PH is defined as a mean pulmonary artery pressure ≥25 mmHg or a systolic pulmonary artery pressure (sPAP) ≥35 mmHg, with the latter being a widely recognized threshold for diagnosis.

Several studies have reported an increased incidence of PH in ESRD patients, both in those undergoing dialysis and in transplant recipients, contributing to heightened morbidity and mortality. Despite this, there is limited prospective research examining changes in sPAP before and after kidney transplantation and their effects on graft function.

This study aims to evaluate sPAP in ESRD patients undergoing dialysis and kidney transplantation, investigate factors influencing pulmonary artery pressure, and assess the impact of transplantation on PH. The findings aim to address a gap in the literature by providing prospective data on PH in this patient population.

METHODS

Approval for the study was obtained from the Ankara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 09.03.2015, Decision No: 2015/12). Between 2013 and 2015, patients aged 18 and older who underwent kidney transplantation at Ankara University Medical Faculty Hospitals' Department of Internal Medicine, Nephrology Division were informed about the study. The study followed the requirements given in the Declaration of Helsinki.

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Informed consent was obtained from 87 participants, and their demographic data, comorbidities (e.g., hypertension, diabetes, sleep apnea), and various clinical parameters were collected. The number of patients were determined by the time frame the study was done. Data included age, sex, etiology of kidney disease, duration of kidney disease, dialysis details, medications, and echocardiographic findings at baseline, 3 months, and 12 months post-transplant. Patients under 18 years of age and patients with a follow-up under 12 months were excluded. Patients with non-renal causes of pulmonary hypertension (e.g., heart disease, COPD, chronic pulmonary embolism) were also excluded.

Echocardiograms were performed using a continuous-wave Doppler (Toshiba Applio 400) probe. The pulmonary artery pressure (PAB) was calculated using the modified Bernoulli equation. Care was taken to ensure patients were in a euvolemic state post-dialysis to avoid elevated PAB due to volume overload. Additionally, high-risk patients underwent preoperative respiratory function tests. Patients who did not sign the consent form or had severe psychiatric disorders were excluded from the study.

Statistical Analysis

Data were analyzed using IBM SPSS for Windows ver. 18.0. Descriptive statistics included mean±standard deviation for normally distributed variables and median (minimum-maximum) for non-normally distributed ones. Counts (n) and percentages (%) were used for categorical variables.

For continuous variables, a paired T test was used for normal distributions; otherwise, the Wilcoxon test was applied. For multiple follow-ups, repeated measures ANOVA or the Friedman test was used based on distribution normality.

For two groups, the T test assessed mean differences, while the Mann-Whitney test evaluated median differences. For more than two groups, ANOVA and the Kruskal-Wallis test were used. Nominal variables were assessed with Pearson chi-square or fisher exact tests.

Spearman correlation was used for non-normally distributed continuous variables, and pearson correlation for normally distributed ones. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 87 patients were included in the study, comprising 40 men (46%) and 47 women (54%). The average age was 42.6±11.7 years, with no significant gender differences. Among the patients, 26 (29.9%) were current smokers, while 61 (70.1%) had never smoked or had quit. Kidney transplants were performed in 63 patients (72.4%) from living donors and 24 patients (27.6%) from cadaveric donors (**Table**).

Table. Demographics	
Male/female	40/47 (46%/54%)
Median age	(31-54)
Median chronic kidney disease duration	118.6 months
Smoking	
Yes/no	26/61 (29.9%/70.1%)
Graft source	Living/cadaveric: 63/24 (72.4%/27.6%)
Comorbidities	
Hypertension	71 (81.6%)
Left ventricular hypertrophy	29 (33.3%)
Diabetes mellitus	24 (27.6%)
Atherosclerotic heart disease	10 (11.5%)
Obstructive sleep apnea	1 (1.1%)
Chronic obstructive pulmonary disease	1 (1.1%)

The Causes of ESRD Included

- **Chronic glomerular and tubular diseases:** 28 (32.4%)
- **Amyloidosis:** 13 (14.9%)
- **Hypertensive nephropathy:** 11 (12.6%)
- **Diabetes mellitus:** 11 (12.6%)
- **Polycystic kidney disease:** 9 (10.3%)
- **Unknown:** 10 (11.5%)

Pretransplant Data

- 24 patients (27.6%) received pre-emptive transplants without dialysis.
- 54 patients (62.1%) underwent chronic hemodialysis, while 2 (2.3%) received peritoneal dialysis. A total of 63 patients (72.4%) were on chronic dialysis.
- Average duration of chronic kidney disease was 118.6 months, with one patient on record for 30 years.
- Average dialysis duration for those on chronic dialysis was 49.1 months.

Preoperative respiratory function tests showed no significant airway obstruction, ruling out COPD. Transplant compatibility based on HLA was recorded, with varying degrees of matches among the recipients.

Patients underwent echocardiographic assessment and sPAB measurements in the early and late post-transplant periods (3 months and 12 months), and laboratory data were recorded. The mean sPAB value for patients in the early post-transplant period (3 months) was found to be 31.7±5.5 mmHg (min 25-max 65 mmHg). The mean sPAB measured during the pre-transplant period was 36.6±7.97 mmHg. A statistically significant difference was found between the mean sPAB values measured during the pre-transplant period and at 3 months post-transplant ($p=0.002$).

In the late post-transplant period (12 months), the average sPAB was found to be 30.1 mmHg±6.2 (min 20-max 65). It was determined that the average sPAB value measured at the 12th month post-transplant was lower than the average 36.6±7.97 mmHg sPAB value measured in the pre-transplant period. As a result, a statistically significant decrease in average sPAB was observed when comparing the pre-transplant period to the average sPAB measured at the 12th month post-transplant ($p<0.05$). The frequency of pulmonary hypertension at 3 months post-transplant was observed in 32 patients (36.7%), while at 12 months post-transplant, it decreased to 20 patients (22.9%) ($p<0.05$).

The average pre-transplant sPAB value for patients who underwent preemptive transplantation was found to be 36.6±8.8 mmHg. In patients receiving chronic dialysis treatment, the pre-transplant average sPAB was 36.61±6.7 mmHg, with no statistically significant difference between the

two groups (p=0.57). When comparing the average pulmonary artery pressures at the third month post-transplant for these two patient groups, values were measured as 30.4±3.51 mmHg and 32.2±6.1 mmHg, respectively. The average sPAB values at the twelfth month post-transplant were also measured as 28.8±3.8 mmHg and 30.7±6.9 mmHg, respectively, with no statistically significant difference found (p=0.1 and p=0.379).

When comparing the average sPAB values pre-transplant, at early post-transplant (3 months), and late post-transplant (12 months) for our patients, regardless of whether the donor was living or cadaveric, it was observed that there was a decrease in average sPAB values during the post-transplant period for all patients, and this decrease progressively increased during the late follow-up at 12 months.

DISCUSSION

End-stage kidney disease is a critical health condition that significantly impacts morbidity and mortality rates, adversely affecting patients' quality of life. The presence of comorbid conditions such as coronary artery disease, diabetes mellitus and chronic kidney disease exacerbates the morbidity and mortality associated with ESRD. Moreover, the high costs of dialysis and kidney transplantation put a considerable financial burden on national healthcare systems.

Recent studies have indicated an increasing prevalence of pulmonary hypertension (PH) among patients with ESRD.[1-3] According to the World Health Organization's (WHOs) 2013 classification of pulmonary hypertension, PH associated with ESRD falls into group 5,[4] which highlights this unique etiology linked to kidney disease. The mechanisms underlying the development of PH in ESRD patients are multifactorial, including left heart failure, increased cardiac output due to arteriovenous fistulas, endothelial dysfunction, anemia, hypervolemia, and various metabolic and hormonal disturbances related to kidney disease.[3,5]

Kidney transplantation is known to improve both the longevity and quality of life for ESRD patients and can significantly reduce treatment costs. Despite the existing literature emphasizing the prevalence and clinical significance of PH in chronic hemodialysis (HD) and renal transplant patients, there remains a lack of prospective studies evaluating the changes in pulmonary artery pressure (sPAB) and the relationship between kidney graft function and PH in post-transplant periods.[6]

The prevalence of PH among CKD patients has been reported varying widely, from 12.5% to 58.6%. For instance, a study by Yigla et al.[7] found an 8% prevalence of PH in patients with ESRD without any cardiac or pulmonary diseases, suggesting a direct link between ESRD and the development of PH, potentially exacerbated by prolonged HD.

In our study, we aimed to prospectively assess the sPAB levels in patients with ESRD awaiting kidney transplantation, using echocardiographic evaluations. We excluded patients with severe heart failure, chronic pulmonary diseases, or any conditions that could cause PH unrelated to kidney disease. Our findings revealed that 72.4% of the patients had PH, indicating that these patients likely belonged to the WHOs group 5 classification of PH.[4]

Consistent with existing literature, our study found no significant correlation between the presence of PH and gender. Furthermore, no significant age difference was noted between patients with and without PH, although this might be attributed to the low proportion of patients with normal sPAB levels.

The relationship between the duration of dialysis and PH has been previously documented, with studies indicating that prolonged HD correlates with increased sPAB values. However, our findings did not show a statistically significant correlation between dialysis duration and the presence of PH.

In terms of the relationship between PH and hemoglobin levels, we observed a significant negative correlation, suggesting that anemia could contribute to the development of high-output heart failure and subsequent PH. Additionally, a correlation between hypoalbuminemia and PH was identified, potentially pointing to an increased risk of hypervolemia or chronic hypervolemic states contributing to PH.

We also evaluated the impact of kidney transplantation on sPAB levels. Our results demonstrated a statistically significant reduction in sPAB values from the pre-transplant phase to the 3rd and 12th months post-transplant, indicating an improvement in pulmonary hemodynamics following transplantation.

Interestingly, no significant difference was observed in graft function between patients with and without PH after transplantation, nor did the source of the donor (living or cadaveric) influence graft function stability.

CONCLUSION

Research has shown that the presence of PH, regardless of its cause, serves as a risk factor that heightens morbidity and mortality among patients with ESRD. PH not only deteriorates the patients' quality of life but also leads to increased treatment costs. Therefore, close monitoring of patients with ESRD who have PH, as well as addressing the underlying causes of PH, is crucial. Our analysis indicated that AV fistula presence correlates significantly with PH, further supporting the hypothesis that high-output heart failure and PH can arise from the increased blood flow through these fistulas. The existing literature also suggests a correlation between PH and echocardiographic indicators of left ventricular dysfunction, but we chose not to include patients with evident left ventricular failure to maintain a homogeneous study group. Ultimately, our study highlights the importance of monitoring PH in ESRD patients, especially those awaiting kidney transplantation. By understanding the dynamics of sPAB levels and their implications on both pre- and post-transplant outcomes, we can better address the management of these patients to enhance their overall health and quality of life. Our results are open to be confirmed by larger prospective studies.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Ankara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 09.03.2015, Decision No: 2015/12).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

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

Author Contributions

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

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Original Article

Magnesium, calcium and vitamin D levels in polycystic ovarian syndrome: a retrospective single center study

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ABSTRACT

Aims: Polycystic ovary syndrome (PCOS); it is an endocrine-gynecological disorder that affects many women of reproductive age, with chronic anovulation and androgen elevation. Although many pathophysiological processes have been discovered in the development of PCOS; the exact etiology and pathophysiology have not been fully elucidated. Vitamin D is synthesized differently from other vitamins and vitamin D has a hormonal function in the body. Vitamin D is known to have a role in many diseases, as well as its effects on bone metabolism in the body. We aimed to examine the relationship between PCOS and serum vitamin D, calcium and magnesium levels.

Methods: A total of 80 cases, including 40 patients diagnosed with PCOS according to the Rotterdam criteria and 40 cases in the normal population, were included in the study. The cases were examined retrospectively. Magnesium, calcium and vitamin D levels of all cases were compared. Additionally, the hemogram parameters, insulin, glucose, insulin resistance, HbA1c (hemoglobin A1c test), high-density lipoprotein, low-density lipoprotein, triglyceride, total cholesterol, parathyroid hormone (PTH) and phosphorus levels of the cases were compared.

Results: There was no difference in magnesium, calcium, vitamin D values between cases with and without polycystic ovary syndrome, and no difference in hemogram parameters, HbA1c, glucose, insulin, PTH and phosphorus levels between the same groups ($p>0.05$). When lipid levels were compared, total cholesterol and low-density lipoprotein levels were found to be significantly higher in those without PCOS ($p=0.042$). The average age was found to be significantly lower in patients with PCOS ($p<0.001$).

Conclusion: In our study, no significant difference was seen between the two groups in terms of magnesium, calcium and vitamin D levels. Prospective observational studies and randomized controlled studies are needed to more clearly explain the relationship between hormonal and metabolic irregularities and magnesium, calcium and vitamin D levels in PCOS.

Keywords: Polycystic ovary syndrome, vitamin D, magnesium, calcium, lipid profile

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a clinical entity characterized by menstrual irregularities and androgen elevation. Most women with PCOS are in their fertile period and Its prevalence worldwide ranges from 6% to 21%. Most women with PCOS have metabolic disorders such as ovarian dysfunction, insulin resistance, androgen elevations, and dyslipidemia.[1] The etiology of PCOS is unknown. However, the high ratio between Luteinizing hormone (LH) and follicle stimulating hormone (FSH) and the increased frequency of gonadotropin-releasing hormone (GnRH) are the main underlying factors in pathophysiology. Current data; genetic-environmental factors, androgen elevation, insulin resistance are suggested to play a serious role in the development of PCOS as internal and external factors.[2]

Approximately 40-80% of women with PCOS are overweight or obese. A high body mass index negatively affects the patient's sexual activity, psychological and metabolic status. PCOS can cause serious complications such as diabetes mellitus, hypertension, coronary artery disease, endometrium cancer and infertility in the long term.[3]

One of the criteria used in the diagnosis of PCOS is the Rotterdam criteria and consists of three criteria. The presence of at least two of the Rotterdam criteria is diagnostic. Rotterdam criteria: 1-ovulatory dysfunction, 2-hyperandrogenism and 3-polycystic ovary morphology.[4]

Currently, there are four commonly recognized phenotypes of PCOS: type A, polycystic ovary (PCO), chronic oligo-anovulation (OA) and hyperandrogenism (HA); type B, OA and HA; type C, PCO and HA; and type D, PCO and OA. Insulin resistance is present in all phenotypes. Insulin resistance is the most common classical phenotype (types A and B) (80%), followed by ovulatory PCOS (65%) and non-hyperandrogenemic PCOS (38%).[5]

Vitamin D deficiency may be a risk factor for PCOS. The prevalence of vitamin D deficiency is 20-48%. However,

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the prevalence of vitamin D deficiency is higher in patients diagnosed with PCOS.[3,4] One of the vitamins produced in the human body is vitamin D. It has effects on calcium and bone metabolism as well as hormonal effects on many systems. Vitamin D deficiency is thought to play a role in the emergence of autoimmune diseases, rheumatoid arthritis, inflammatory bowel disease, DM, multiple sclerosis, some malignancies and heart diseases.[6,7] In a study, low vitamin D levels were found to be associated with metabolic syndrome, but high vitamin D levels were positively correlated with insulin sensitivity.[8] A positive effect has been detected in women with PCOS who receive calcium and vitamin D treatment in cases such as menstrual disorders, weight loss, and androgen elevation.[9] In this study, we aimed to determine whether PCOS is related to calcium, magnesium and vitamin D levels. In case of a negative correlation, we predict that giving calcium, magnesium and vitamin D to women with PCOS for treatment may reduce some negative effects.

METHODS

Ethics

The study was carried out with the permission of the Van Yüzüncü Yıl University Non-interventional Clinical Researches Ethics Committee (Date: 19.08.2022, Decision No: 2022/08-06). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

In our study, the files of individuals who applied to the Internal Medicine and Endocrinology polyclinic of Van Yüzüncü Yıl University Faculty of Medicine Dursun Odabaş Training and Research Hospital between 01.01.2020 and 30.09.2022 were retrospectively examined.

80 cases (40 cases with PCOS and 40 cases without PCOS) were included in the study. Patients diagnosed with PCOS in previous examinations and imaging studies and a healthy control group were included in the study, regardless of age range.

Inclusion criteria for the study: Being over 18 years of age, being female, not being pregnant, and having been diagnosed with PCOS according to the Rotterdam criteria.

Exclusion criteria for the study: Lack of laboratory results such as vitamin D, calcium, magnesium and parathyroid hormone; using steroids, sex hormones or drugs that may cause electrolyte imbalance in the last six months; having diseases such as congenital adrenal hyperplasia, Cushing syndrome, thyroid gland diseases and cancer.

Methods

Demographic characteristics of all individuals (age and gender), calcium, magnesium, vitamin D, phosphorus, parathyroid hormone (PTH), mean platelet volume (MPV), erythrocyte distribution volume (EDV), white blood cells (WBC), neutrophil count, lymphocyte count , platelet count, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), glucose, insulin, HbA1c (hemoglobin A1c test), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG) and total cholesterol (TK) levels was recorded.

The Architect i2000 system (Abbott Laboratories, USA) uses chemiluminescent microparticle immunoassay (CMIA) technology. The hormone assays performed by this analyser included tests for cortisol, follicle-stimulating hormone (FSH), carcinoembryonic antigen (CEA), α-fetoprotein (AFP), ferritin, PTH, free thyroxin (FT4), vitamin B12, folic acid, prolactin, progesterone, luteinising hormone (LH), and β-human chorionic gonadotrophin (βhCG).

In our total laboratory automation (TLA) setting, two Architect c16000 platforms are used to automatically measure HIL indices on all plasma and serum samples on which chemistry and immunochemistry tests are requested. Both instruments are used and maintained by strictly following the manufacturer's instructions. In particular, the Architect c16000 analyzer measures HI through the dilution of samples with saline solution and the polychromatic photometric detection of the interferent.

Statistical Analysis

Descriptive statistics for the features emphasized; expressed as mean, standard deviation, minimum and maximum values. Independent samples T test was performed to compare group means in terms of continuous variables. To determine the relationship between these variables, Pearson correlation coefficients were calculated separately in the groups. In the calculations, the statistical significance level was taken as 5% and the SPSS (ver: 21) statistical package program was used for the calculations.

RESULTS

A total of 80 cases, 40 cases diagnosed with PCOS and 40 healthy cases, were included in the study. The average age of PCOS cases was 27±6 years, and the average age of healthy cases was 36±12 years. The average age of patients with PCOS was found to be significantly lower (p=0.001). While the average magnesium of PCOS cases was 1.9±0.36 mg/dl, calcium 9.4±0.5 mg/dl, phosphorus 3.42±0.58 mg/dl, PTH 73±25 pg/ml and vitamin D 14.09±8.2 pg/ml; The average magnesium of the control group cases was 1.9±0.29 mg/dl, calcium 9.4±0.4 mg/dl, phosphorus 3.38±0.46 mg/dl, PTH 76±42 pg/ml and vitamin D 10.16±8.4 pg/ml. No statistically significant difference was detected between the groups. Magnesium, calcium, phosphorus, PTH and vitamin D levels of the cases are given in **Table 1**.

Table 1. Comparison of magnesium, calcium, phosphorus, vitamin D and parathormone levels of cases with and without polycystic ovary sendrome

Parameters	With PCOS cases (n=40)	PCOS without (n=40)	p
Magnesium (mg/dl)	1.9±0.36	1.9±0.29	0.124*
Calcium (mg/dl)	9.4±0.5	9.4±0.4	0.652*
Phosphorus (mg/dl)	3.42±0.58	3.38±0.46	0.713*
Parathormone (pg/ml)	73±25	76±42	0.659*
Vitamin D (pg/ml)	14.09±8.2	10.16±8.4	0.061*

*Student T test, PCOS: Polycystic ovary syndrome

While the average total cholesterol of the PCOS cases was 165.6±44.4 mg/dl and LDL was 92.5±32.6 mg/dl, the total cholesterol of the control group cases was 186.3±36.7 mg/dl and LDL was 109.0±29.2 mg/dl. Total cholesterol and LDL levels of patients without PCOS were found to be statistically significantly higher. While the average triglyceride of the PCOS

cases was 125.3±85.3 mg/dl and HDL was 51.4±16.2 mg/dl, the triglyceride of the control group cases was 119.6±71.6 mg/dl and HDL was 54.3±13.4 mg/dl. No statistically significant difference was detected between the groups (Table 2).

Table 2. Comparison of lipid levels in cases with and without polycystic ovary sendrome

Parameters	With PCOS cases (n=40)	PCOS without (n=40)	p
TG (mg/dl)	125.3±85.3	119.6±71.6	0.776*
LDL (mg/dl)	92.5±32.6	109.0±29.2	0.04*
HDL (mg/dl)	51.4±16.2	54.3±13.4	0.444*
TK (mg/dl)	165.6±44.6	186.3±36.7	0.05*

*Student T test, PCOS: Polycystic ovary syndrome, TG: Triglyceride, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TK: Total cholesterol

No statistically significant difference was detected between the groups in hemogram parameters, HbA1c, glucose, insulin and insulin resistance level (Table 3).

Table 3. Comparison of laboratory values in cases with and without polycystic ovary sendrome

Parameters	With PCOS cases (n=40)	PCOS without (n=40)	p
WBC (mm^3)	7.552±2.332	7.201±1.844	0.458*
Neutrophil (mm^3)	4.447±1.541	3.976±1.163	0.127*
Lymphocyte (mm^3)	2.445±778	2.469±782	0.894*
Platelet ($10^3/mm^3$)	293.800±63.450	299.080±60.456	0.704*
Neutrophil/lymphocyt	2.08±1.65	1.69±0.56	0.158*
Platelet/lymphocyte	138.3±87.7	128.7±35.5	0.521*
MPV (fL)	10.3±0.90	10.3±0.88	0.940*
EDV (fL)	13.07±1.17	13.5±1.14	0.141*
Insulin (IU/lt)	12.8±8.0	12.3±10.6	0.818*
Glucose (mg/dl)	95.3±32.4	97.6±33.0	0.749*
HbA1c (mmol/mol)	5.4±0.5	5.5±1.4	0.563*

*Student T test, PCOS: Polycystic ovary syndrome, WBC: White blood cells, MPV: Mean platelet volume, EDV: Erythrocyte distribution volume, HbA1c: Hemoglobin A1c test

DISCUSSION

Infertility, oligo-amenorrhea, and abnormal menstrual bleeding due to ovarian dysfunction may occur in PCOS. Moreover, symptoms such as hirsutism and skin acne due to high androgen levels are common. The prevalence of obesity in women with PCOS is approximately 40-60% (Table 4). Menstrual cycles of at least 45 days or fewer than 9 periods per year; presence of findings related to high androgen levels such as acne, hirsutism, alopecia, acanthosis nigrigans; or laboratory findings of high androgen levels are considered criteria for chronic oligo-amenorrhea.[10]

Table 4. Frequency of signs and symptoms of polycystic ovary sendrome

Signs and symptoms of polycystic ovary syndrome	Frequency
Hirsutism	60-90%
Oligomenorrhea	50-90%
Infertility	55-75%
Polycystic ovary	50-75%
Obesity	40-60%
Amenorrhea	25-50%
Dysfunctional uterine bleeding	30%
Acne	25%
Normal menstrual pattern	22%

Several scientific studies have examined the nutrition of women with PCOS. It was observed that 25% of these women had insufficient magnesium consumption.[11] Asemi et al.[12] showed that women with PCOS consumed an average of 233 mg/day of magnesium. This is below the average of 320 mg/day for women ≥19 years of age.

Vitamin D deficiency emerges as a significant health problem for patients with PCOS. Various studies show that vitamin D plays a critical role in hormonal balance and insulin metabolism. In individuals with PCOS, deficiency of this vitamin can increase insulin resistance, which can lead to complications such as metabolic syndrome, obesity and other hormonal imbalances. Demographic characteristics of PCOS women diagnosed with DM and metabolic syndrome were evaluated. A negative correlation was observed between magnesium and insulin resistance in these patients. It has been observed that low magnesium is associated with insulin resistance. Insulin resistance is believed to be the main pathogenic factor associated with the increased rate of metabolic disorders among women with PCOS.[13]

In 2016, Rajeswari et al.[14] 80 premenopausal women with PCOS were compared with 40 women without PCOS. Women with PCOS (116.65±11.15 compared to 89.10±5.89 mg/dl, p=0.0001) had lower magnesium levels (1.210 mg/dl) than the control group. Moreover, found significantly higher fasting blood sugar levels in women with PCOS. (While it was 2.087±0.189 mg/dl in the control group, it was 1.210±0.239 in the PCOS group, p=0.0001). In this study, there was an inverse relationship between glucose and magnesium (r=-0.412, p=0.0001) levels in patients with PCOS. In our study, blood magnesium levels were found to be similar between patients with PCOS and the other group (p>0.05).

Some studies have shown that vitamin D deficiency is a problem in patients with PCOS.[15,16] It has been observed that there is a relationship between low vitamin D levels and the criteria of metabolic syndrome in women with PCOS.[17] The regulatory effect of vitamin D on insulin-mediated intracellular and extracellular calcium plays a critical role in many biological processes. Vitamin D can strengthen the effects of insulin by increasing insulin resistance at the cellular level. Additionally, vitamin D can regulate calcium levels both inside and outside cells by increasing calcium absorption from the gastrointestinal tract and urinary tract. Calcium is vital for cellular signaling and various metabolic pathways. This regulatory role of vitamin D becomes even more important, especially in diseases with hormonal imbalances such as PCOS.[18] Another hypothesis is examining the effects of vitamin D on gene expression related to insulin receptors. Research shows that vitamin D may promote the transcription of certain genes and increase the production of insulin receptors by these genes. Thus, vitamin D deficiency can lead to increased insulin resistance and metabolic risk.[19] In summary, many studies have shown that there may be a relationship between PCOS and vitamin D and calcium. In our study, no significant difference was found between the group with PCOS and the group without PCOS in terms of parameters such as vitamin D, calcium, PTH and phosphorus (p>0.05 for all).

In individuals with PCOS, hormonal imbalances and metabolic disorders, combined with factors such as treatment and oxidative stress, may cause adverse vascular disorders.

Additionally, high androgen levels may further increase the risk of blood pressure increases. These metabolic abnormalities can increase the risk of diseases such as heart disease and stroke.[20] Dyslipidemia is also common in PCOS. In a meta-analysis conducted by Wild et al.,[21] it was reported that TG levels were 26 mg/dl and LDL 12 mg/dl higher, and HDL concentrations were 6 mg/dl lower in women with PCOS compared to the control group. In some studies, the prevalence of dyslipidemia was found to be up to 50-70% higher in women with PCOS.[22] Another study found that 72.4% of non-hispanic women with PCOS had dyslipidemia and elevation in LDL was most common, with important predictors being age, insulin, and testosterone.[23] In our study, LDL and TC values of the group without PCOS were found to be higher and significant ($p<0.05$ for all). There was no difference between the two groups in terms of insulin.

C-reactive protein (CRP) is a disease-produced protein produced by the liver and is an acute phase reactant that is increased in inflammatory events. CRP levels rise when an infection or inflammatory response occurs in the body. The normal range of neutrophil lymphocyte ratio (NLR) is considered to be 1-2. In adults, values higher than 3.0 and lower than 0.7 are pathological. Values between 2.3-3.0 may be a sign of chronic inflammatory diseases. NLR has been shown to positively correlate with CRP levels.[24] NLR is a cost-effective marker that could be an alternative to CRP for inflammation. There are also several studies reporting increased leukocyte counts in patients with PCOS.[25,26] In some studies, MPV can be used as a marker to detect inflammation. It has been shown that the MPV value in patients with PCOS is higher than in the control group.[25] In our study, when PCOS cases were compared with the control group, leukocyte count, neutrophil count, lymphocyte count, NLR, platelet count, platelet-lymphocyte ratio and MPV value were similar ($p>0.05$ for all).

Limitations

One of the most ımportant lımıtatıons was that our study was sıngle center and thıs was one of the maın factors of the low number of patıents. Another limitation was that our study was retrospective. Therefore, most patients diagnosed with PCOS did not have the laboratory parameters we wanted. We believe that prospective and multicenter studies on PCOS will contribute more to the literature.

CONCLUSION

In our study, magnesium, calcium and vitamin D levels were found to be similar between the PCOS group and the other group. More studies are needed to clarify the relationship between PCOS and magnesium, calcium and vitamin D levels. However, we think that there may be a significant difference when done with a larger number of patients.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was approved by the the Van Yüzüncü Yıl University Non-interventional Clinical Researches Ethics Committee (Date: 19.08.2022, Decision No: 2022/08-06).

Informed Consent

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

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

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

Author Contributions

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

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Original Article

Comparative analysis of human telomerase reverse transcriptase mutation in transitional epithelial cell bladder cancer in high and low grade tumors

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ABSTRACT

Aims: We aimed to evaluate the presence of telomerase reverse transcriptase (TERT) promoter region mutations in transitional epithelial cell bladder cancer by analyzing the h-TERT mutation in high and low-grade tumors and to compare it with the clinicopathological data of the patients.

Methods: A total of 90 patients diagnosed with bladder cancer, 60 with low-grade tumors and 30 with high-grade tumors were included in this study. To detect mutations in the TERT gene in the DNA samples obtained, the most frequently mutated region of the gene was amplified by PCR using forward and reverse primers and mutation analysis was performed by sequence analysis.

Results: TERT promoter mutation was positive in 38 and negative in 22 out of 60 patients in the low-grade group, and positive in 26 and negative in 4 out of 30 patients in the high-grade group. Patients with high-grade tumors were 1.940 times more likely to be TERT promoter mutation positive than low-grade patients (p=0.027).

Conclusion: When interpreted together with other studies that have obtained significant results that TERT mutation analysis can be used in diagnosis, tumor grading and follow-up, it is thought that it can be used in the grading and follow-up algorithm.

Keywords: Bladder cancer, telomerase activity, TERT mutation

INTRODUCTION

Bladder cancer is the most common cancer of the urinary tract.[1] Its prevalence increases with age and is influenced by individual or environmental factors such as gender, race, genetics, smoking, industrial chemical exposure, bladder infection, bladder stones or catheterization. It is predicted that the incidence will continue to increase in the next 10 years worldwide.[1] Considering that approximately 75% of newly diagnosed urothelial bladder cancer cases are detected at the non-invasive stage,[2] the importance of early diagnosis becomes evident.

The diagnosis is made by urine cytologic examination and histopathologic specimens obtained by cystoscopic examination. Histopathologic evaluation is based on the presence of muscle invasion. TNM staging is performed with radiologic imaging and histopathologic data. Stage is the most important independent prognostic marker for progression and overall survival in invasive bladder cancer.[3] While the 5-year survival rate for invasive tumors that have not spread outside the bladder is up to 70%, the survival rate for tumors confined to the inner layer of the bladder has been reported to reach 96%. It is known that most of the cases are detected at these stages.[4]

Many genetic factors play a role in the development of urothelial cancer. Deletion of chromosome fragments, epigenetic alterations, gene mutations and mRNA alterations are the most common disorders that can cause carcinogenesis. These changes become more pronounced with loss of control of the cell cycle, genomic instability and telomere dysfunction.[5,6]

Telomeres are specialized heterochromatin structures located at the ends of linear chromosomes that protect chromosomes from random DNA breakage, preventing their unwanted ends from joining or fragmenting and additionally

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involved in replication and cell proliferation.[7] Telomerase is a ribonucleoprotein DNA polymerase enzyme that prevents the loss of DNA material after the replication cycle by ensuring the formation of "TTAGGG" repeats at the chromosome ends. This activity of telomerase is essential for long-term replicative survival in cells. Mice lacking telomerase showed signs of impaired tissue homeostasis.[8]

Telomerase reverse transcriptase (TERT), known as h-TERT in humans, is one of the subproteins of the telomerase enzyme. Although this enzyme complex is known to be involved in the elongation of the DNA G3' end, its functions have not been fully elucidated.[8] There are data suggesting that it is closely related to cell immortality and cancer formation.

TERT promoter region mutations have been found to be common in many tumor types. These mutations result in an increase in TERT expression and telomerase reactivation by creating new binding sites for many cellular transcription factors. TERT mutation has been reported in bladder cancer cases with a frequency between 53-83% in different studies. These mutations have been found to be more common in non-muscle invasive bladder cancer cases than other genetic mutations considered as risk. In addition, it has been reported to be seen not only in urothelial carcinomas but also in other histologic subtypes.[9-15]

Bladder cancer patients require repeated cystoscopies during diagnosis, treatment and post-treatment follow-up. Although the role of cystoscopy in these processes is very important, it should not be forgotten that it is an invasive, complicated and expensive procedure. Therefore, the search for an alternative non-invasive method continues and some biomarkers are frequently studied for this purpose. The frequency of TERT mutations up to 80% in bladder cancer suggests that it may become a tool and target in follow-up and treatment. In our study, we aimed to analyze the h-TERT mutation in high- and low-grade tumors in bladder cancer with variable epithelial cells, to elucidate its relationship with tumor grade and to compare it with clinicopathological data.

METHODS

Ethics

This study was conducted in accordance with the decisions of the Declaration of Helsinki and the patient rights regulation, with the approval of the University of Health Sciences Hamidiye Clinical Researches Ethics Committee (Date: 06.09.2021, Decision No: 4).

Patient Selection

This study included 90 patients diagnosed with bladder cancer who were followed up in the Urology Department of Sultan 2. Abdülhamid Han Training and Research Hospital between 2016 and 2021. 60 patients had low grade tumors and 30 patients had high grade tumors. Our study was performed by taking 10 µm thick tissue sections from paraffin blocks in the archive of the pathology department and applying the following procedures.

Mutation Analysis

TERT gene promoter region mutations were analyzed by PCR-based direct sequencing method (sanger sequencing). DNA was extracted from dissected tumor sections after standard deparaffinization using QIAamp DNA FFPE tissue kit (catolog no: 56404) (QIAGEN, Hilden, Germany). To detect mutations in the TERT gene in the DNA samples, HotStarTaq DNA polymerase kit (catolog no: 203205) (QIAGEN, Hilden, Germany) and forward (CAGCGCTGCCTGAAACTC) and reverse (GTCCTGCCCCCCTTCACCTTT) primers, PCR mixtures were prepared in sterile biocabins with a final volume of 50 µl and the most frequently mutated region of the gene was amplified by PCR in a thermal cycler (ABI, Applied Biosystems, USA) and mutation analysis was performed by sequence analysis. PCR mixture consisted of 5 µl 10xPCR buffer, 10 µl Q solution, 1.5 µl dNTP mixture (10 mM), 14 µl primers (7 µl fwd + 7 µl rev) (4 pmol/µl), 0.25 µl HotStartTAq DNA polymerase, 1 µl DNA (50 ng) and distilled water. PCR conditions consisted of a cycle of activation at 95˚C for 15 min followed by denaturation for 30 s, primer annealing at 55˚C for 30 s, chain extension at 72˚C for 42 cycles for 45 s, and final extension for 1 cycle for 10 min, followed by removal to +4˚C for electrophoresis. PCR products were loaded onto a 1.5% agarose gel stained with ethidium bromide (Et-Br) and electrophoresed. The samples were then examined in a UV-transluminator with a wavelength of 312 nm and recorded in a gel imaging system. After it was observed that the samples were PCR amplified to the expected length, the control was working and there was no contamination, the purification of the PCR products was started chain extension at 72˚C for 45 s, final extension for 1 cycle for 10 min, and then removal to +4ºC for electrophoresis.

PCR products were purified for sanger sequencing using QIAquick PCR Purification Kit (catalog #: 28106) (QIAGEN, Hilden, Germany) by adding binding buffer, placing in spin column, washing with spin column wash buffer and eluting in a 1.5 ml tube. Purified PCR products were sequenced bidirectionally (forward and reverse) with BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) according to the manufacturer's protocol on an ABI-3730 (48 capillary) DNA sequencer (Applied Biosystems, USA). Samples were denatured at 95˚C for 3 minutes and immediately cooled rapidly on ice. The plate was then loaded into an ABI-3730 (48 capillary) DNA Analyzer (Applied Biosystems, USA) after appropriate programming. Forward and reverse sequence electrophoregrams were analyzed using SeqScape Software v3.0.

Statistical Analysis

All data analyses were performed with SPSS version 20.0 (SPSS Inc., IBM Corp). Compliance with normal distribution was evaluated by Kolmogorov-Smirnov test. Student t test was used for data conforming to normal distribution and nonparametric Mann-Whitney U test was used for comparison of quantitative variables in case of non-normal distribution. Chi-square test was applied for qualitative variables between groups. Survival was tested using the Kaplan-Meier method. Receiver operating curve (ROC) curves were additionally plotted. The p value <0.05 was considered statistically significant. All data were analyzed by rstudio (2022.02.1 Build461). Descriptive statistics (frequency distributions, percentages) were generated, and logistic regression analysis was used as appropriate (**Table**).

RESULTS

This study included 90 patients with bladder cancer who were followed up in the Urology Department of Sultan Abdülhamid Han Training and Research Hospital between 2016 and 2021.

Table. Logistic regression analysis of risks affecting TERT promoter region mutation

Population patients		n=90	OR (95% CI)	p
Recurrence (TERT) mutation (negative/positive)				
Positive				
Risk	Reference is low risk	26		
	High risk	64	1.940 (1.077-3.493)	0.027
Recurrence (TERT) mutation (negative/C228T/C250T)				
Recurrence of TERT status	Reference is negative			
C228T				
Risk	Reference is low risk	35		
	High risk	24	0.265 (0.081-0.867)	0.028
C250T				
Risk	Reference is low risk	3		
	High risk	2	0.273 (0.034-2.188)	0.221

TERT: Telomerase reverse transcriptase, OR: Odds ratio, CI: Confidence interval

60 of the patients were followed up with low grade tumors and 30 with high grade tumors. Out of 60 patients in the low-grade group, TERT promoter mutation was positive in 38 patients and negative in 22 patients. Among the 30 patients in the high-grade group, TERT promoter mutation was positive in 26 patients and negative in 4 patients (**Figure 1**). The TERT mutation rate was 71.1% in all bladder cancer cases. The most common TERT promoter mutations are a single cytosine change to thymine at chromosome 5 base position 1,295,228 (C228T) or less frequently at base position 1,295,250 (C250T) (-124 and -146 bp from the ATG start site, respectively). Of the 26 positive mutations in the high-grade group, 24 were C228T positive and 2 were C250T positive; of the 38 positive mutations in the low-grade group, 35 were C228T positive and 3 were C250T positive.

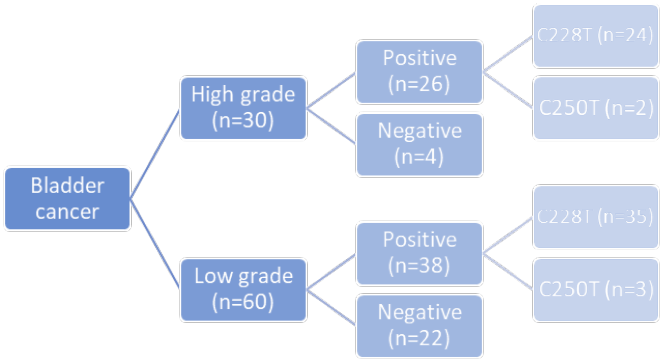

Figure 1. Distribution of bladder cancer cases according to grade and TERT mutations

TERT: Telomerase reverse transcriptase

The mean age of 64 patients who were positive for TERT promoter region mutation (C228T+C250T) was 70.09±10.45 and the mean age of 26 patients who were negative was 68.58±10.61. ROC analysis was performed to determine the cut-off point of the age continuous variable for TERT promoter region mutation. The cut-off point for the mutation analysis of the TERT promoter region mutation was 70, which was not statistically significant [p=0.381; AUC=0.559; 95% CI (0.424-0.694)] (**Figure 2**).

Patients with high grade tumors were 1.940 times more likely to be TERT promoter mutation positive than low grade patients [p=0.027; exp (b) = 1.940 95% CI (1.077-3.493)] (**Table**).

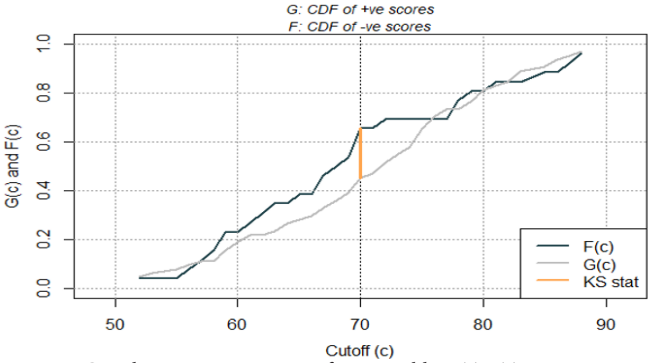

Figure 2. Curvilinear representation of age variable in TERT promoter region mutation

TERT: Telomerase reverse transcriptase

If the patient's bladder cancer is high grade, the probability of TERT recurrence mutation analysis being C228T is 0.265 times higher than if the patient's bladder cancer is low grade [p=0.028; exp (b)=0.265 95% CI (0.081-0.867)], and the probability of being C250T is 0.273 times higher than if the patient has low grade [p=0.221; exp (b)=0.273 95% CI (0.034-2.188)] (**Figure 3**).

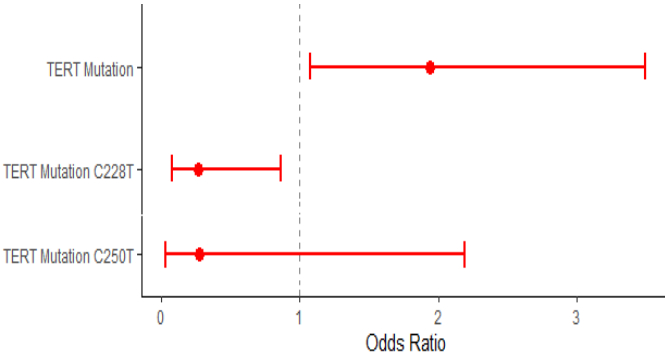

Figure 3. Probabilistic distribution of risks affecting TERT promoter region mutation

TERT: Telomerase reverse transcriptase

DISCUSSION

There is an ongoing search for alternative non-invasive methods to reduce the repeated exposure of bladder cancer patients to an invasive procedure such as cystoscopy and the risks and complications associated with the procedure during follow-up and to intelligently manage increased healthcare costs. Laukthina et al.[16] found that urinary biomarkers can reduce the frequency of cystoscopy by approximately 74% in the follow-up of non-muscle invasive bladder cancer cases.

Based on the discovery of telomerase activity and TERT mutation and the data obtained afterwards, there are studies suggesting that it may be a prognostic marker in various cancer types.[17,18] There is information that hTERT expression is a rate-limiting determinant of the enzymatic activity of human telomerase and that increased expression of hTERT may play a critical role in human carcinogenesis.[19]

It is known that approximately 84% of bladder cancer cases have TERT promoter mutations and the mutant sequence can be detected in urine samples of mutation positive bladder cancer cases.[20-22] Given this high prevalence, TERT promoter mutation analysis would have a high sensitivity in diagnosing bladder cancers. In addition, since these mutations are only seen in cancer cases, it can be predicted that the specificity will also be high.[22] In a study by Pakmanesh et al.,[23] TERT

promoter mutation in urine was found to be 100% sensitive and 88% specific for detecting first diagnosed bladder cancers, while it was 50% sensitive and 88% specific for detecting recurrent bladder cancers. The overall sensitivity and specificity of TERTpm for detecting bladder cancer were 67.7% and 88.0%, respectively, which were consistent across different tumor stages and grades.

In addition to urothelial carcinomas, these mutations have also been reported in other rare histologic variants of primary bladder cancers such as squamous cell carcinoma,[14] small cell carcinoma,[15] non-enteric type adenocarcinoma[13] and plasmacytoid urothelial carcinoma.[11] Furthermore, TERT promoter mutations may serve as biomarkers to differentiate subtypes of urologic malignancies.[12]

Carrasco et al.[24] conducted genetic studies in muscle invasive bladder cancer patients before and after radical cystectomy. In 39 bladder cancer patients, TERT mutation was analyzed in blood samples taken preoperatively and one, four and twelve months postoperatively. TERT c.-124C> T mutation was found to be particularly sensitive in showing tumor recurrence at twelve months. Wang et al.[25] 2015 in Chinese patients, TERT promoter was identified in 47.8% of patients and urine samples were obtained from some of the patients carrying the mutation and analyzed. The mutant promoter was detected in the urine of 52% of patients before the operation and disappeared in most urine samples (80%) examined 1 week after the operation. The results are promising that TERT mutation analysis can be used in postoperative recurrence screening.

In our study, the TERT mutation rate in bladder cancer was 71% and the probability of TERT mutation was 1.94 times higher in cases with high grade tumors, which is consistent with many studies in the literature. Although there are a limited number of studies examining the relationship between grading and TERT mutation in bladder cancer, different results have been obtained in these studies.[20,23,26] Allory et al.[20] reported a TERT promoter mutation frequency of 83% in bladder tumors, independent of stage or disease-related risk. The mutation frequency was almost identical for low-risk non-muscle invasive bladder cancer (NMIBC) (73%) and high-risk NMIBC (74%). Muscle invasive bladder cancer (MIBC) was found to be 53%.[20] In a study by Morozov et al.,[27] the TERT promoter mutation rate in bladder cancer was up to 80% in both tissue and urine, resulting in a sensitivity of 62-92% for primary tumors and 42% for recurrence. Specificity ranged between 73% and 96%, with no correlation with stage. There are also studies indicating that telomerase activity is related to the pathological grade and clinical stage of the tumor, and that telomerase activity is higher in more advanced grade and deep invasive tumors.[26] Our study also supported these data.

In the future, the search for alternative non-invasive methods to cystoscopy is expected to play a key role in the reduction of morbidity and mortality related to bladder cancer with early diagnosis or early recurrence detection, and the results of TERT mutation analysis used in this direction are predicted to provide valuable information in the initial diagnosis and follow-up of recurrence after treatment.

CONCLUSION

Studies are still ongoing to discover a non-invasive method that can achieve similar accuracy to cystoscopy in follow-up. There are studies that have obtained predictions that telomere activity and TERT mutation analysis can be used for cancer diagnosis, grading, typing, recurrence follow-up and evaluation of treatment efficacy in various tissue types. Considering the high prevalence of TERT mutation in bladder cancer, it is thought to be used as a marker in bladder cancer follow-up. In our study, TERT mutation positivity was found to be associated with the presence of high-grade tumors in bladder cancer cases. Accordingly, it is thought that TERT mutation analysis in these patients may affect the follow-up algorithm of the patient. In the light of previous studies, obtaining significant data that TERT mutation analysis can be used in the diagnosis of bladder cancer cases, tumor grading and follow-up of recurrence after treatment indicates that more intensive and numerous studies should be conducted on this subject.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the University of Health Sciences Hamidiye Clinical Researches Ethics Committee (Date: 06.09.2021, Decision No: 4).

Informed Consent

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

Referee Evaluation Process

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

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

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Original Article

Epstein-Barr virus RNA percentage and its impact on prognosis in patients with diffuse large B-cell lymphoma

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ABSTRACT

Aims: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphomas. This study aimed to investigate the impact of Epstein-Barr virus RNA (EBER) positivity on the prognosis of patients diagnosed with DLBCL.

Methods: This retrospective study was conducted on 105 patients diagnosed with DLBCL at Sivas Cumhuriyet University Hospital between June 1, 2009, and May 31, 2022.

Results: Of the 105 patients, 55.2% were male, and the mean age was 56.89 years. EBER positivity was detected in 4.8% of the patients. While 71% of EBER-negative patients achieved a complete response, overall survival and progression-free survival were shorter in EBER-positive patients. However, no significant relationship was observed between EBER positivity and prognostic indices (IPI, R-IPI, NCCN-IPI).

Conclusion: The findings of this study indicate that EBER positivity could act as a prognostic indicator, given its association with unfavorable survival rates and diminished response to treatment in individuals with DLBCL.

Keywords: Diffuse large B-cell lymphoma, EBER, prognosis

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most commonly observed subtype of non-Hodgkin lymphomas (NHL), accounting for approximately 30-40% of all NHL cases.[1] Its etiology includes genetic mutations, immune deficiencies, and viral infections. Epstein-Barr virus (EBV) targets B cells, establishing latent infections and, in some cases, exhibiting oncogenic effects.[2,3]

EBV positivity is associated with poor prognosis and clinical heterogeneity in DLBCL patients. Studies have reported that the prevalence of EBV in DLBCL ranges between 3% and 16.9%.[4,5] While EBV-positive DLBCL was initially thought to affect elderly patients predominantly, recent findings indicate that it can also occur in younger individuals.[6] EBV positivity is recognized as an independent prognostic factor affecting overall survival (OS) in DLBCL patients, along with variables such as older age, advanced disease stage, and activated B-cell-like characteristics.[5] The presence of EBV in DLBCL cases is typically detected using in situ hybridization techniques for EBV-encoded RNA (EBER).[6] Further research is needed to understand EBV's impact on DLBCL prognosis fully and to develop tailored treatment strategies for this subgroup of patients.[4] The role of EBV in DLBCL remains a topic of interest both diagnostically and prognostically. This study evaluates the relationship between EBV RNA positivity and the prognosis of DLBCL.

METHODS

Ethics

In this retrospective study, 105 patients diagnosed with DLBCL at the Hematology Clinic of Sivas Cumhuriyet University Health Services Application and Research Hospital between June 1, 2009, and May 31, 2022, were evaluated. The study was carried out with the permission of the Sivas Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date: 25.05.2022, Decision No: 2022-05/27). The Sivas Cumhuriyet University Scientific Research Projects (CÜBAP) unit supported the study with project number T-2022-977. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Data Collection and Analysis

The patients included in the study were between 18 and 90 years old, with sufficient pathology tissue samples and accessible treatment data. A reactive lymph node section previously considered EBER-positive was used as a positive control.

Patient information was obtained from e-Nabız, the Hospital Information Management System, the Death Notification System, the MEDULLA Physician Application, discharge summaries, outpatient clinic notes, pathology reports, and imaging reports. Clinical parameters such as Eastern Cooperative Oncology Group (ECOG) performance scores,

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Ann-Arbor staging, extranodal involvement, and B symptoms were recorded. The scores for the International Prognostic Index (IPI), Revised International Prognostic Index (R-IPI), and National Comprehensive Cancer Network-International Prognostic Index (NCCN-IPI) were determined.

Treatment response was evaluated according to the Lugano criteria, and the duration of OS and progression-free survival (PFS) were calculated.

Histopathological Evaluation and In Situ Hybridization

Hematoxylin-eosin-stained slides were re-examined, and suitable tissues were selected for in situ hybridization. The presence of EBV was assessed using the chromogenic in situ hybridization (CISH) method. Blue reactions in target cells confirmed positivity, graded on a scale from 1 to 3 based on intensity (**Figure 1**).

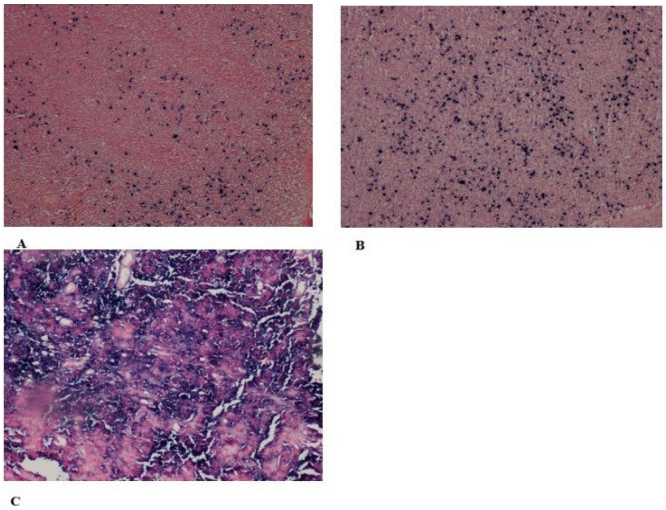

Figure 1. A) EBV grade 1, B) EBV grade 2, C) EBV grade 3

EBV: Epstein-Barr virus

Statistical Analysis

Data analysis was conducted using SPSS 26.0 software, with a 95% confidence interval. Categorical variables are presented as frequencies and percentages, while numerical variables are reported as mean, standard deviation, minimum, maximum, and median values.

Independent T tests, Mann-Whitney U tests, one-way ANOVA, and Kruskal-Wallis tests were utilized for group comparisons, while chi-square tests were applied to analyze categorical variables; Pearson and Spearman correlation tests were applied to assess relationships between numerical variables. Dependent T tests, Wilcoxon signed-rank test, and McNemar tests were conducted for repeated measurements. The Kaplan-Meier method was used for survival analyses, while ROC analysis assessed the predictive capability of measurements for exitus status.

RESULTS

Analysis of Clinical and Pathological Findings

In this study, 55.2% of the DLBCL patients were male, and 47% were female. Pathological examination revealed that 44.9% of the patients had germinal center B-cell (GCB) type, 100% were Ki67 positive, 80.2% were CD5 positive, 34.1% were CD10 positive, 100% were CD20 positive, 66.3% were Bcl-6 positive, 70.5% were Mum-1 positive, and 79.3% were Bcl-2 positive. Additionally, 4.8% of the patients tested positive for EBER.

The patients' interim and post-treatment positron emission tomography/computed tomography (PET/CT) imaging were evaluated according to the Lugano response criteria, with 61% showing a complete response at interim imaging and 68.6% showing a complete response at post-treatment imaging. At the time of diagnosis, 47.6% of the patients were in stage 4, 39% had extranodal involvement, 88.6% had lymph node involvement, 28.6% had bone marrow involvement, 61% had organ involvement, and 46.7% had B symptoms. Of the patients, 67.6% had an ECOG score of 1, 50.5% were in the low-medium risk group based on the IPI score, 46.7% were in the good-risk group according to the R-IPI score, and 39.0% were in the low-medium risk group based on the NCCN-IPI score (**Figure 2**).

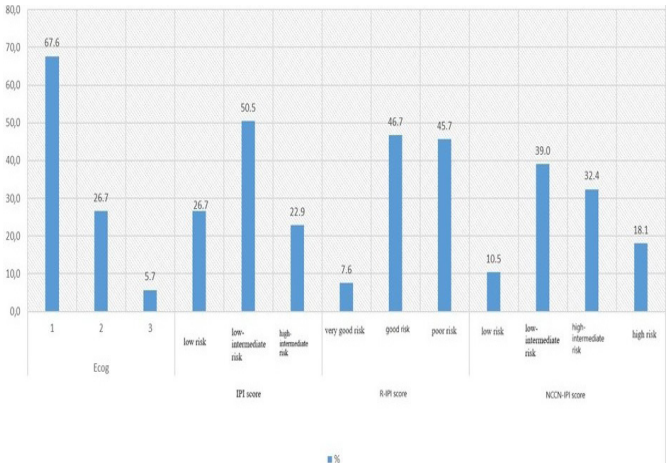

Figure 2. Distribution of patients according to ECOG, IPI, R-IPI, and NCCN-IPI scores

ECOG: Eastern Cooperative Oncology Group, IPI: International Prognostic Index, R-IPI: Revised International Prognostic Index, NCCN: National Comprehensive Cancer Network

Treatment and Survival Analysis

As first-line treatment, 79% of patients received R-CHOP (Rituximab-Cyclophosphamide-Doxorubicin-Vincristine-Prednisone), 9.5% received second-line R-DHAP (Rituximab-Dexamethasone-Cisplatin-Cytarabine), 2.9% received third-line R-GIFOX (Rituximab-Gemcitabine-Ifosfamide-Oxaliplatin), and 1% received fourth-line Pixantron. Autologous stem cell transplantation was performed in 9.5% of patients, radiotherapy in 17.1%, and central nervous system (CNS) prophylaxis in 38.1%. No statistically significant relationship was found between the patients' pathological classification as GCB or non-GCB lymphoma and their ECOG, IPI, R-IPI, or NCCN-IPI scores ($p>0.05$). The average OS time was determined to be 41.49 months, while the PFS period averaged 36.99 months.

Relationships with EBER Positivity

A statistically significant correlation was observed between EBER positivity and germinal center versus non-germinal center B-cell status ($p=0.037$), with all EBER-positive cases classified as germinal center B-cells. However, no significant associations were identified between EBER positivity and the expression of Ki67, CD5, CD10, CD20, Bcl-6, Mum-1, or Bcl-2 ($p>0.05$) (**Table 1**). A significant relationship was observed between EBER positivity and post-treatment imaging status

(p=0.041), with a higher complete response rate in EBER-negative patients (71%). No statistically significant association was found between EBER positivity and ECOG, IPI, R-IPI, or NCCN-IPI score levels (p>0.05) (Table 2).

Table 1. Relationship between EBER positivity and germinal center, Ki67, CD, Bcl-6, Mum-1, Bcl-2

		EBER		
		Positive	Negative	p
Germ cell	Germinal	4 (100)	31 (41.9)	0.037*
	Non-germinal	0 (0)	43 (58.1)	
Ki67	Positive	5 (100)	100 (100)	x
	Negative	0 (0)	0 (0)	
CD5	Positive	4 (100)	69 (79.3)	0.581
	Negative	0 (0)	18 (20.7)	
CD10	Positive	3 (75)	27 (32.1)	0.113
	Negative	1 (25)	57 (67.9)	
CD20	Positive	5 (100)	100 (100)	x
	Negative	0 (0)	0 (0)	
Bcl-6	Positive	4 (100)	51 (64.6)	0.295
	Negative	0 (0)	28 (35.4)	
Mum-1	Positive	1 (25)	54 (73)	0.074
	Negative	3 (75)	20 (27)	
Bcl-2	Positive	2 (50)	63 (80.8)	0.188
	Negative	2 (50)	15 (19.2)	

*A p-value of <0.05 indicates a significant relationship, while a p-value of >0.05 indicates no considerable relationship; the chi-square test, EBER: Epstein-Barr virus RNA

Table 2. Association between EBER positivity and interim and post-treatment imaging outcomes

		EBER		
		Positive	Negative	p
Interim PET/CT	Stable disease	0 (0)	2 (2)	0.356
	Partial response	1 (20)	22 (22)	
	Complete response	2 (40)	62 (62)	
	Progression	0 (0)	2 (2)	
	No imaging	2 (40)	12 (12)	
Post-treatment PET/CT	Stable disease	0 (0)	0 (0)	0.041*
	Partial response	1 (20)	5 (5)	
	Complete response	1 (20)	71 (71)	
	Progression	1 (20)	9 (9)	
	No imaging	2 (40)	15 (15)	

*A p-value of <0.05 indicates a significant relationship, while a p-value of >0.05 indicates no considerable relationship; the chi-square test, EBER: Epstein-Barr virus RNA, PET/CT: Positron emission tomography/computed tomography

Survival Durations and Factors

Overall survival (p=0.015) and PFS (p=0.031) showed a significant difference between patients who were EBER-positive and those who were EBER-negative. EBER-positive patients had shorter OS (14.38 months) and PFS (12.74 months) than EBER-negative patients (Table 3, Figure 3, 4). However, no significant differences in OS or PFS durations were noted based on gender or germinal/non-germinal B-cell status (p>0.05).

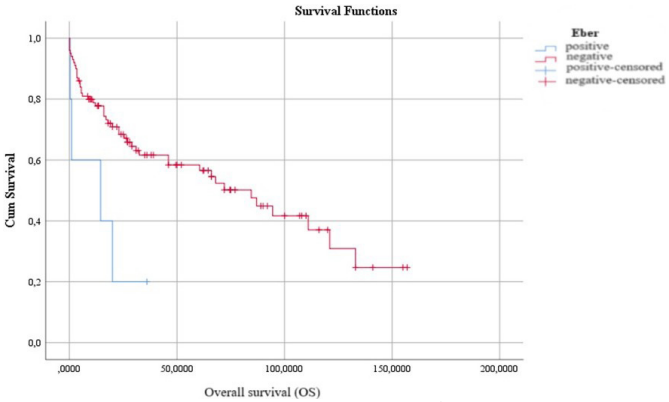

Figure 3. Association Between EBER Positivity and OS

EBER: Epstein-Barr virus RNA, OS: Overall survival

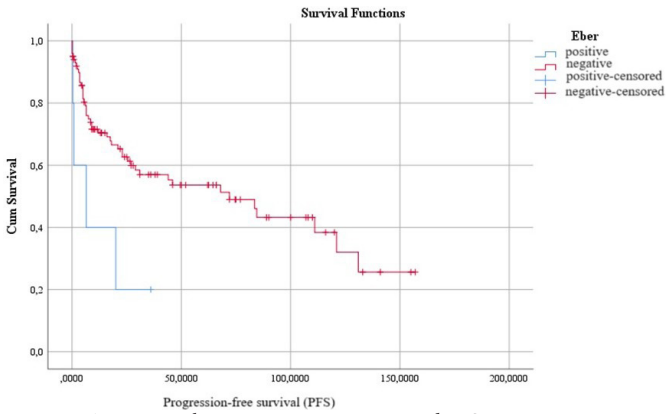

Figure 4. Association between EBER Positivity and PFS

EBER: Epstein-Barr virus RNA, PFS: Progression-free survival

DISCUSSION

DLBCL represents approximately one-third of all non-Hodgkin lymphoma cases, ranking as one of the most common subtypes. The role of EBV in the biological and clinical characteristics of DLBCL has not been fully clarified.[7] In this context, many studies have examined the prognostic impact of EBV infection. Specifically, identifying prognostic factors during diagnosis could be crucial in determining the need for early and intensive treatment in DLBCL patients. Some studies have indicated that EBER positivity holds significant prognostic value in DLBCL, while others have not supported these findings.[5,8-16] Numerous studies have reported variability in the frequency of EBER positivity among patients with DLBCL. For instance, in Asian countries (Korea and Japan), the frequency of EBER positivity ranges from 8-9%, while in Western countries, this rate is 1-3%.[8,11,17,18] In a study by Park et al.[14] involving 380 DLBCL patients, 9% were EBER positive.[19] Furthermore, in a meta-analysis of 13 studies, including 4111 DLBCL patients, the frequency of EBER positivity was reported to range from 1.4% to 14.9%. In our study, 4.8% of patients diagnosed with DLBCL were found to be EBER positive. Among the 5 EBER-positive patients, 1% were classified as grade 1, 1.9% as grade 2, and 1.9% as grade 3 based on staining intensity.

Table 3. Relationship between EBER Positivity and overall survival and progression-free survival

		Overall survival				Progression-free survival			
Factor	Group	n (%)	Mean	95% CI Mean	p	n (%)	Mean	95% CI Mean	p
EBER	Positive	1 (20)	14.380	2.795-25.965	0.015*	1 (20)	12.746	0.812-24.68	0.031*
	Negative	53 (53)	78.732	64.299-93.164		53 (53)	75.717	60.643-90.791	

*p<0.05 indicates a significant effect, p>0.05 indicates no significant effect; Kaplan-Meier test, EBER: Epstein-Barr virus RNA, CI: Confidence interval

Many studies have reported a negative association between EBER positivity and OS durations.[5,10,13] Tracy and colleagues, through their research, revealed that neither EBV positivity nor immunosuppression status significantly influences the treatment approaches or survival rates in patients diagnosed with diffuse large B-cell lymphoma (DLBCL).[12] Hong and colleagues highlighted that serum survivin positivity serves as an independent adverse prognostic factor influencing disease progression in DLBCL patients treated with R-CHOP. However, they noted that the expression of EBER in tumor tissues does not have a significant impact on clinical outcomes.[16] Jarrett et al.[20] conducted a study with 437 classical Hodgkin lymphoma patients, identifying EBER positivity as an independent factor influencing survival. Similarly, our study found that EBER-positive patients had shorter OS and PFS durations compared to EBER-negative patients, with this difference being statistically significant. Most research has indicated that EBER-positive patients have higher IPI scores, with a statistically significant relationship.[11] However, in another study involving 89 patients, no significant difference was found between EBER positivity and IPI scores.[5] Contrary to the findings in the literature, our study did not detect a statistically significant relationship between EBER positivity and IPI scores. A study conducted with 362 patients in North America found a significant association between EBER positivity and GCB and non-GCB subtypes of DLBCL.[12] However, some studies did not find this association statistically significant.[13,15] Our study identified a statistically significant relationship between EBER positivity and GCB and non-GCB DLBCL subtypes.

The prognostic and biological effects of EBER positivity on DLBCL have been reported with varying results in the literature. Our study has contributed to the existing knowledge in this field and confirmed the negative impact of EBER positivity on survival durations. Additionally, the relationships between EBER positivity, IPI score, and GCB subtypes suggest that further studies are needed to better understand these associations.

CONCLUSION

Our study found a statistically significant relationship between EBER positivity and OS and PFS durations. We concluded that EBER-positive patients had lower OS and PFS than EBER-negative patients. EBV-positive patients should be closely monitored during treatment planning. In future studies, a more detailed investigation of the molecular mechanisms of EBV may contribute to developing new strategies for managing DLBCL.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Sivas Cumhuriyet University Non-interventional Clinical Researches Ethics Committee (Date: 25.05.2022, Decision No: 2022-05/27).

Informed Consent

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

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support

Author Contributions

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

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Case Report

Vulvar and vaginal graft versus host disease in a patient with chronic phase chronic myeloid leukemia after allogeneic stem cell transplantation

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ABSTRACT

Graft versus host disease (GvHD) is one of the serious complications of allogeneic stem cell transplantation in the treatment of hematological malignancies. Skin, liver, and eyes are frequently affected areas. In addition to frequently affected areas, genital involvement can also be seen. Allogeneic stem cell transplantation is one of the curative treatments for hematological malignancies seen at the young age group. And its use for therapeutic purposes in young patients is increasing daily. Vulvovaginal GvHD is a disease type that concerns female patients of reproductive age. In this case, we wanted to reportin the literature a case that underwent allogeneic stem cell transplantation after chronic myeloid leukemia diagnosis and tyrosine kinase inhibitory resistance and then developed vulvovaginal GvHD. In vaginal involvement, in addition to many genitourinary complaints, many negativities in sexual life and deterioration in quality of life are experienced. The patient is currently continuing her symptomatic local topical treatment and systemic immunsupressive treatment for GvHD with response at following up. Our aim in presenting this case is to emphasize that GvHD should be considered in the differential diagnosis of female patients with allotransplanted hematological disease and vulvovaginal sign and symptoms.

Keywords: Chronic vulvovaginal graft versus host disease, chronic myeloid leukemia, allogeneic stem cell transplantation

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INTRODUCTION

Graft versus host disease (GvHD) is a serious complication with acute and chronic stages, categorized according to the various symptoms that develop after allogeneic stem cell transplantation (allo SCT). The pathogenesis of GvHD is a complex T cell-mediated immune response; In this mechanism, grafted donor cells react against histocompatibility antigens in the recipient. GvHD may affect various organs, including skin, liver, and intestines. GvHD also occurs in the female genitals, especially the vulva and vagina, where it is known as vulvo-vaginal GvHD (VVGvHD).

A few studies have specifically addressed VVGvHD and its effects on patients' sexual health and quality of life. We observed that female patients did not focus on health problems involving their genital area when they considered a serious and life-threatening procedure such as bone marrow transplantation (BMT) and these symptoms may be overlooked by physicians

Female genital GvHD affects the vulva and vagina in approximately 25-50% of allogeneic SCT survivors, 68% in the vulva and 26% in the vulva and vagina. Onset is on average 7-10 months after allogeneic SCT. However, after a few years, vaginal GvHD may develop. The main symptoms of VVGvHD are dryness, burning, itching, pain to touch, pain during sexual intercourse, and, in a condition known as vestibular dysuria, burning when urine touches the entrance to the vagina. Vaginal atrophy must be excluded because after transplantation, estrogen deficiency occurs as most patients enter menopause. Physical findings consistent with VVGvHD include purulent discharge, mucosal erosion, vaginal stenosis, and loss of elasticity, which is graded from mild to severe.

CASE

42-year-old-female patient was diagnosed as chronic phase chronic myeloid leukemia in 2015. She was treated with imatinib 400 mg/day. After 6 months molecular response was not obtained and treatment changed to dasatinib 100 mg/day, however after 3 months of dasatinib treatment molecular and hematologic progression occurred and treatment was changed to nilotinib, and bone marrow transplantation was planned. Imatinib resistance mutations could not be investigated in the patient. After 4 months the patient transplanted successfully with HLA-matched sibling stem cell donor. Tyrosine kinase inhibitory was used till 1 year after transplantation, and Bcr/abl was negative after transplantation and until now.

During 2 months of transplantation, acute GvHD occurred and healed without any serious complication, but after 10

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months symptoms and signs of chronic GvHD developed. Dry skin, itching, and dark hyperpigmentation occurred in generalized of the body, especially in the upper extremities and ocular GvHD was the main symptoms of the patient. She used cyclosporin and steroids for prevention and treatment of GvHD, also she uses ursodeoxycholic acid for liver protection.

Chronic GvHD sustained more than 2 years especially ocular findings (drying, itching, and scarring of conjunctiva and eyelid). After 5 years of transplantation, she told her symptoms of genitalia such as vulvodinia, pain during sexual intercourse, and decreased sexual function to our nurse. She has problems with her husband for this reason. In gynecological examination, there were findings consistent with vulvodynia, but there was no genital atrophy. We prescribed 2% amitriptyline plus 2% baclofen cream two times a day for the treatment of vulvodynia.

When she came for control 1 month later of local treatment, she stated that sign and symptoms were better in terms of sexual function but could not urinate completely. Bacteriuria, pyuria, and hematuria were observed in urinalysis. Pelvic ultrasonography was normal. We treat her for a urinary tract infection. Since the patient's genital atrophy was not evident, we did not prescribe vaginal estrogen during both examinations. The symptoms and signs of the patient were healed near completely when we call to patient for evaluated last situation of disease.

DISCUSSION

Hematopoietic stem cell transplantation (HSCT) is a treatment method for malignant and benign hematological diseases as well as in the treatment of some non-hematological disorders such as autoimmune diseases.[1] Graft-versus-host disease (GVHD) is an immunity-related disease which affects 30-70% of patients after hematopoietic stem cell transplantation (allo HSCT) and is a significant contributor of morbidity and non-relapse mortality (NRM).[2] Chronic GVHD is a mucosal disease of the mouth, eyes, genitals, intestines, and lungs. It includes inflammation and fibrosis of membranes. There are some evidence that indicates clinical symptoms and pathogenesis of GVHD are similar to various autoimmune disorders such as Scleroderma, Sjögren's syndrome, and lichen planus.[3,4] Female genital GVHD was first described by Corson et al.[5] by observing Sclerosing vaginitis and structure problems in 5 women in 1982. Nowadays, it is an underdiagnosed condition and affects the quality of life which occurs in one-quarter of long-term surviving women after allogeneic stem cell transplantation.[6] The rates of genital GVHD vary widely, with rates ranging from 24.9-69%.[7] The wide variation in the incidence of genital GVHD is due to a variety of abnormalities, including the time at which incidence is calculated, the systematic and time-dependent gynecological evaluations, and the useddiagnostic criteria (findings of examination with or without symptoms, etc.).[8] The main risk factor for the development of chronic genital GVHD is using of peripheral blood as a source of stem cells; It represents a risk of three times higher than that obtained from bone marrow stem cells.[9-11] The presence of GvHD in other organs is also considered one of the other risk factor.[12] While one study found that 79% of patients with VVGvHD were previously treated for GvHD in a different organ, another study reported that almost all patients with VVGvHD had active chronic GvHD in the skin, mouth, and eyes.[13,14] Our patient was receiving immunsupresive treatment for skin and liver involvement caused by chronic GVHD. It is supported by various studies that it develops after an average of 10.2 months after transplantation.[6] In our patient, VVGvHD was diagnosed approximately 5 years after allogeneic transplantation. The clinic may be asymptomatic; the main signs and symptoms are vulvar tenderness to palpation of openings of the mucosa, erosion of the mucosa, cracks, leukokeratosis, labial or clitoral fusion, fibrous vaginal ring, vaginal shortening, vaginal adhesions, and complete vaginal stenosis. Other symptoms include dryness, burning, itching, pain to touch, dysuria, dyspareunia, and resulting sexual dysfunction takes place.[5] She has vulvodynia, pain during sexual intercourse, and decreased sexual function. Although symptoms are similar to primary ovarian insufficiency which occurs after allogeneic stem cell transplantation, synechia and adhesive bands are not encountered in primary ovarian failure. In addition, studies have shown that hormone replacement therapy is used for the prophylaxis of this condition does not affect the development rate of vulvovaginal GVHD.[11] The National Institutes of Health (NIH) Consensus Development Project proposed guidelines for screening, diagnosing, and preventing genital GVHD in HSCT survivors.[15]

CONCLUSION

Incidence and severity of genital GVHD in women should be included in GVHD intervention studies. Treatment goals for Female genital GVHD include symptom relief, disease control, and prevention of further damage. In its treatment, various patient-specific treatment modalities are advocated such as topical estrogens, topical steroids, topical immunosuppressive agents (such as cyclosporine, tacrolimus), vaginal dilators, and surgical lysis. Diagnosis and treatment of post-transplant genital GVHD require a systematic approach and collaboration between bone marrow transplant physicians, coordinators and gynecologists.

ETHICAL DECLARATIONS

Informed Consent

The patient signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

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Author Contributions

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

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