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Original Article

Use of Dapsone in Chronic/Refractory Immune Thrombocytopenic Patients: A Single Center Experience

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ABSTRACT

Background Dapsone is a second-line therapy for immune thrombocytopenia (ITP). It is cost-effective, with a response rate comparable to other drugs used as second-line therapy, such as azathioprine, danazol, cyclophosphamide, cyclosporine, vincristine, rituximab, and eltrombopag.

Material and Methods This retrospective study analysed ten adult patients who presented to our hematology division outpatient clinic between March 2013 and July 2021, was diagnosed with chronic/refractory ITP, did not respond to first-line therapy, and used dapsone.

Results Eight (80%) patients were female, and 2 (20%) were male. The median age was 50 (range, 24-64) years. The mean pre-treatment platelet value was $12.8x10^9$/L (range: 4-22.1x109/L). The median duration of symptoms before dapsone treatment was 60 (6-360) months. The median number of treatments received before dapsone was 4 (range: 3-6). All patients were routinely treated with oral dapsone 50 mg for two weeks, followed by 100 mg. The median time to treatment response was 39 (range: 14-90) days. The response rate was 60% (complete response 40%, partial response 20%). Asymptomatic anaemia was observed as a side effect in only one patient.

Conclusions Based on these results, it can be speculated that dapsone is an effective, inexpensive, and well-tolerated treatment option. Considering the economic status of developing countries, it seems very attractive to use dapsone as the second-line therapy for chronic/refractory ITP. To the best of our knowledge, this is the first study in Turkey on the use of dapsone for chronic/refractory ITP.

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INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune disease characterised by low platelet count and mucocutaneous bleeding. Patients diagnosed with ITP are classified into three categories: acute ITP (<3 months), persistent ITP (3-12 months), and chronic ITP (>12 months).[1] Life-threatening bleeding is rare in ITP (0.16%-0.38% in adult patients) and is mostly observed in elderly patients. Steroids are the main drug used in first-line therapy. Seventy per cent of adult patients respond to steroid therapy, of whom 50% showed sustained response after treatment discontinuation.[2] The other half of the patients either do not respond to steroids or become steroid dependent. Treatment options for these patients include pulse dexamethasone, azathioprine, cyclosporine, danazol, or vincristine, with response rates ranging from 10% to 30%, and splenectomy, to which approximately 70% of the patients respond.[2] New treatment options such as rituximab or thrombopoietin receptor agonists (TPO-RA) are promising in patients with contraindicated splenectomy.[3] The high cost of these drugs and their side effects, such as thrombosis and bone marrow reticulin fibrosis, should be considered in the long-term use of TPO-RA.[4] Dapsone (4,4-Diaminodiphenyl sulfone) is an antibiotic from the group of sulfones as well as a folate antagonist with anti-inflammatory and anti-parasitic effects. It is also used for the treatment of leprosy. Since it is both inexpensive and well tolerated, it is a good option for second-line therapy.

A response rate of 62% was achieved in 21 steroid-dependent patients reported to be using dapsone for the first time in ITP.[5] A response rate of 50% was also reported in a case series of 66 patients.[6] Similar rates were found in another reported publication.[7] The mechanism of action of dapsone in ITP is not fully understood. One possible mechanism is the induction of hemolysis due to the conversion of dapsone to hydroxylamine, which leads to erythrophagocytosis in the reticuloendothelial system. Thus, sequestration and destruction of platelets are prevented. Another hypothesis is that dapsone is an immunomodulatory drug.[8] Methemoglobinemia and hemolysis are the most common side effects of dapsone therapy. Other rarer side effects may include peripheral neuropathy, agranulocytosis secondary to bone marrow suppression, hepatitis, dermatitis, and psychosis. Response to dapsone is slow, sustained, and on treatment, relapses are rare. Because of the slow response to treatment, patients should be treated for at least three months. There is no consensus on when to discontinue dapsone therapy. Most patients develop relapse after the discontinuation of dapsone. The present study retrospectively evaluated our patients on dapsone, considering that it may be an option in chronic/refractory ITP due to its acceptable response rates, side effect profile, and cost advantage.

MATERIAL AND METHODS

The study included ten patients who were followed up in the Adult Hematology Outpatient Clinic of Bursa Uludag University Faculty of Medicine Hospital, diagnosed with chronic/refractory ITP between March 2013 and July 2021, and used dapsone as second-line therapy. The data of patients were obtained from the hospital information system and patient files. Patients under the age of 18 and patients with acute ITP were not included in the study. Haemoglobin and platelet levels at diagnosis, age at diagnosis, number of lines of treatment received before dapsone, duration of dapsone therapy, treatment responses, and treatment complications of all patients were evaluated. All patients were initiated on oral dapsone 50 mg/day for two weeks. The dose was increased to 100 mg after that. Conditions such as human immunodeficiency virus (HIV), hepatitis C virus (HCV), H. pylori, systemic lupus erythematosus (SLE), and lymphoma that may cause secondary thrombocytopenia were tested and excluded. Bone marrow biopsy was performed in patients with an indication.[9] A platelet count ≥30,000/μL or twice the baseline and no signs of bleeding were defined as the response. A platelet count greater than $100x10^9$/L was considered a complete response.[10] The study was approved by Bursa Uludag University's local ethics committee.

RESULTS

Ten patients receiving dapsone were previously treated with at least three lines of treatment (steroid, intravenous immunoglobulin, splenectomy, danazol, rituximab, etc.). Splenectomy was the most common treatment in the second line (40%) (Figure 1). The data of 10 patients and their response to dapsone therapy are shown in Table 1. All patients were initiated on steroid therapy as the first-line treatment. Intravenous immunoglobulin was administered to 8 patients who had bleeding or an emergency such as surgery (80%). Although there are treatment options such as cyclosporine, cyclophosphamide, and vincristine for the second-line treatment, these agents were not

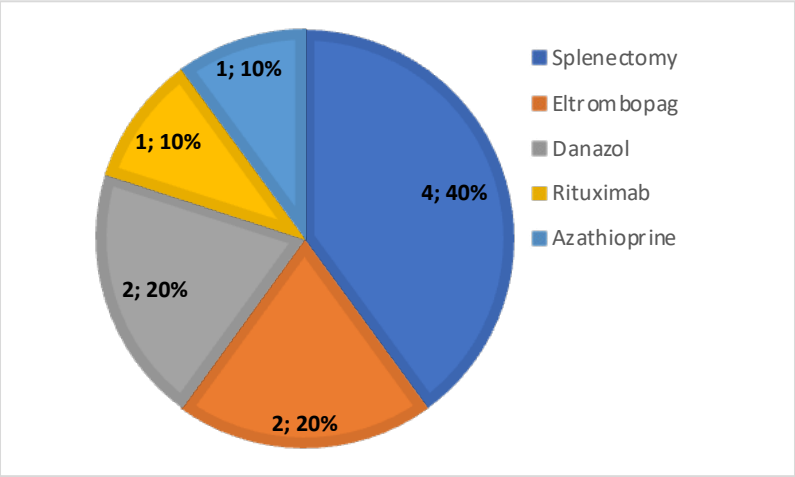

Figure 1. Treatments of choice as second-line treatment in patients with chronic/refractory ITP.

administered to the patients. Despite the absence of any side effects, dapsone therapy of one patient was discontinued in a short period of 1.5 months since their platelet count did not increase. Response status could not be evaluated in another patient due to loss of follow-up during dapsone therapy.

Of the patients, eight were female (80%), and two were male (20%). The median age was 50 (range: 24-64) years. The pre-treatment mean haemoglobin and platelet values were 12.6 g/dL (range: 9.1-16.4) and 12.8x10^9/L (range: 4-22.1), respectively. The median duration of symptoms before dapsone was 60 (range: 6-360) months. The median follow-up period of the patients was 6 (range: 2-29) months (Table 2). The initial admission complaint was bleeding findings (nasal, gingival, and subcutaneous). Severe bleeding findings such as intracranial haemorrhage were not observed in any patients. Asymptomatic anaemia was observed in only 1 (10%) female patient as a side effect during dapsone therapy (from 12.3 g/dL to 8.8 g/dL). This side effect was noted during the routine tests performed during the second week of the treatment.

Seven of the ten patients diagnosed with chronic ITP underwent bone marrow biopsy. Their pathology reports revealed that six patients had increased megakaryocyte count, while one had decreased

Table 1. Patients' data and response to dapsone therapy.

Patient number	1	2	3	4	5	6	7	8	9	10
Age/gender	39/M	55/F	24/F	30/F	50/M	31/F	64/F	60/F	57/F	48/F
Disease duration before dapsone (months)	12	360	168	180	NA	6	60	8	24	84
Previous treatments										
Steroid	+	+	+	+	+	+	+	+	+	+
Immunoglobulin	+	+	+	+	-	+	-	+	+	+
Splenectomy	+	+	+	+	+	+	+	+	-	+
Danazol	+	-	+	-	+	+	+	+	-	-
Azathioprine	+	-	-	+	+	-	-	-	-	-
Cyclosporine	-	-	-	-	-	-	-	-	-	-
Cyclophosphamide	-	-	-	-	-	-	-	-	-	-
Vincristine	-	-	-	-	-	-	-	-	-	-
Rituximab	+	-	+	+	+	-	-	-	-	+
TPO-RA	+	+	+	+	-	-	-	-	+	+
Pre-dapsone platelet count (x10^9/L)	10.3	5.15	13.5	13	NA	22.1	15.4	4	17	15.4
Duration of treatment (months)	6	3	17	1.5	NA	6	NA	25	3	24
Response[a]	Yes	No	Yes	No	No	Yes	NA	Yes	Yes	Yes
Complete response[b]	Yes	No	Yes	No	No	Yes	NA	No	No	Yes

a platelet count ≥30x10^9/L as response; b platelet count ≥100x10^9/L as complete response.
TPO-RA: thrombopoietin receptor agonist; NA: not available.

Table 2. Characteristics of ten patients on dapsone.

Median age (years)	50 (24-64)
Gender (M/F)	2/8
Hemoglobin (g/dL)	12.6 (9.1-16.4)
Platelet count (10^9/L)	12.8 (4-22.1)
Median duration of symptoms before dapsone (months)	60 (6-360)
Median follow-up (months)	6 (2-29)

Table 3. Results of dapsone therapy.

Complete response n (%)	4 (40%)
Response n (%)	6 (60%)
Non-response n (%)	3 (30%)
Not evaluated n (%)	1 (10%)
Relapse n (%)	5 (83.3%)
Time to response (days)	39 (14-90)
Treatment duration (months)	6 (3-25)
Response time after discontinuation of dapsone therapy (months)	3.5 (1-12)
Number of patients with sustained response at 6 months after discontinuation of dapsone	1 (10%)

megakaryocyte count. The median time to respond to dapsone therapy was 39 (range: 14-90) days, and the median duration of treatment was 6 (range: 3-25) months. While six patients responded to dapsone therapy, 4 had a complete response (>10 x109/L). Four of the six patients who had a response developed a relapse in the first six months after discontinuing dapsone therapy, and one patient developed a relapse at 12 months. One patient is currently on dapsone therapy (>25 months). The median duration of response was 3.5 (range: 1-12) months after discontinuing dapsone therapy. Only one patient had a remission period of more than six months after treatment discontinuation (Table 3).

Patient number one was reinitiated on dapsone therapy after relapse. A response was regained with this treatment. The patient, who received dapsone therapy for seven months as the initial treatment and remained in remission for 12 months after treatment discontinuation, was given treatment for the second time for 12 months. While the response was maintained during the follow-up of the patient whose treatment was discontinued, the patient developed a soft tissue infection in the surgical site associated with aseptic necrosis of the femoral head and died after two days of follow-up at intensive care due to septic shock secondary to this infection. The patient's dapsone therapy dose, duration, and response are shown in Figure 2.

DISCUSSION

The response to dapsone therapy is usually slow. Administering treatment for at least three months is recommended to see the treatment response. Two patients (20%) responded on day 14 and the others on days 39, 45, 70, and 90, respectively. The median response time was 39 (14-90) days. The study by

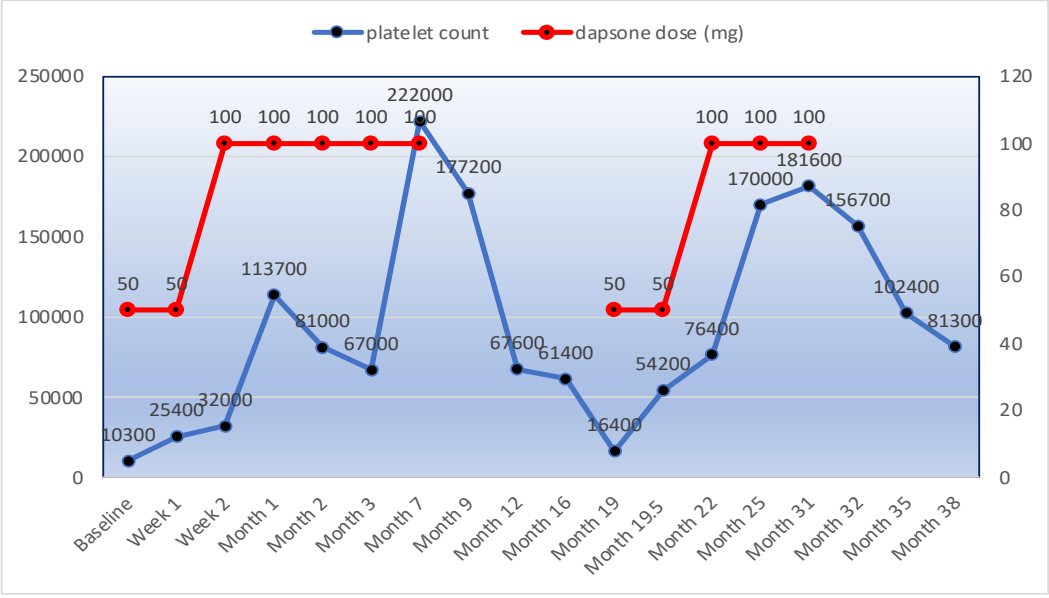

Figure 1. Relationship of a platelet count of patient number one with dapsone dose and duration.

Table 4. Summary of studies on dapsone therapy in patients with ITP.[15]

Reference number	Durand et al.[15]	Godeau et al.[5]	Hernandez et al.[17]	Godeau et al.[6]	Damodar et al.[13]	Vancine et al.[18]	Zaja et al.[11]	Present
Country	France	France	Spain	France	India	Brazil	Italy	Turkey
Number of patients	5	21	15	66	90	52[a)]	20[b)]	10
Median age (years)	76 (68-87)	37 (22-79)	58 (16-84)	43 (26-68)	21 (3-61)	38 (13-78)	51 (27-74)	50 (24-64)
Disease duration before dapsone (months)	34 (6-60)	14 (2-240)	29 (12-131)	52 (3-240)	CR: 21 (6-120) PR: 31 (6-84) NR: 25 (6-120)	R: 6 (1-21) NR: 5 (1-30)	46 (3-274)	60 (6-360)
The median number of treatments before dapsone	2 (0-4)	2 (1-9)	1 (1-2)	3 (0-10)	NA	NA	NA	4 (3-6)
Mean platelet count before dapsone ($x10^9$/L)	36 (23-43)	16 (2-49)	16 (7-48)	23 (2-49)	CR: 18 (1-49) PR: 13 (2-35) NR: 10 (2-46)	R: 26±14 NR: 18±11	19 (NA)	12.8 (4-22.1)
Daily dapsone dose	75 mg	100 mg	100 mg	100 mg	1-2 mg/kg	100 mg	100 mg	100 mg
Median duration of treatment, months	14 (2-48)	3 (1-11)	6 (1-31)	R: 13 (1-48) NR 3 (1-9)	CR: 13 (3-18) PR: 9 (3-14) NR: 6 (3-8)	R: 39 (1-91) NR: 3 (1-29)	9 (4-56)	6 (2-29)
Response rate[c] (%)	100	29	40	50	63	44	55	60[d)]
Combination therapy	No	Yes	No	Yes	No	No	No	No

[a] Forty patients with a diagnosis of primary ITP.
[b] Sixteen patients with a diagnosis of primary ITP.
[c] A platelet count ≥50,000/μL was considered a response to dapsone.
[d] A platelet count ≥30,000/μL was considered a response to dapsone in our study.[6]
CR: complete response (>$10x10^9$/L), ITP: immune thrombocytopenia, NR: non-responder (<$5x10^9$/L), PR: partial response (5-$10x10^9$/L), R: responder (>$5x10^9$/L), NA: not available.

Zaja *et al*.[11] reported a mean time to response of more than one month. While the response to danazol and azathioprine, the other second-line treatment options, is slow, the response to cyclophosphamide, cyclosporine, vincristine, splenectomy, rituximab, and thrombopoietin receptor agonist (TPO RA) eltrombopag is faster.[9]

Anaemia may develop secondary to bleeding in most patients with ITP. Since dapsone will induce hemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PDH) deficiency, the level of the G6PDH enzyme in male patients using this drug is a parameter that should be checked before treatment. In our study, two male patients received dapsone. Although the G6PDH test was not performed before treatment, low haemoglobin levels were not observed in these patients. Dapsone may rarely reduce haemoglobin oxidation by inhibiting the haemoglobin reductase enzyme in erythrocytes. Side effects such as toxic hepatitis, anaemia, and skin lesions may be seen in patients using dapsone.[10] The acceptable safety profile of dapsone has been previously reported in several articles.[9,11-13] Several studies have reported that dapsone is an effective drug regardless of previously used treatments. The study by Zaja *et al*.[11] in 20 patients reported a response rate of 55% and a complete response rate of 20%, Damodar *et al*.[13] found a response rate of 61.8% and a complete response rate of 48% in 55 patients, and Colella *et al*.[14] reported a response rate of 66% and a complete response rate of 24% in 122 patients. The results of our study revealed a response rate of 60% and a complete response rate of 40% in 10 patients.

A few studies are performed to determine the duration of response after discontinuation of dapsone therapy. Godeau *et al*.[6] reported that 1 out of 13 patients who responded to dapsone therapy had a sustained response, and Patel *et al*.[10] said that 2 out of 18 patients with treatment response had a sustained response. Zaja *et al*.[11] reported that 1 out of 20 patients who responded to treatment had a sustained response for more than six months after discontinuing the drug. In our study, 1 of the six patients with a response had a more than six months response duration. There is a need for studies with more significant numbers of patients to understand better the response time after discontinuing dapsone therapy. The study published by Lee *et al*.[15] summarised the results of different numbers of patients from other countries diagnosed with chronic ITP and treated with dapsone. All

analyses were conducted with a few patients (Table 4). Table 4 shows that the dapsone dose was generally 100 mg orally in those studies, as in our study. A notable detail in this table is that Godeau *et al*.[6] used dapsone and danazol combination therapy in some non-responders. In the study of Rattanathammethee *et al*.[17], dapsone was administered with colchicine therapy to patients with chronic/refractory ITP. In the present study, we did not administer a combination therapy to any patient. Moreover, as is seen in this table, a platelet count ≥50,000/μL was considered a response to dapsone therapy. We, however, considered a platelet count ≥30,000/μL as a response to dapsone.

CONCLUSIONS

In conclusion, using dapsone as second-line therapy is appealing for several reasons. Among these are its cost-effectiveness and comparable efficacy with other drugs used as second-line treatment options, such as azathioprine, cyclosporine, vinca alkaloids, mycophenolate mofetil, cyclophosphamide, danazol, rituximab, and eltrombopag. Dapsone has a good safety profile and rarely requires discontinuation due to side effects. Discontinuation of dapsone therapy, as with the TPO agonist eltrombopag, leads to relapse in many patients. Although the TPO agonist has a high response rate, its higher cost is a disadvantage. In contrast, the cost of treatment with dapsone is considerably low. Considering the economic status of developing countries, it seems very attractive to be used as second-line therapy for ITP. As an oral agent, dapsone provides an option in cases of ITP with no response after steroid and splenectomy. Considering its tolerability, it should be considered for refractory ITP patients.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical Approval

Ethics approval was obtained from the non–invasive clinical research ethics committee of the medical faculty (date: 08.12.2021, number: 2021-18/28). All aspects of the study, including periodical clinical and laboratorial checkups, were performed according to the principles of the declaration of Helsinki (64th, 2013).

Authors' Contribution

Study Conception: ÖC, VÖ, TGK; Study Design: ÖC, VÖ, FÖ, TE; Supervision: ÖC, TZT, FÖ; Literature Review: ÖC, VÖ, FÖ, TE; Critical Review: ÖC, FÖ, VÖ; Data Collection and/or Processing: ÖC, FÖ, VÖ, VA; Analysis and/or Data Interpretation: ÖC, VÖ, FÖ, TE; Manuscript preparing: ÖC, VÖ, FÖ.

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Original Article

What is the main reason of erectile dysfunction in lymphoma patients: Chemotherapy or Depression?

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ABSTRACT

Background Erectile dysfunction (ED) may be associated with chemotherapy and depression in lymphoma patients. The role of depression in developing ED in lymphoma patients may be more critical than chemotherapy. This study aimed to determine which plays a more important role in ED.

Material and Methods This study included 20 patients aged under 60 years who were admitted to the Hematology Outpatient Clinic between March 2015 and March 2016 and diagnosed with lymphoma. While the Beck Depression Inventory (BDI) was used to assess depression severity before (T1), during (T2) and after (T3) chemotherapy, the International Index of Erectile Function (IIEF) was used to assess sexual function. The Mann-Whitney U and Wilcoxon signed-rank tests were used for statistical analysis. A p-value of <0.05 was considered statistically significant.

Results Twenty male lymphoma patients (14 [70%] patients with non-Hodgkin lymphoma and 6 [30%] patients with Hodgkin lymphoma) were included in the study. The mean BDI score was 11.75±1.44 at T1, 6.60±3.61 at T2, and 3.25±2.12 at T3, respectively ($p<0.01$). The mean IIEF score was 15.25±6.12 at T1, 12.95±6.03 at T2, and 20.40±8.59 at T3, respectively ($p<0.01$). There was a significant decrease in the mean BDI and IIEF scores between T1 and T2. However, the mean BDI score decreased between T2 and T3, while the mean IIEF score tended to increase.

Conclusions It is impossible to suggest a single cause when considering the multifactorial aetiology of ED in lymphoma patients. However, our study showed that depression and related psychological factors are the leading cause of ED in lymphoma patients.

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INTRODUCTION

Erectile dysfunction (ED) is the persistent and/or recurrent inability to achieve and/or maintain an erection sufficient for satisfactory sexual activity.[1] Akkus *et al*.[2] reported that the prevalence of ED was 69.2% in 1,982 male individuals from Turkey (mild 33.2%, moderate 27.5%, severe 8.5%). They found that its prevalence was 7.6% in men aged 40-49 years, 33.3% in men aged 50-59 years, 70.2% in men aged 60-69 years, and 90.1% in men aged 70 years and older.[2]

ED is divided into organic and psychogenic impotence and usually has a multifactorial aetiology. While the prevalence of psychogenic ED is approximately 10% in men over 50 years, 45% of all patients with ED have psychogenic problems.[3] Psychogenic causes may include emotional problems (such as depression, anxiety, previous sexual traumatic experiences, low self-esteem, doubts in the sexual role), physical disabilities or loss of physical attraction to one's sexual partner, as well as socioeconomic factors (such as familial discordance or cultural differences, sexual myths or work-related stress).[3,4] The lifetime prevalence of major depression in the general population has been reported as 4.8-17.1%.[5] It is difficult to identify and distinguish the relationship between ED and depression. It is not clear whether depression leads to ED or vice versa. A Finnish study involving 1683 patients aged 50-70 years who were treated for depression and not treated found a strong relationship between depression and ED.[6]

Combining adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) is the most commonly used chemotherapy regime for Hodgkin lymphoma (HL). Neutropenia is the most common complication after the ABVD chemotherapy regimen.[7] Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) or rituximab plus cyclophosphamide, vincristine, and prednisone (R-CVP) chemotherapy regimens are first-line treatment for non-Hodgkin lymphoma (NHL). Complications of peripheral neuropathy/paresthesia occur in patients treated with vincristine.[8]

Drug-induced ED is very common. Several studies have reported its average incidence as 25%.[9] Chemotherapeutic drugs may cause a loss of sexual desire and a decline in the frequency of sexual intercourse.[10] Systemic side effects (such as fatigue, nausea, vomiting, taste and smell changes, diarrhoea, constipation, weight changes, insomnia, fear, anxiety, and stomatitis) after chemotherapy can lead to an individual to feel like asexual. Erectile dysfunction occurs predominantly in patients receiving chemotherapeutic agents (especially cyclophosphamide, chlorambucil, bleomycin and cisplatin).[11] This study aimed to determine which factor plays a more important role in ED in young lymphoma patients under 60 years.

MATERIAL AND METHODS

Study design and population

This study included 20 patients aged 25-60 admitted to the Haematology Outpatient Clinic of Dr Lütfi Kırdar Kartal Training and Research Hospital between March 2015 and March 2016 and diagnosed with lymphoma. ED was evaluated cross-sectionally. All patients enrolled in the study were married or had a regular sexual life, were at least primary school graduates, and could read and understand the survey questions. In addition, patients who had previously been diagnosed with ED had diseases leading to ED and had depression previously were excluded from the study. While the Beck Depression Inventory (BDI) was used to assess depression severity before (T1), during (T2) and after (T3) chemotherapy, the International Index of Erectile Function (IIEF) was used to assess sexual function. ED was evaluated by IIEF scores: 0-10 (severe), 11-16 (moderate), 17-21 (mild-to-moderate), 22-25 (mild) and 26-30 (none). Patients whose lymphomas did not improve or progress at T2 were excluded from the study. A physician recorded the answers during face-to-face interviews. A haematologist performed the medical follow-up of patients, and their information was recorded in the study file.

Statistical analysis

Statistical analysis was performed with the Number Cruncher Statistical System (NCSS) 2007 statistical software program (NCSS, LLC, Kaysville, Utah, USA). Descriptive statistics (mean, standard deviation, median, frequency, rate) were used to summarize the data. When analyzing non-normally distributed variables, the Mann-Whitney U test was used for intergroup comparisons, whereas the Wilcoxon signed-rank test was used for intragroup comparisons. A p - value < 0.05 was considered statistically significant.

RESULTS

Twenty male lymphoma patients (14 [70%] patients with NHL and 6 [30%] patients with HL) were included in the study. The mean age was 46.90 ± 10.56 years. The number of patients aged under 50

Table 1. Sociodemographic and clinical data of patients.

	Mean ± SD/median (min:max)
Mean age (years)	46.90 ± 10.56/50.5 (26-59)
Disease duration (years)	1.60 ± 3.10/0 (0-10)
Smoking duration (years)	26.00 ± 16.43/25 (0-60)
Age groups (< 50/> 50 years) n (%)	10 (50)/10 (50)
Marital status (married) n (%)	20 (100)
Smoking (no/yes) n (%)	3 (15)/17 (85)
Alcohol consumption n (%)	20 (100)
Occupation n (%)	
Officer	3 (15)
Worker	12 (60)
Self-employed	5 (25)
Education n (%)	
Primary school	10 (50)
Secondary school	3 (15)
High school	6 (30)
University	1 (5)
Additional disease n (%)	6 (30)
Drug intake n (%)	6 (30)
Non-Hodgkin/Hodgkin lymphoma n (%)	14 (70)/6 (30)
Stage 1-2/Stage 3-4 n (%)	9 (45)/11 (55)

years was 10 (50%). NHL groups included eight diffuse large B-cell lymphomas, two T-cell-rich B-cell lymphomas, one mantle-cell lymphoma, one follicular lymphoma, one primary mediastinal large B-cell lymphoma, and one marginal zone lymphoma, respectively. ABVD chemotherapy regimens had been received with HL patients. R-CHOP, R-CVP, dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab) chemotherapy regimens had received with NHL patients. The median number of chemotherapy cycles was 6 (range: 4-6) for HL groups and 6 (range: 2-8) for NHL groups. While 10 (50%) patients graduated from primary school, only 1 (5%) graduated from university. All the patients were married-smoking at diagnosis (85%). Abdominal bulky lesions and testicular mass were not observed in the pre-treatment evaluation. Grade 3 neutropenia was seen in 1 patient with HL groups. Vincristine was discontinued in 1 patient with peripheral neuropathy in NHL groups. Of them, 9

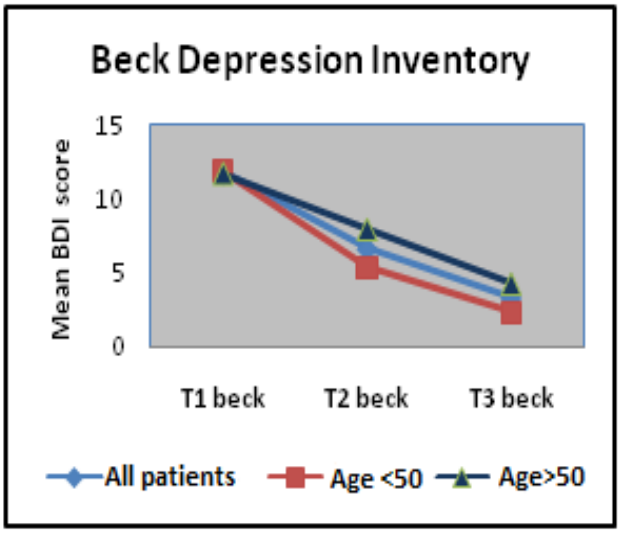

Figure 1. Evaluation of BDI scores according to follow-ups.

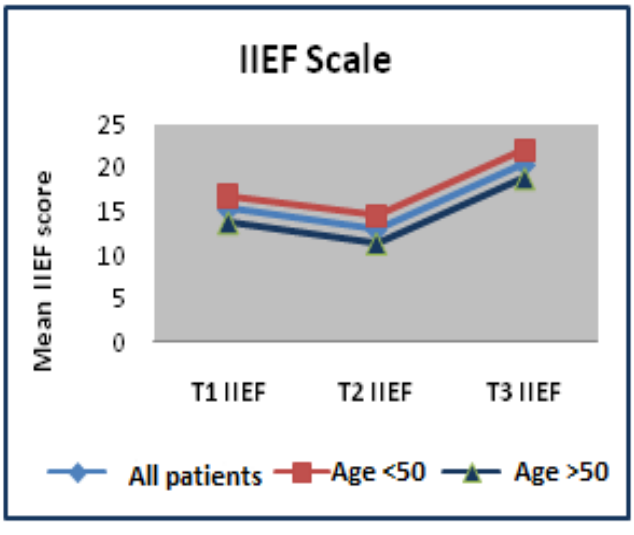

Figure 2. Evaluation of IIEF scores according to follow-ups.

(45%) had early-stage cancer (Stage 1-2), and 11 (55%) had advanced-stage cancer (Stage 3-4) (Table 1).

Comorbidities were three patients with essential hypertension, two patients with diabetes mellitus, and one patient with asthma. Only one of the patients with hypertension used a selective beta-1-blocker (atenolol). Patients with diabetes mellitus were used metformin. Age, diabetes mellitus, hypertension, smoking history, and disease stage distribution were similar in both groups.

The mean BDI score was 11.75 ± 1.44 at T1, 6.60 ± 3.61 at T2, and 3.25 ± 2.12 at T3, respectively ($p < 0.01$). The mean BDI score significantly decreased from T1 to T2 ($p = 0.001$; $p < 0.01$) and from T2 to T3 ($p = 0.001$; $p < 0.01$) (Figure 1). The mean IIEF score was 15.25 ± 6.12 at T1, 12.95 ± 6.03 at T2, and 20.40 ± 8.59 at T3, respectively ($p < 0.01$). The mean IIEF score significantly decreased from T1 to T2 ($p = 0.009$; $p < 0.01$). However, the mean IIEF score significantly increased from T1 to T3 ($p = 0.002$; $p < 0.01$) and from T2 to T3 ($p = 0.001$; $p < 0.01$) (Figure 2).

IIEF measurements at T1 ($p = 0.191$), T2 ($p = 0.256$) and T3 ($p = 0.161$) did not show statistically significant differences according to age groups. In cases under 50, the mean decrease of 2.20 ± 3.26 in IIEF measurements from T1 to T2 was statistically significant ($p = 0.042$; $p < 0.05$). The mean increase of 5.20 ± 4.02 in IIEF measurements from T1 to T3 was statistically significant ($p = 0.009$; $p < 0.01$). The mean increase of 7.40 ± 5.46 in IIEF measurements from T2 to T3 was found to be statistically significant ($p = 0.007$; $p < 0.01$) (Table 2). In cases over 50 years of age, the mean decrease of 2.40 ± 4.09 in IIEF measurements from T1 to T2 was not found to be statistically significant ($p = 0.068$; $p > 0.05$). The mean increase of 5.10 ± 6.77 in IIEF measurements from T1 to T3 was statistically significant at borderline ($p = 0.050$; $p < 0.05$). The mean increase of 7.50 ± 8.24 in IIEF measurements from T2 to T3 was found to be statistically significant ($p = 0.022$; $p < 0.05$) (Table 2).

DISCUSSION

Although ED is not life-threatening, it negatively affects the quality of life. The survival of HL and NHL is significantly prolonged by current treatments.[12,13] Mental disorders such as anxiety and depression caused by lymphoma and chemotherapy are frequently seen in lymphoma patients, similar to all cancer patients.[14] Kornblith *et al*.[15] reported that male patients with HL had more frequent and longer-lasting sexual problems than leukaemia patients of similar age. However, it should be noted that approximately half of the patients in this study received radiotherapy and that radiotherapy was one of the reasons. In our study, patients were newly diagnosed and did not receive radiotherapy since it is less frequently indicated in current treatment guidelines.

Predictably, lymphoma patients have increased rates of psychological problems (especially depression).[15,16] It is usual to experience anxiety in this period when negative emotions such as stressful thoughts of everyday life and fear of death are predominant. Our study found that patients had higher depression scores at the first assessment. There was a significant decrease in depression scores at the mid-term evaluation (usually after 3 or 4 cycles of chemotherapy) in patients receiving treatment. This can be explained by the fact that 17 of 20 patients in our study were informed that lymphoma responds well to treatment and is likely to improve. It was observed that depression scores continued to decrease during follow-up and reached their lowest value at the end of the treat-

Table 2. Evaluation of IIEF measurements according to age groups.

	Age <50 (n: 10) Mean ± SD (median)		Age >50 (n: 10) Mean ± SD (median)	*P* value
T1 IIEF	16.80 ± 6.94 (19.5)		13.70 ± 5.05 (15.5)	0.197[a]
T2 IIEF	14.60 ± 6.20 (15.0)		11.30 ± 5.68 (11.0)	0.256[a]
T3 IIEF	22.00 ± 9.26 (26.0)		18.80 ± 8.02 (21.0)	0.161[a]
Pairwise comparisons	Difference mean ± SD	P value	Difference mean ± SD	
T1-T2	2.20 ± 3.26	0.042*[b]	2.40 ± 4.09	0.068[b]
T1-T3	-5.20 ± 4.02	0.009**[b]	-5.10 ± 6.77	0.050*[b]
T2-T3	-7.40 ± 5.46	0.007**[b]	-7.50 ± 8.24	0.022*[b]

IIEF T1: before chemotherapy, T2: during chemotherapy, T3: after chemotherapy.
[a]Mann-Whitney U test, [b] Wilcoxon signed-rank test (adjustment for Bonferroni).

ment. The fact that psychological fear and anxiety experienced by patients at the time of initial diagnosis significantly decreased and partially disappeared is mainly responsible for this. Previous studies demonstrated that psychological problems improved during long-term follow-up after treatment and that chronic fatigue and aesthetic concerns/social issues related to physical changes continued.[17,18] No such evaluation was performed in our study. Long-term follow-up was not planned because our study was a cross-sectional study.

Alkylating drugs are known to cause germ cell damage with the dose increase. Cyclophosphamide (total dose > 6-10 g), chlorambucil, procarbazine, busulfan, nitrogen mustard, and nitrosoureas have been shown to cause azoospermia. Testicular atrophy occurs in 80% of HL patients receiving the MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone) treatment regimen. Reversible azoospermia was reported in 35% of HL patients receiving the ABVD treatment regimen.[19]

Considering the pathogenesis and aetiology of ED, the frequency of ED is expected to increase over time because the negative effects of the disease and treatment would occur over time. The leading causes are the accumulation of chemotherapeutic drugs (such as anthracycline and bleomycin) in the body over time, increased side effects, and treatment-related fatigue and strength loss. In our study, ED scores decreased at the mid-term assessment (indicating that the frequency and severity of ED were increased). This increase was in contrast with the improvement in depression. However, depression scores continued to decrease after the mid-term assessment, while ED scores tended to increase (indicating that the frequency and severity of ED decreased). This decrease was found to be higher than expected. Our results showed that ED seen during treatment often have a psychological origin and are not organic. Long-term follow-up studies revealed that sexual disorders (particularly chronic fatigue) increased over time.[16-18]

Depression decreases significantly during treatment in lymphoma patients, whereas ED shows a slight increase in the early stage of treatment. However, depression decreased after the mid-term assessment while ED improved. The most important reason for this is that patients were informed about the improvement in lymphoma at the mid-term evaluation, and the psychological effects of this situation appeared immediately. It is known that there are organic and psychogenic causes in the aetiology of ED and that psychogenic ED may occur acutely and improve in a short time.[20]

The frequency of ED increases with age. This is explained by decreased testosterone levels, increased frequency of chronic diseases, and atherosclerosis.[21] Because our patients were generally concentrated within a certain age range, a statistical analysis was performed as under and over 50. As a result, it was observed that the age range did not reveal a significant difference for both depression and ED.

CONCLUSIONS

Maintaining the quality of life during treatment in male lymphoma patients is very important. Sexual life is considerable, especially in young patients and is one of the main factors determining the quality of life. Therefore, it is necessary to provide support and treatment for these patients to have a healthy sex life during the treatment period. Erectile function is essential for a healthy sexual life. The correct identification of ED causes is the basis of the treatment in these patients. It is impossible to suggest a single cause when considering the multifactorial aetiology of ED in lymphoma patients. However, our study showed that depression and related psychological factors are the leading cause of ED in lymphoma patients. Organic causes are often put forward in the long-term follow. These patients should be closely monitored psychologically during treatment and evaluated in detail for anxiety and depression. Starting pharmacological or psychological treatment based on this evaluation and maintaining the chemotherapy process with this support would significantly improve quality of life.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical Approval

This study was approved by Dr Lütfi Kırdar Kartal Training and Research Hospital ethics committee (Decision number: 514/87/11 dated 29.06.2019). All male patients who participated in the study signed a

consent form.

Authors' Contribution

Study Conception: GY, CY,; Study Design: GY, CY,; Literature Review: CY,; Critical Review: GY, CY,; Data Collection and/or Processing: CY, AE,; Analysis and/or Data Interpretation: CY, AE,; Manuscript preparing: CY.

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Original Article

Evaluation of Hearing and Auditory Pathways in Fabry Disease Patients

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ABSTRACT

Background Hearing and the auditory pathway are affected in Fabry diseases (FD). There is limited data on hearing and auditory pathways in this population. Therefore, we aimed to investigate auditory functions and auditory pathways using auditory brainstem responses (ABR), otoacoustic distortion emission (DPOAE), pure tone audiometry (PTA), and tympanometry in patients with FD and to compare these results with those of healthy individuals.

Material and Methods This study included 16 patients with FD (F/M: 8/8, age: 33.5 ± 15.4 years) and 16 healthy controls (F/M: 5/11, age: 33.6 ± 6.3 years). Hearing functions and auditory pathways were assessed with ABR, DPOAE, PTA, and tympanometry.

Results According to the results of PTA, conductive hearing loss was detected in 4 (25%) of the patients with FD. When the 500-4,000 Hz frequencies were assessed, the bone pathway hearing threshold in both ears was significantly higher in the patients with FD than in the control group ($p = 0.014$ and$p = 0.014$, respectively). When we compared the DPOAE measurements of the patients with FD and the control groups, the dB value measured at 2.8 kHz was significantly lower in the patient group than in the control group ($p = 0.018$). When we compared the ABR measurements, the right ear's 3-5 interpeak latency at 60 dB was significantly lower in the patient with FD than in the control group (1.8 ± 0.3 ms vs 2 ± 0.2 ms, $p = 0.033$).

Conclusions We found that the hearing loss rate and hearing threshold were statistically significantly higher in FD patients than in the control group. Hearing screening should be systematically performed in these patients.

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INTRODUCTION

The most common diseases among lysosomal storage diseases are Gaucher and Fabry diseases. The disease results from the deposition of globotriaosylceramide (Gb3) in various tissues due to insufficiency of the enzyme α-galactosidase A. Numerous signs of the disease may occur due to the deposition of Gb-3 in autonomic and spinal ganglia, renal tubulointerstitial cells and, renal glomerular, vascular smooth muscle cells, cardiac myocytes, vascular and lymphatic endothelial cells in the cornea.[1] Early clinical manifestations of the disease include hypohidrosis, gastrointestinal symptoms (GIS), angiokeratomas, distal joint pain, and tinnitus. The most important predictor of the likelihood of developing Fabry-related complications is enzyme activity.[2] However, even if distinctive signs and symptoms occur, there is a significant delay in diagnosis up to 20 years from the onset of symptoms. This is probably due to a lack of awareness and the wide range of clinical manifestations, especially in women.[3,4] Therefore, recognizing the signs and symptoms of Fabry disease (FD) is closely associated with disease awareness among paediatricians, pediatric metabolic specialists, pediatric geneticists, cardiologists, neurologists, dermatologists, nephrologists, trained pathologists, and ophthalmologists. In particular, after diagnosis, it is possible to change the disease's natural course and progression and improve patients' quality of life through treatment.[5,6] Nowadays, there is no curative treatment for FD. Recombinant α-Gal A and migalastat are treatment options for suitable patients.[7]

Patients with FD experience progressive hearing problems. These hearing problems, especially hearing loss, may develop gradually or occur suddenly. Symptomatic hearing loss happens in %18-55 of patients with FD, but sudden hearing loss occurs in %6-36 and tinnitus in %17-53 of patients. Typically, it is observed more frequently and to a greater extent in male FD patients. Recent studies have shown that the developing hearing loss in these patients is predominantly sensorineural.[8] However, this population has limited data on hearing and auditory pathways. Therefore, we aimed to investigate auditory functions and auditory pathways using auditory brainstem responses (ABR), pure tone audiometry (PTA), otoacoustic distortion emission (DPOAE), and tympanometry in patients with FD and to compare these results with those of healthy individuals.

MATERIAL AND METHODS

The protocol of the study was approved by the Medical Ethics Committee of Necmettin Erbakan University (NEU) (Faculty of Medicine, Konya, Turkey). The patients and healthy volunteers signed the written informed consent. This study included 16 patients with FD (F/M: 8/8, age: 33.5 ± 15.4 years) and 16 healthy controls (F/M: 5/11, age: 33.6 ± 6.3 years). In addition, we categorised patients according to whether they received treatment. Patient's medical records (information on patients' age, medications taken, duration and course of disease, and otologic history) were reviewed. Inclusion criteria were 1) 18-70 years of age, 2) decreased (< 2.5 nmol/mL/hour) α-gal-A activity in dried blood spots (DBS) in male patients, and 3) presence of GLA gene mutation associated with FD in female patients.

The screening of FD was performed by assessing α-gal-A activity < 2.5 nmol/mL/hour in DBS and was confirmed by GLA gene mutation analysis. The criteria for the diagnosis of FD were α-gal-A activity < 2.5 nmol/mL/hour in male patients and a genetic mutation associated with FD in female patients. The screening of FD was performed by assessing α-Gal A activity in dried blood spots (DBS) and was confirmed by GLA gene mutation analysis. GLA gene was sequenced using the MiSeq next-generation sequencing (NGS) platform, an FDA-approved diagnostic system (Illumina, San Diego, CA, USA). Plasma lyso-Gb3 levels were measured via tandem mass spectrometry method from DBS before ERT at the beginning and end of the study. Diagnostic procedures have been rearranged by adding them to the material method section.

A complete oto-rhino-larygologic examination was performed on all participants. Otologic history was obtained from all patients, including inherited deafness, otologic symptoms, otologic trauma or surgery, use of ototoxic agents, and noise exposure. All patients underwent otoscopy. None of the patients was found to have comorbidities related to otolaryngology in their history or on examination. Our patients had no middle ear problem, and no issues were found in the external auditory meatus and inner ears in the otolaryngologic test. In addition, otolaryngologic anamnesis and history showed no abnormality in their ears. Hearing functions and auditory pathways were assessed with ABR (Eclipse Interacoustics), DPOAE

(Otodynamics Echoport ILO 288 USB), PTA (Interacoustics AC33 Audiometer), and tympanometry (Interacoustics AT235). Tympanograms of all patients were also found to be Type A-Normal.

Air conduction and bone conduction hearing thresholds were calculated for each ear using a 5-dB stepwise method at 250, 500, 1000, 2000, 4000, and 8000 kHz. The severity of hearing loss was determined as PTA for 0.5, 1, 2, and 4 kHz. In addition, a high-frequency hearing loss (hFhl) was considered at 4 and 8 kHz to assess the inner ear's involvement better.

ABR measurements were performed on both ears separately with single-channel electrode placement. While subjects were in the supine position, measurements were made in the alternate mode, 1200 sweep, 11.7 rates, and click stimulus. Stimulus intensities used were 60 dBnHL, 40 dBnHL, 20 dBnHL, and 10 dBnHL. The amplitudes of the I./III./V. waves, the latencies of the I./III./V. waves and the latencies of the I- III/I-V/III-V interpeak were recorded.

During DPOAE measurements, the frequency density within the stimulus was determined as L1 (65 dB-SPL) for frequency f1 and L2 (55 dB-SPL) for frequency f2. The DPOAE results were recorded twice for each frequency, 1000, 1500, 2000, 3000, 4000, 6000, and 8000 Hz. In the DPOAE results, the "signal-to-noise ratio" (SNR) values determined for each frequency are read from the table generated by the test system.

Venous blood samples were collected for biochemical analysis without ingestion of drugs and after at least 10 hours of fasting. Biochemical analyzes were performed at the Central Biochemical Laboratory of our hospital NEU Meram Faculty of Medicine. Analyzes were performed using the oxidase-based technique with a modular system from Roche and Hitachi (Mannheim, Germany).

Statistical Analysis

Windows version 12.0 (SPSS Inc. Chicago/Illinois/USA) was used to analyse clinical and experimental data. Descriptive statistics were determined for each variable individually. Numeric or categorical variables were expressed as mean ± standard deviation and number per cent. Whether or not the data were normally distributed was examined using the Shapiro-Wilk test. Parametric statistics (t-test for independent samples) and nonparametric statistics (Mann-Whitney U test) were used for continuous variables. Fisher's Exact and Chi-square tests were used to compare categorical data between independent groups. A p - value of < 0.05 was considered statistically significant.

Table 1. Demographic, clinic and biochemical features of the patients with FD and healthy subjects.

Parameters	Patients with Fabry disease (n: 16)	Healthy subjects (n: 16)	*P* value
Gender (F/M)	8/8	5/11	0.280
Age (years)	33.5 ± 15.4	33.6 ± 6.3	0.976
eGFR (mL/min)	108.3 ± 49.9	103.9 ± 13.3	0.736
Glucose (mg/dL)	89.3 ± 6.3	91 ± 8.3	0.530
Urea (mg/dL)	29.6 ± 18.1	27.1 ± 7.3	0.509
Creatinine (mg/dL)	1.2 ± 1.9	0.8 ± 0.1	0.203
Sodium (mmol/L)	139.3 ± 1.8	139.1 ± 1.8	0.703
Potassium (mmol/L)	4.6 ± 0.4	4.3 ± 0.3	0.029
Total protein (g/L)	68.9 ± 3.8	72.4 ± 3	0.008
Albumin (g/L)	42.9 ± 4.1	46.7 ± 2.8	0.005
AST (U/L)	16.2 ± 3.5	20.9 ± 12.1	0.749
ALT (U/L)	13.8 ± 7.1	28.1 ± 20	0.001
CRP (mg/dL)	2.5 ± 3.9	3.4 ± 3.6	0.118
Protein/creatinine ratio in spot urine (mg/dL)	741.1 ± 1329	88.1 ± 38.6	< 0.001
White blood cell count (10^3/uL)	7.4 ± 1.3	8.3 ± 1.6	0.100
Neutrophil count (10^3/uL)	4.3 ± 1.1	4.4 ± 1.2	0.755
Lymphocyte count (10^3/uL)	2.4 ± 0.6	2.9 ± 0.6	0.033
Hemoglobin count (g/dL)	13.6 ± 1.3	14.4 ± 1.5	0.173
Platelet count (10^3/uL)	256.6 ± 58.3	270.7 ± 66.3	0.523

RESULTS

Table 1 shows the demographic data, clinical characteristics, and biochemical results of 16 patients with FD and 16 healthy control subjects. When the patients and healthy subjects were evaluated, it was found that there were no significant differences concerning the following variables: sex, age, serum levels of glucose, creatinine, sodium, CRP, haemoglobin, and platelet count. In addition, serum levels of total protein, albumin, alanine aminotransferase (ALT) and lymphocyte count were higher in the control group ($p = 0.008$, $p = 0.005$, $p = 0.001$ and p = 0.033, respectively). Serum potassium levels and protein/creatinine ratio were significantly higher in patients with FD than in control subjects ($p = 0.029$ and $p < 0.001$, respectively) (Table 1).

After the diagnosis of FD in 4 patients, 12 more affected family members were identified through family screening. The pedigree analysis provided an early diagnosis for two male relatives (nephews) of a male patient and four male relatives (2 sons and two nephews) of the other male patient. The diagnosis was made in 3 female relatives of a female patient (1 daughter, two nieces) and three relatives (2 daughters and a niece) of the other female patient. The mean duration of diagnosis was 67.69 ± 13.29 months, and the mean duration of treatment was 62.44 ± 15.21 months in patients with FD (Table 2). Nine patients were using agalsidase alfa, and seven were using agalsidase beta. In addition, the patients' enzyme levels and lyso Gb-3 levels were given in Table 2.

When the hearing functions of the participants were evaluated, it was found that the rate of hearing loss was higher in the patients with FD than in the healthy control group ($p = 0.033$). According to the results of PTA, conductive hearing loss was detected in 4 (25%) of the FD patients, while it was not detected in the control group. The mean value of hearing thresholds obtained with PTA in the patients with FD and the control groups was within the normal range in the bone and airway for both ears. However, when the frequencies of 500-4,000 Hz were assessed, the bone pathway hearing threshold in both ears was higher in the FD patients than in the healthy control group ($p = 0.014$ and $p = 0.014$, respectively) (Table 3).

When we compared the otoacoustic distortion product measurements of the FD patients and the control group, the dB value measured at 2.8 kHz was lower in the patient group ($p = 0.018$). When we compared the ABR measurements, the right ear's 3-5 interpeak latency at 60 dB was lower in the patients with FD (1.8 ± 0.3 vs 2 ± 0.2 ms, $p = 0.033$).

When patients were divided into groups after agalsidase treatment, there were no significant differences in sex, age, serum glucose, serum creatinine, sodium, potassium, C-reactive protein, lymphocyte count, and platelet count between the treated and untreated groups; only the spot urine protein/creatinine ratio was higher in the treated group ($p = 0.039$). Hearing thresholds determined with PTA in the treated and untreated patients were within the normal bone and airway range at all frequencies between 250-8,000 Hz in both ears. No significant difference

Table 2. Diagnosis, treatment dates and enzyme levels of patients with Fabry disease.

No	Gender	Age (years)	Dignosis date	FD duration (months)	Treatment date	Treatment duration (month)	Enzyme level	Lyso Gb-3	Treatment
1	Female	19	01.01.2017	72	01.01.2018	60	2.5	5.2	Agalsidase alfa
2	Male	48	01.01.2017	72	01.01.2018	60	0.2	4.6	Agalsidase alfa
3	Female	21	01.03.2017	69	01.06.2017	67	0.8	1.8	Agalsidase alfa
4	Female	33	01.06.2019	43	17.02.2020	35	2.1	4.2	Agalsidase beta
5	Male	37	11.11.2015	86	01.01.2016	84	0.9	39.3	Agalsidase beta
6	Male	39	23.11.2015	86	01.01.2016	84	1.4	27.5	Agalsidase beta
7	Male	47	08.06.2016	79	01.10.2016	75	0.2	1.7	Agalsidase beta
8	Female	36	11.01.2019	48	01.11.2019	38	2.0	7.9	Agalsidase beta
9	Male	25	18.10.2016	74	23.02.2017	71	1.2	60.8	Agalsidase alfa
10	Female	66	03.01.2017	71	23.02.2017	71	2.4	11.5	Agalsidase alfa
11	Male	38	01.02.2017	70	23.02.2017	71	1.2	23.3	Agalsidase beta
12	Female	43	11.04.2017	68	03.08.2017	65	2.3	14.7	Agalsidase alfa
13	Female	19	04.03.2019	45	01.09.2019	40	2.5	5.2	Agalsidase alfa
14	Male	48	01.03.2018	58	01.12.2018	49	0.2	4.6	Agalsidase alfa
15	Female	21	09.10.2017	63	01.03.2018	58	0.8	1.8	Agalsidase alfa
16	Female	33	19.06.2016	79	17.02.2017	71	2.1	4.2	Agalsidase beta

Table 3. Comparison of audiometry findings of the patients with FD and healthy subjects.

Parameters	Patients with Fabry disease (n: 16)	Healthy subjects (n: 16)	*P* value
Right ear 500-4000 Hz (air)	9.1 ± 4.8	6.6 ± 1.7	0.253
Right ear 4000-8000 Hz (air)	12.9 ± 8.7	8.4 ± 1.8	0.474
Right ear 500-4000 Hz (bone)	6.5 ± 3.8	3.8 ± 2.1	0.014
Left ear 500-4000 Hz (air)	8.4 ± 3.7	6.5 ± 1.7	0.200
Left ear 4000-8000 Hz (air)	12.3 ± 7.1	8.3 ± 1.7	0.361
Left ear 500-4000 Hz (bone)	6.3 ± 3.4	3.8 ± 2.1	0.014
Hearing loss n (%)	4 (25)	0	0.033

was found between the two groups.

When the measurements of the otoacoustic distortion product of the groups were evaluated, it was found that the dB value at the right ear SNR 1.4 and 2.8 kHz and at the left ear SNR 1.0, 1.4, 2.0, and 2.8 kHz was significantly higher in the untreated patient group ($p = 0.027$, $p = 0.050$; $p = 0.014$, $p = 0.022$, $p = 0.08$, and $p = 0.029$, respectively) (Table 4). When the ABR measurements were evaluated between the groups, it was found that there was no statistical difference.

DISCUSSION

As a result of the study, we came to three important conclusions. First, proteinuria was significantly increased in FD patients compared with the control group. Second, the proportion of patients with hearing loss was higher in FD patients. Third, the average hearing threshold of patients with FD was higher than that of the healthy group at the frequencies of 500-4,000 Hz in the bone tract for both ears.

FD is a genetic storage disease that plays a role in chronic kidney disease but can be treated. Renal findings occur in at least 20% of females and about 50% of male patients.[9] The primary renal finding is usually proteinuria.[10] In a study by Turkmen *et al*.[11], the incidence of proteinuria in 30 patients with FD was 23.3%. According to the literature, the protein-to-creatinine ratio in our study's puncture urine was higher in patients with FD than in the control group. When we divided the patients with and without treatment into groups, the ratio of protein to creatinine detected in the puncturing was higher in the treatment group. This result may be attributed to treated patients having more severe diseases and being more likely to develop organ damage.

The effects of FD on hearing have also been described in the last 15 years. Several studies have reported that progressive sensorineural and episodic hearing loss is more common than the average population, especially at high frequencies.[12,13] Sergi *et al*.14

Table 4. Autoacoustic emission findings of treated and nontreated FD patients with agalsidase.

Parameters	FD patients with treatment (n: 9)	FD patients without treatment (n: 7)	*P* value
Right ear SNR 1.0 kHz	4.6 ± 7.8	8.6 ± 8.3	0.339
Right ear SNR 1.4 kHz	5.9 ± 6.1	13 ± 5.2	0.027
Right ear SNR 2.0 kHz	2.4 ± 5.8	8.2 ± 6.1	0.074
Right ear SNR 2.8 kHz	-7.4 ± 13.2	2.0 ± 9.2	0.050
Right ear SNR 4.0 kHz	0.3 ± 8.2	0.7 ± 8.1	0.791
Right ear SNR 6.0 kHz	-6.0 ± 12.9	-2.7 ± 9.8	0.588
Right ear SNR 8.0 kHz	-15.1 ± 6.9	-13.7 ± 9.7	0.740
Left ear SNR 1.0 kHz	1.1 ± 9.2	12 ± 4.8	0.014
Left ear SNR 1.4 kHz	6.5 ± 4.5	12.3 ± 4.3	0.022
Left ear SNR 2.0 kHz	0.6 ± 6.4	10.2 ± 5.8	0.008
Left ear SNR 2.8 kHz	-3.4 ± 7.7	5.1 ± 5.8	0.029
Left ear SNR 4.0 kHz	1.1 ± 7.6	8.2 ± 5.8	0.060
Left ear SNR 6.0 kHz	-4.4 ± 11.5	2.9 ± 10.2	0.205
Left ear SNR 8.0 kHz	-15.7 ± 8.2	-23.3 ± 6.7	0.067

investigated the involvement of the inner ear in 20 patients with FD receiving enzyme replacement therapy (ERT). This study's patients were audiologically evaluated every six months using audiometry, OAE, and ABR methods. The mean follow-up time was 51.5 months (range: 25-73). Audiometry detected a hearing loss in 18 ears (45%) (13 patients) at pretreatment evaluation. These hearing losses were reported to be sensorineural, and the site of the lesion was the cochlea, as indicated by the OAE and ABR findings. When the planned follow-up times were reached in the study, the number of ears with hearing loss increased to 21 (52.5%), but it was found that the difference was not statistically significant. The authors suggested that inner ear involvement remains stable with ERT, so treatment should be started without waiting for hearing loss to develop. In our study, the rate of patients with hearing loss was higher than in control subjects. No difference in hearing loss was found when the treated and untreated patients were examined. But a conductive hearing loss in 4 patients could not be associated with any condition. However, it was thought that impedance changes due to FD-related deposit accumulations in the sound conduction path starting from the tympanic membrane and continuing with the malleus incus stapes and their ligaments and ending in the fenestra ovale might be a factor. However, because our study was cross-sectional, it may be misleading to make a statement about the effect of ERT on hearing loss.

In addition, hearing loss due to neurological involvement in patients with FD is another reason for blaming. In a study by Koeping *et al*.[15], %74 of FD patients were found to have sensorineural hearing loss by audiometry. In the ABR evaluation of these patients, the interpeak wave latencies I-III/III-V/I-V were within the normal range. In our study, the ABR test performed to evaluate the patients' auditory pathways found that the right ear of the patients with FD was significantly lower at 60 dB than the control group with 3-5 interpeak latencies. However, the results of ABR were found to be normal in all our patients.

In a study by Bitirgen *et al*.[16], corneal sensitivity, density, and nerve fibre length were lower in FD patients than in control subjects when evaluated by corneal confocal microscopy. Although FD-associated nerve damage was directly detected by microscopy in this study, the presence of auditory nerve damage in our study could not be seen by the ABR test. This could be because the head pairs were affected by FD at different rates or because a method that assessed electrical activity, such as the ABR test, was inadequate. Studies are needed to evaluate more patients and use other ways, such as autopsy studies.

Another question that needs to be addressed regarding hearing loss in patients with FD is whether it is possible to halt or even reverse hearing loss with treatment. To investigate this question, Palla *et al*.[17] audiometrically assessed the hearing of 47 patients before starting ERT and 60 months after treatment. The authors, who found no difference between the two evaluations, claimed that although the hearing loss was not reversed by ERT, at least hearing functions were stabilized. One of the major shortcomings of our study is that the periods before and after treatment were not evaluated to examine the effect of ERT on hearing loss.

CONCLUSIONS

As a result, we found that the hearing loss and hearing threshold rates were higher in FD patients than in the healthy group. Although the effects of pathogenesis and enzyme replacement therapy are not yet fully known, hearing screening should be systematically performed in these patients and included in this screening in asymptomatic patientsWe believe that possible hearing loss can cause a severe deterioration in the quality of life, even if it does not cause life-threatening situations, and that adverse effects on quality of life can be prevented by early diagnosis/treatment.

Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

The protocol of the study was approved by the Medical Ethics Committee of Necmettin Erbakan University (NEU) (Faculty of Medicine, Konya, Turkey). (Decision number: 2018/1179, date: 19.01.2018).

Authors' Contribution

Study Conception: FY, MAD, KT; Study Design:

FY, MAD, IB, KT; Supervision: FY, CKY, HO, YO, IB; Literature Review: FY, CKY, MAD, HO, YO; Critical Review: MAD, IB, KT; Data Collection and/or Processing: FY, CKY, MAD, HO, YO; Statistical Analysis and/or Data Interpretation: FY, IB, YO; Manuscript preparing: FY, CKY, MAD, KT. Oto-rhino-larygologic examination and Hearing functions and auditory pathways: MAD.

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Original Article

Mel-200 or Mel-140, Which One is More Advantageous? Retrospectively Analysis of The Multiple Myeloma Patients Treated with Autologous Hematopoetic Stem Cell Transplantation

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ABSTRACT

Background Autologous hematopoietic stem cell transplantation (AHSCT) is one of the standard treatment modalities for patients with multiple myeloma (MM) under 65 years of age. Renal failure, significant disease comorbidity, significantly affects treatment choices. There are conflicting data in the literature regarding the dose of melphalan to be used for AHSCT in patients with renal failure and comorbid conditions. This study aimed to compare the efficacy and side effect data of different melphalan doses in patients with renal failure.

Material and Methods The study included 107 patients older than 18 years of age with a diagnosis of MM who underwent AHSCT in our centre between January 2010 and January 2019. The data of the patients were analyzed retrospectively. Patients were grouped according to estimated glomerular filtration rate (eGFR: < 60 or ≥ 60 mL/min) and melphalan doses (140-200). In addition to renal failure, patients with low-performance scores (ECOG 3 and above) or severe systemic comorbid disease were included in the Mel-140 group.

Results Comparative analysis of MEL-140 and MEL-200 doses used for AHSCT showed no significant difference between the two groups regarding side effects, disease-free survival, and overall survival. Engraftment times were similar in both groups. When the patients were analyzed according to eGFR level, the incidence and severity of mucositis were higher in the group with low eGFR levels (p = 0.016). The duration of engraftment, complication with a febrile neutropenic attack, and development of septic shock were similar in both groups.

Conclusions In addition to renal failure, MEL-140 emerges as a preferable transplant preparation regimen considering its efficacy and side effect profile in patients with low-performance scores or severe systemic comorbidities.

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INTRODUCTION

Autologous hematopoietic stem cell transplantation (AHSCT) is the standard treatment approach in patients diagnosed with multiple myeloma (MM) aged < 65 years.[1,2] Although different criteria for transplant candidates are used in different centres, AHSCT following induction therapy in young patients with good performance status is the standard treatment. Although the term "young patient" is used for patients who are not older than 65 years of age, patients who are older than 65 years of age without comorbidities and with good performance status can also be considered transplant candidates. Renal function is one of the critical factors, along with age, for AHSCT candidacy in MM patients.1 Renal failure is observed in 20-30% of newly diagnosed MM patients, and hemodialysis may be required in 10% of the patient group.[3] Although a high treatment response rate is obtained with AHSCT with high dose melphalan following remission induction therapies, including the bortezomib-immunomodulatory agent (Imid)-dexamethasone in current studies, there is not enough consensus regarding the use of high-dose melphalan in patient groups with renal failure.[4,5] Studies have shown that AHSCT can be used in all stages of renal failure, including the need for dialysis and does not cause post-transplant engraftment failure.[5] Although studies performed in patients with advanced renal failure are limited and retrospective, studies show that melphalan dose adjustment should be performed mainly in dialysis-dependent patients and transplant-related mortality rates in these patients may be high.[6]

Melphalan used for autologous transplantation in patients with MM is a bifunctional alkylating agent, and the myeloablative dose of 200 mg/m^2 is generally used for transplantation.[7,8] Although spontaneous destruction is the most crucial step in eliminating melphalan from the body, some renal excretion also plays a role in elimination.[9-11] Although the rate of renal excretion is low, it is known that melphalan pharmacokinetics is affected in patients with renal failure.[12] In patient groups with renal failure, some centres aimed to prevent toxicity by reducing the dose of melphalan to 140 mg/m^2.[13] We aimed to contribute to the literature on the effective and safe dose in patient groups with renal failure and vulnerable patients by evaluating the data of patients who were followed up in our centre due to limited and contradictory data in studies on melphalan dose.

MATERIAL AND METHODS

The study included 107 patients older than 18 years of age with a diagnosis of MM who underwent AHSCT in Bursa Uludag University Faculty of Medicine, Department of Hematology, between January 2010 and January 2019. The data of the patients were retrospectively analyzed from their files. The patients were evaluated according to Kidney Disease Improving Global Outcomes (KDIGO) estimated glomerular filtration rate (eGFR: < 60 or ≥ 60 mL/min) levels and melphalan doses (140-200). Patients with low-performance scores (ECOG 3 and above) or severe systemic comorbidities other than renal failure were included in the Mel-140 group. The Mel-140 group included 11 patients, and the Mel-200 group included 96 patients. Age, gender, primary diagnosis, stage, pre-transplant treatment regimens, stem cell collection regimen, transplant preparation regimen, serum creatinine level, need for hemodialysis before transplantation, eGFR level, post-transplant complications, history of febrile neutropenic attack, presence of septic shock, neutrophil and platelet engraftment times, presence of recurrence, presence of mortality in the first 100 days were analyzed. Our study was conducted under the institutional research committee's ethical standards and according to the 1964 Helsinki Declaration.

Statistical Analysis

The compatibility of the variables with normal distribution was analyzed by the Shapiro-Wilk test. Continuous variables were expressed as median (minimum: maximum) and mean ± standard deviation. Categorical variables were expressed as n (%). Mann-Whitney U test was used for comparisons between two groups according to the normality test results. Pearson chi-square, Fisher's exact chi-square, and Fisher-Freeman-Halton tests were used to compare categorical variables between groups. Kaplan-Meier analysis was performed to investigate differences in overall and disease-free survival, and survival curves were compared using the log-rank test. For statistical analyses, SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) programme was used, and $p < 0.05$ was considered statistically significant.

RESULTS

Of 107 patients with MM, 41 (38.3%) were male,

Table 1. Patient characteristics according to estimated glomerular filtration rate (eGFR) level.

	eGFR < 60 mL/min (n: 18)	eGFR ≥ 60 mL/min (n: 89)	*P* value
Age (years)	58 (49:70)	55 (20:71)	0.040[a]
Gender (Male/Female)	5/13	36/53	0.224[b]
ISS phase I	5 (27.8)	28 (31.8)	0.038[b]
ISS phase II	2 (11.1)	32 (36.4)	
ISS phase III	11 (61.1)	28 (31.8)	
Number of treatment received before transplant	2 (1:3)	2 (1:4)	0.700[a]
Lenalidomide based	10 (47.6)	30 (25.6)	0.041[b]
Bortezomib based	18 (85.7)	85 (72.6)	0.205[b]
Stem cell G-CSF	11 (61)	50 (56.8)	0.818[b]
Collection regime chemotherapy+G-CSF	7 (39)	39 (43.2)	
Transplant preparation melphalan 200 mg/m^2	10 (10.4)	86 (89.6)	< 0.001[c]
Regime* melphalan 140 mg/m^2	8 (72.7)	3 (27.3)	
Serum creatinine (mg/dL)	1.60 (1.2:8)	0.70 (0.5:1.2)	< 0.001[a]
eGFR <50 (mL/min)	1 (5.6)	3 (3.38)	0.487[d]
eGFR ≥50 (mL/min)	17 (94.4)	86 (96.62)	

G-CSF: granulocyte colony-stimulating factor. Data were expressed as median (minimum: maximum) or n (%).
*percentages in brackets were calculated according to transplant preparation regime groups.
[a]Mann-Whitney U test, [b] Chi-square test, [c] Fisher Freeman-Halton test, [d] Fisher's exact chi-square test.

and 66 (61.7%) were female. Patient characteristics according to eGFR level were shown in Table 1. 18 patients (16%) were in the group with eGFR < 60 mL/min, while 89 patients (84%) were in the eGFR ≥ 60 mL/min group. The age range was 20-71 years, and the median age was 58 in the low eGFR group and 55 in the normal eGFR group. The subgroup analysis of patients with MM was shown in Table 2.

When melphalan doses used in transplant preparation were compared according to eGFR level, it was found that the melphalan 140 mg/m2 dose was used more in patients with low eGFR. It was statistically significant ($p < 0.001$). Two doses were used in the transplant preparation regime to evaluate the melphalan dose regarding side effects and engraftment time. No significant difference was found between the two groups regarding toxicity or engraftment time. A comparison of the groups according to melphalan dose was shown in Table 3.

In the analysis of the complications that developed during transplantation, the incidence and severity of mucositis were higher in the low eGFR group ($p = 0.040$, $p = 0.012$). No significant difference was found in hepatotoxicity, nephrotoxicity, frequency of febrile neutropenic attacks, septic shock incidence, and diarrhoea development according to eGFR level. No significant difference was found between the two groups in evaluating neutrophil engraftment times according to eGFR level. Median platelet > 20,000/mm3 engraftment time was found to be the 14th day in the eGFR < 60 mL/min group and the 12th day in the eGFR ≥ 60 mL/min groups, but no statistically significant difference was found between the two groups ($p = 0.117$). Median platelet > 50,000/mm^3 time was longer in the group with eGFR < 60 mL/min ($p = 0.006$). Complication evaluation according to eGFR level was summarized in Table 4.

According to the melphalan dose, median disease-free survival (DFS) was 37 months in the Mel-140 mg/m^2 group and 41 months in the Mel-200 mg/m^2 group, and no significant difference was found between the two groups ($p = 0.882$) (Figure 1A). No median value was reached in the overall survival (OS) analysis, and 12, 36, and 60-month OS in the Mel-200

Table 2. Subgroup analysis of patients diagnosed with multiple myeloma.

	eGFR <60 mL/min (n: 18)	eGFR ≥60 mL/min (n: 89)
IgG	9 (50)	43 (48.3)
IgA	2 (11.1)	23 (25.8)
Kappa light chain	6 (33.3)	7 (7.9)
Lambda light chain	0	11 (12.4)
Plasmacytoma	0	4 (4.5)
IgE	1 (5.6)	0
Plasma cell leukemia	0	1 (1.1)

Data were expressed as n (%).

Table 3. Comparison of side effects and engraftment times according to melphalan dose.

Transplant preparation regime	Melphalan 140 mg/m^2 (n: 11)	Melphalan 200 mg/m^2 (n: 96)	*P* value
Transaminase elevation	1 (9.1)	10 (10.4)	> 0.99[a]
Serum bilirubin elevation	2 (18.2)	13 (13.5)	0.651[a]
Mucositis			> 0.99[b]
Grade 1-2	6 (54.5)	51 (53.1)	
Grade 3-4	1 (9.1)	9 (9.4)	
None	4 (36.4)	36 (37.5)	
Diarrhoea			0.337[b]
Grade 1-2	7 (63.6)	38 (39.6)	
Grade 3-4	4 (36.4)	54 (56.3)	
None	0	4 (4.2)	
Neutrophil engraftment (days)	11(10:12)	11 (8:40)	0.322[c]
Platelet 20,000 engraftment (days)	13 (11:28)	12.50 (8:54)	0.297[c]
Platelet 50,000 engraftment (days)	21 (14:35)	17 (10:90)	0.065[c]
Progression free survival (PFS) (months)	39 ± 8.42	54.38 ± 6.76	0.882
Overall survival (OS) (months)	68.47 ± 3.98	124.43 ± 11.27	0.665

Data were expressed as median (minimum: maximum), mean ± standard deviation or n (%).
[a] Fisher's exact chi-square test, [b] Fisher Freeman-Halton test, [c] Mann-Whitney U test.

mg/m^2 group was 97.9%, 88.7%, and 70.5%, respectively. In the Mel-140 mg/m^2 group, 12-36-60 months OS was determined as 100%, 100%, and 53%, respectively. There was no significant difference in OS between the two groups ($p = 0.665$) (Figure 1B).

DISCUSSION

AHSCT performed in combination with high-dose chemotherapy is the standard treatment approach in patients with MM with good performance who

Table 4. Evaluation of complications during transplantation and duration of engraftment according to estimated glomerular filtration rate (eGFR) level.

	eGFR < 60 mL/min (n: 18)	eGFR ≥ 60 mL/min (n: 89)	*P* value
Renal failure in transplantation			0.132[a]
Yes hemodialysis none	7 (38.9)	18 (20.2)	
Yes hemodialysis yes	1 (%5.6)	3 (3.4)	
None	10 (%55.6)	68 (76.4)	
Transaminase elevation	0	11 (12.4)	0.205[b]
Serum bilirubin elevation	2 (11.1)	13 (14.6)	> 0.99[b]
Mucositis			0.016[c]
Grade 1-2	10 (55.6)	47 (52.8)	
Grade 3-4	5 (27.8)	5 (5.6)	
None	3 (16.6)	37 (41.6)	
Diarrhoea			0.297[a]
Grade 1-2	5 (27.8)	40 (44.9)	
Grade 3-4	13 (72.2)	45 (50.6)	
None	0	4 (4.5)	
Febrile neutropenia attack	16 (88.9)	86 (96.6)	0.196[b]
Septic shock	1 (5.60)	3 (3.40)	0.527[b]
Neutrophil engraftment (days)	11 (9:14)	11 (8:40)	0.002[d]
Platelet 20,000 engraftment (days)	14 (10:33)	12 (8:54)	0.117[d]
Platelet 50,000 engraftment (days)	21 (14:39)	17 (10:90)	0.006[d]
Progression free survival (PFS) (months)	42.87 ± 6.33	53.95 ± 6.90	0.331
Overall survival (OS) (months)	81.03 ± 7.49	121.11 ± 11.59	0.387

Data were expressed as median (minimum: maximum), mean±standard deviation or n (%).
[a] Fisher Freeman-Halton test, [b] Fisher's exact chi-square test, [c] Chi-square test, [d] Mann-Whitney U test.

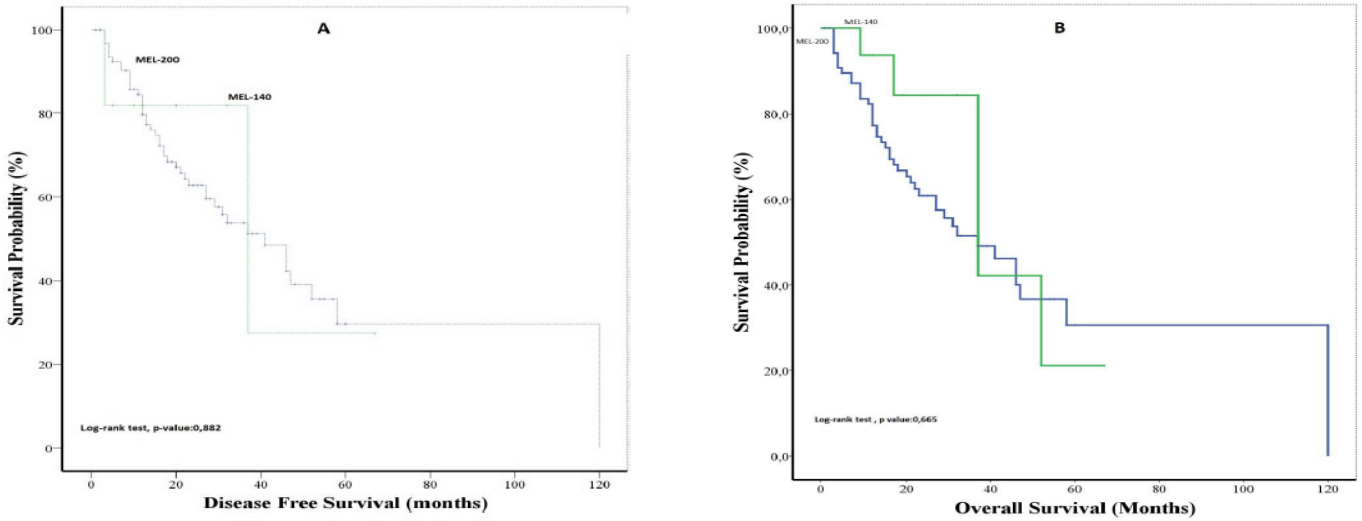

Figure 1. Kaplan-Meier survival analysis according to melphalan doses: (A) disease-free survival; (B) overall survival.

achieve remission with induction therapies.[1,14] However, there is still no consensus about the application of AHSCT in patients with renal failure, especially in patients with MM, and the dose of melphalan to be used as a preparatory regimen. In our survey analysis of the melphalan dose, no significant difference was found between both melphalan doses regarding disease-free survival and overall survival. A study conducted in 2018 on 55 MM patients with renal failure showed that melphalan could be used as an effective treatment option in all stages of renal failure, including patients on dialysis, and that the 140 mg/m^2 dose was safer in terms of side effect management in renal failure. Regarding efficacy, 140 mg/m^2 dose also influenced survival analysis results.[15] Similarly, in a study in which EBMT data were analyzed according to melphalan dose, no significant difference was found between Mel-140 and Mel-200 doses in the survey analysis.[13] In another study in which elderly MM patients were analyzed, it was observed that patients who used Mel-140 mg/m^2 in the transplant preparatory regimen had lower progression-free survival and overall survival rates compared to the Mel-200 mg/m^2 group.[16] In our study, no statistically significant difference was detected between the two groups. The difference between the two studies may be because patients with lower performance scores and lower survey expectations were included in the Mel-140 group and the difference between the number of patients and the induction therapy used before transplantation.

No significant difference was detected between the two groups in the rate of mucositis, development of hepatotoxicity, and engraftment times in the comparison results we performed according to melphalan doses. Similarly, in comparing melphalan doses, side effects and toxicity-related mortality rates were detected between the two groups.[13] As another result of our study, the incidence and severity of mucositis were higher in patients with low GFR levels. Similarly, a study was conducted on 381 newly diagnosed MM patients during AHSCT. Low GFR and high melphalan dose were observed as risk factors for severe mucositis.[17] The two groups had no significant difference regarding non-mucositis side effects and engraftment times compared to melphalan doses. A 1996 study showed that although melphalan was renally excreted, the main route of elimination was spontaneous hydrolysis and melphalan half-life and clearance did not change significantly even in patients with severe renal failure. In the same study, no difference was detected between the groups with and without renal failure in hematopoietic recovery, the frequency of transfusion requirement, and the incidence of severe mucositis (grade 3 and above).[18]

There are few case reports in the literature about lenalidomide-induced hepatotoxicity. One patient presented with a cholestatic injury pattern[19], another patient had lenalidomide-associated hepatitis[20], the third patient had a mixed pattern of liver injury[21], and the fourth patient had asymptomatic transaminase elevation.[22] Most of cases, patients had pre-existing renal failure. Lenalidomide is mainly excreted by kidneys, so patients with renal failure may be more prone to developing hepatotoxicity.[21] In our study results, there were no cases of lenalidomide-induced hepatotoxicity.

Study Limitations

The small number of patients with renal failure, the exclusion of patients with incomplete data due to the study's retrospective design, and the fact that patients with MM constitute a heterogeneous patient population can be considered as the limiting factors of our study. Prospective randomised controlled studies on homogeneous groups of transplant candidates with renal failure will contribute to the literature in the future.

CONCLUSIONS

In conclusion, Mel-140 appears to be an effective and safe transplantation preparatory regimen in frail

patients with renal insufficiency, low-performance scores, or severe systemic comorbid disease. The fact that melphalan undergoes spontaneous hydrolysis along with renal excretion allows it to be used safely in patients with low GFR without a significant increase in side effects.

Conflict of Interest

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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Ethical Approval

Our study was conducted under the institutional research committee's ethical standards and according to the 1964 Helsinki Declaration. The protocol of the study was approved by the Medical Ethics Committee of Bursa Uludag University (Faculty of Medicine, Bursa, Turkey). (Decision number: 2019-6/39, date: March 2019).

Authors' Contribution

Study Conception, Supervision, Critical Review: FCH, FO, VO; Study Design, Fundings: FCH, FO; Data Collection and/or Processing: FCH, FO, VG; Analysis and/or Interpretation: FCH,FO,VG; Materials: FCH, FO, VG, VO; Literature Review, Writer: FCH.

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Original Article

Risk Factors and Outcomes for Carbapenem-resistant Klebsiella Pneumoniae Infection in Haematological Patients

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ABSTRACT

Background Prolonged hospitalization, prolonged neutropenia, and immunosuppressive treatments increase bloodstream infections in haematological patients. Identifying risk factors for carbapenem-resistant Klebsiella pneumoniae (CRKP) infection will shed light on controlling the spread of CRKP. Our retrospective study aimed to determine the clinical features, antimicrobial susceptibility, and mortality risk factors of patients who developed CRKP in patients followed up for haematological cancer in the Izmir University of Economics Haematology Department.

Material and Methods 19,170 blood-urine-sputum cultures were delivered from the patients, 1,595 (8.31%) of which presented growth. CRKP comprised 302 (1.57%) of such growth cases. The study included 72 patients with haematological malignancy who presented CRKP growth in 302 cultures obtained during the neutropenic fever period.

Results The mean age of patients was 51 (18-75 years). Acute myeloid leukaemia was the most common disease (n: 26, 36.11%). As to the antibiotic sensitivity of CRKP, 44 patients (61.1%) were colistin sensitive, 28 patients (38.9%) were colistin-resistant, 47 patients (65.3%) were tigecycline sensitive/medium sensitivity, 25 patients (34.7%) were tigecycline resistant, there was no statistically significant difference between antibiotic sensitivities and survival.

Conclusions Today, early detection of CRKP colonization in high-risk haematological patients, taking rectal culture, and if the patient presents rectal colonization of CRKP or had CRKP bacteremia during prior hospitalizations, early initiation of treatment with antibiotics acting against CRKP during NPF would significantly reduce mortality.

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INTRODUCTION

In recent years, total life expectancy in haematological patients has been extended by the development of effective chemotherapy treatments, increased frequency of autologous and allogeneic stem cell transplantation, and improved supportive treatments. However, prolonged hospitalization, long neutropenia time, invasive medical procedures and repeated intensive immunosuppressive treatments increase bloodstream infections.[1] The frequency of bloodstream infection among cancer patients varies between 11% and 38%, and the mortality rate rises to 40%.[2] Carbapenems (meropenem or imipenem/cilastatin) are used in the first place in hemodynamically unstable patients with neutropenic fever, comorbid diseases, and neutrophil < 100/mm3.[3,4] The use of long-term carbapenem increases the prevalence of meropenem-resistant gram-negative bacteria. Multi-drug-resistant (MDR) gram-negative bacteria are reported at an increasing rate in many countries worldwide.[5] The most frequently isolated factor in carbapenem-resistant bacterial infections is *Klebsiella pneumoniae*.[6] Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is one of the nosocomial pathogens that can cause outbreaks where high mortality rates are observed and frequently isolated, especially from intensive care patients.[7] CRKP bacteremia is also a bacterium with increasing prevalence and can cause significant morbidity and mortality in immunosuppressed patients. In this group of patients, prolonged use of broad-spectrum antibiotics during neutropenic fever increases the frequency of colonization of MDR gram-negative bacteria in different body parts.

The control and treatment of CRKP is a critical problem worldwide and in Turkey.[8] CRKP's MDR and limited antibiotic responsiveness reduce the chances of treatment.[9,10] The optimal treatment approach for Enterobacteriaceae infections with carbapenem-resistance has not yet been determined. Treatment options for *Enterobacteriaceae* infections resistant to carbapenem include polymyxin B, colistin, tigecycline, fosfomycine, aminoglycosides and ceftazidime-avibactam.[11] With its bactericidal effect depending on concentration and ability to reach an adequate concentration in serum, colistin represents an important treatment option, especially in CRKP infections in blood circulation.[12] With the widespread use of colistin, colistin-resistance has increased.[13,14] Determining the risk factors for CRKP infection will shed light on controlling the spread of CRKP. In patients with a haematological malignancy, there is limited information on the epidemiology of *Klebsiella pneumoniae* bacteremia, development risk factors, and disease prognosis. In our retrospective study, we aimed to identify the clinical characteristics, laboratory findings, antimicrobial sensitivities, disease development and mortality risk factors of patients that developed CRKP among those who have been followed up in the Izmir University of Economics, Faculty of Medicine, Haematology Department with haematological cancer, who received chemotherapy or underwent allogeneic or autologous stem cell transplantation.

MATERIAL AND METHODS

In this retrospective study, among the patients who were hospitalized and followed up at the Haematology Clinic and Bone Marrow Transplantation Unit of Izmir University of Economics, Faculty of Medicine from 1 January 2015 to 31 August 2019, the patients who presented single or repeated CRKP growth through the neutropenic period in catheter and/or peripheral blood, urine and sputum cultures were included. The characteristics of patients, epidemiological and clinical findings, underlying diseases, antimicrobial susceptibility profiles, laboratory findings, and additional interventional procedures were evaluated. The ethics committee approved the study. No informed consent was received from patients due to the study's retrospective design.

Microbiological tests

When the axillary fever of neutropenic patients was >38 °C, the patients' catheter, peripheral blood, urine cultures and sputum cultures (if they presented sputum) were taken. (BACTECTM FX 200, Becton Dickinson). The bacterial identification and antibiotic susceptibility tests were performed with a microflex-TM LT/SH mass spectrometer (Bruker Daltonik, Bremen, Germany) and a VITEK® system (bioMérieux, Hazelwood, MO, USA) according to the manufacturer's instructions. Cefazolin, cefoperazone-sulbactam and tigecycline were determined by the Kirby-Bauer disk diffusion method.

Definitions

It was defined by the Infectious Diseases Society of America.15 This definition defines fever as an axillary temperature of at least 38.3 °C measured at once or above 38 °C continuing for more than an hour. Later, body temperature rising to 38 °C and above twice within 12 hours was added to this definition. According to the 2003 guidelines of the Febrile Neutropenia

Working Group in our country, neutropenic fever is defined as orally measured body temperature > 38.3 °C at once or >38 °C for more than one hour in neutropenic patients.16 Neutropenia is when the absolute number of neutrophils is less than 500/mm^3 or the number of neutrophils initially less than 1,000/mm3 drops to 500/mm^3 or less within 24-48 hours. Septic shock is defined as the condition in which systolic blood pressure is < 90 mmHg for a patient with fever or the need to use inotropic agents to maintain blood pressure at normal levels.

Klebsiella pneumoniae bacteremia was diagnosed when at least one of the blood sample cultures was positive for *Klebsiella pneumoniae.* Empirical antibiotic therapy was considered appropriate if at least one drug was active against the strain of Klebsiella pneumoniae (as determined by in vitro susceptibility tests). Antibiotic susceptibility was determined according to the Clinical and Laboratory Standards Institute 2015 recommendations.[17] MDR was defined as non-susceptible to at least one agent in ≥ 3 antimicrobial categories, according to Magiorakos *et al*.[18] Initial treatment for patients with neutropenic fever starts with meropenem treatment. Then, after CRKP growth, aminoglycosides, colistin and tigecycline were added to the treatment with antibiotics administered according to the antibiogram. If the patient had CRKP infection in previous neutropenic fever periods or rectal CRKP colonization, combined antibiotic therapy was started without waiting for culture in resistant fever.

Statistical Analysis

The data were expressed as mean±SD for normally distributed continuous variables, median (minimum:-maximum) for skew-distributed continuous variables, and frequencies for categorical variables. Pearson's chi-square test was performed to compare the categorical variables. ANOVA compared means of normally distributed continuous variables. The Mann-Whitney U test compared skew-distributed continuous variables. Cox regression analysis was used for multivariate analyses. The Statistical Package for Social Sciences (SPSS) for Windows version 15.0 (SPSS Inc., Chicago) was used for the analysis, and a two-sided *p* - value of <0.05 was considered significant.

RESULTS

Patient Characteristics

Nineteen thousand one hundred seventy blood-urine-sputum cultures were delivered from the patients hospitalized at Izmir University of Economics, Medicalpark Hospital, Clinic of Haematology and Bone Marrow Transplantation Unit, 1,595 (8.31%) of which presented growth. CRKP comprised 302 (1.57%) of such growth cases. The study included 72 patients with haematological malignancy who presented CRKP growth in 302 cultures obtained during the neutropenic fever (NPF) period. Table 1 showed the basic characteristics of 72 patients. The mean age of patients was 51 years (range: 18-75); 50 (69.44%) of them were male, and 22 (30.56%) were female. Acute myeloid leukaemia (AML) was the most common disease (n: 26, 36%). Other diseases were acute lymphoblastic leukaemia (n:18, 25%), non-Hodgkin lymphoma (n: 18, 25%), multiple myeloma (n: 5, 7%), aplastic anaemia (n: 3, 4.16%) and myelodysplastic syndrome (n: 2, 2.84%).

When patients are examined, CRKP growth was observed in 41 patients (56.95%) during remission induction treatment, 9 (12.5%) during consolidation treatment, 7 (9.72%) during peripheral hematopoietic stem cell transplantation, 12 (16.66%) during allogeneic stem cell transplantation, and 3 (4.17%) during hospitalization for acute graft versus host disease (GVHD) treatment. While 47 (65.28%) patients presenting growth had a treatment-resistant disease, 25 (34.72%), patients were in remission. Eight patients received the first remission induction, 16 received the second, 12 received the third, 7 received the fourth, one received the fifth, and three received the sixth induction treatments with resistant diseases. Ten patients had related allogeneic stem cell transplantation, and six underwent unrelated transplantation. Six of the patients with allogeneic transplants underwent transplantation with resistant disease. Six patients had CRKP rectal colonization while undergoing allogeneic transplantation, 12 presented CRKP growth during chemotherapy, and 4 presented it during hospitalization for acute GVHD treatment. CRKP growth was detected in the first month of transplantation in 11 patients, 30-100 days in 3 patients and 100-365 days in 2 patients. Acute GVHD developed in 6 of 15 patients during the follow-up. Five patients were treated with methylprednisolone, and cyclosporine, while one was treated with multiple immunosuppressive treatments (methylprednisolone, mycophenolate mofetil, tacrolimus, and mesenchymal stem cell infusion).

Eight of the ten patients with allogeneic transplants from sibling donors who had CRKP growth during

Table 1. Characteristics of patients and their impact on survival.

Parameters	Subgroup	Survival		P value	χ^2
		Live	Exitus		
Gender	Woman	6	16	0.815	0.055
	Man	15	35		
Diagnosis	Acute myeloid leukaemia*	3	23	0.003	17.743
	Acute lymphoblastic leukaemia	4	14		
	non-Hodgkin lymphoma	7	11		
	Multiple myeloma	5	0		
	Myelodysplastic syndrome	1	1		
	Aplastic anemia	1	2		
Amikacin	Sensitive	2	15	0.125**	3.262
	Resistant	19	36		
Colistin	Sensitive	13	31	0.929	0.008
	Resistant	8	20		
Tigecycline	Sensitive	5	14	0.613	0.979
	Medium sensitivity	10	18		
	Resistant	6	19		
Meropenem	Resistant	21	51	NC	NC
Gentamicin	Sensitive	2	10	0.489**	1.089
	Resistant	19	41		
Chemotherapy	None	1	2	0.002	19.182
	Remission induction*	7	34		
	Consolidation	4	2		
	Autologous SCT	6	1		
	Allogeneic SCT	3	9		
	GVHD treatment	0	3		
Resistant disease	No	12	13	0.010	6.575
	Yes	9	38		
Allogeneic transplantation	None	18	38	0.577	1.101
	Allo-sibling	2	8		
	Allo-unrelated	1	5		
Prior transplantation	No allo-autologous SCT	20	32	0.019	7.932
	Allogeneic SCT	1	13		
	Autologous SCT	0	6		
CRKP growth location	Catheter blood	1	2	0.961	0.081
	Peripheral blood	1	3		
	Catheter-peripheral blood	12	34		
Quinolon oral prophylaxis	None	5	8	0.504**	0.663
	Positive	16	43		
Use of meropenem	No	1	4	1.000**	0.219
	Yes	20	47		
Use of meropenem in the last 4 weeks	No	2	6	1.000**	0.076
	Yes	19	45		
Neutrophil during infection	500-700/mm^3	4	4	0.332	2.205
	<100-500/mm^3	16	42		
	<100/mm^3	1	5		
Transferred from another centre	No	12	21	0.217	1.527
	Yes	9	30		
Hospitalization at ICU	No	20	46	0.664**	0.495
	Yes	1	5		
Invasive procedure	Non-catheter	2	10	0.488**	0.944
	Catheter	18	41		
Mucositis	Grade 1-2	19	43	0.713**	0.472
	Grade 3-4	2	8		
Prior NPF colonization	None	15	45	0.095**	3.025
	Positive	6	6		
Prior NPF bacteremia	None	15	46	0.131**	2.742
	Positive	5	5		
CRKP bacteremia 30-day mortality	None	21	3	< 0.001	59.294
	Positive	0	48		

Continuation of Table 1

Parameters	Subgroup	Survival		P value	χ^2
		Live	Exitus		
Empirical treatment	M	0	1	0.243	9.142
	M-A	1	0		
	M-A-C	0	3		
	M-A-C-T	13	28		
	M-G-C-T	3	5		
	M-C-T	2	13		
	M-C	1	1		
	M-T	1	0		
IPA	None	17	37	0.454	0.560
	Positive	4	14		
IPA Treatment	None	7	17	0.894	0.613
	Caspofungin	11	25		
	Voriconazole	1	4		
	Liposomal amphotericin B	2	7		
Colonization at hospitalization	Unexamined	7	30	0.685**	0.286
	None	10	13		
	Positive	6	6		
GVHD	None	19	43	0.662**	0.515
	Acute GVHD	1	5		
Immunosuppressive treatment	No	16	32	0.322	0.980
	Yes	4	15		
Septic shock	None	13	3	< 0.001**	27.011
	Positive	8	48		
Inhaler treatment	None	17	32	0.132	2.268
	Positive	4	19		
Mechanical ventilation	None	20	20	< 0.001	18.908
	Positive	1	31		
Cause of death	Other	21	38	0.011**	7.430
	Klebsiella bacteremia	0	13		

$P < 0.05$ was considered significant and * indicates significant subgroup. Pearson's Chi-Square and **Fisher's Exact Chi-Square tests were used. NC: not calculated. SCT: stem cell transplantation, GVHD: Graft versus host disease, CRKP: carbapenem-resistant *Klebsiella pneumoniae*, ICU: intensive care unit, NPF: neutopenic fever, M: meropenem, A: amikacin, G: gentamisin, C: colistin, T: tigecycline, IPA: invasive pulmoner aspergillosis.

hospitalization and 5 of the six patients with unrelated transplants were lost at the follow-up. Six of the lost patients with allogeneic transplants underwent transplantation with resistant disease. Twelve patients (16.7%) presented rectal CRKP colonization; ten patients (14.1%) had CRKP bacteremia in previous NPF periods. The patients presenting growth were hospitalized five times on average (1 to 12 times), 39 patients (54.2%) were transferred to our hospital from another clinic, and six patients (8.3%) stayed in the intensive care unit. Sixty patients (83.3%) had a temporary central venous catheter. The most common invasive procedure for patients was the insertion of the temporary central venous catheter, and other less frequent methods were shown in Table 2. CRKP growth was detected in the catheter and peripheral blood cultures in 47 patients (65.28%), peripheral blood culture only in 4 patients (5.5%), and catheter blood culture only in 4 patients (5.5%) (Table 3). On average, CRKP growth was observed to be 1.94 (1-6) for peripheral blood culture and 1.73 (1-5) for catheter blood culture. It was observed that 64 patients (88.9%) with growth have been receiving meropenem in the last four weeks, 67 patients took meropenem due to NPF (93.1%) during growth, while 60 patients (83.3%) received quinolone prophylaxis. On average, they took meropenem for 9.05 days between 0-30 days.

Considering the empirical antibiotic treatments given to this patient group, 41 patients (56.94%) received meropenem, amikacin, colistin, and tigecycline in combination, 15 (20.83%) meropenem, colistin, tigecycline and eight patients (11.1%) took meropenem, gentamycin, colistin treatment (Table 1).

As to the antibiotic sensitivity of CRKP, 44 patients (61.1%) were colistin sensitive (31 patients lost on follow-up, 84%), 28 patients (38.9%) were colistin-resistant (20 patients lost on follow-up, 71%), 47 patients (65.3%) were tigecycline sensitive/medium sensitivity (32 patients lost, 65%), 25 patients (34.7%) were tigecycline resistant (19 patients lost, 76%), 17 patients (23.61%) were amikacin sensitive (15 patients lost, 88%), 55 patients (76.39%) were amikacin resis

Table 2. Distribution of 72 invasive procedures applied to patients.

Procedure	n (%)
Central and venous catheter	60 (83.2)
Endoscopy-colonoscopy	2 (2.8)
Rectal abscess drain	2 (2.8)
Abdomen exploration	2 (2.8)
Splenectomy	1 (1.4)
Bronchoscopy	1 (1.4)
Prostate abscess drain	1 (1.4)
Pancreas cyst drain	1 (1.4)
No procedure	2 (2.8)

Table 3. CRKP growth locations.

Locations	n (%)
Catheter-peripheral blood culture	47 (65.28)
Blood-urine culture	6 (8.3)
Sputum-blood culture	6 (8.3)
Catheter blood culture	4 (5.5)
Peripheral blood culture	4 (5.5)
Urine culture	3 (4.32)
Sputum culture	1 (1.4)
Catheter-peripheral blood and BOS culture	1 (1.4)

tant (36 patients lost, 65%), 12 patients (16.7%) were gentamicin sensitive (10 patients lost, 83%), 60 patients (83.3%) were gentamicin resistant (41 patients lost, 68%); and there was no statistically significant difference between antibiotic sensitivities and survival (Table 4).

Rectal swabs were taken from patients during hospitalization as of January 2018. According to the hospitalization data of 72 patients with CRKP growth, it was observed that no rectal swab was taken from 37 patients, while rectal swabs were taken from 35 patients. Rectal colonization was detected in 12 patients, six patients with rectal colonization survived, but six patients were lost.

During the CRKP growth, 38 patients (52.8%) had grade 1-2 mucositis, ten patients (13.9%) had grade 3-4 mucositis, and 24 patients (33.3%) had no mucositis. Among the patients with CRKP growth, 54 patients (75%) had no invasive pulmonary aspergillosis (IPA), while 12 patients (16.7%) presented probable and six patients (8.3%) presented proven IPA at that time of hospitalization. Ten patients received liposomal amphotericin B. Six patients received voriconazole, 36 received caspofungin, and 20 had no antifungal.

Among the patients with CRKP growth, 23 patients (31.9%) had steroids and beta-agonist, and 32 (44.4%) were followed up with ventilator support. In the follow-up, 48 patients (66.7%) died in the first 30 days after CRKP growth, and 51 (70.83%) died in 60 days. A total of 13 patients (18.05%) died due to CRKP bacteremia, 35 patients (48.61%) were lost due to disease progression and CRKP infection, while three patients (4.16%) were lost due to GVHD and disease progression.

Factors influencing survival were shown in Table 1. Given the factors influencing survival, the mortality rates of patients diagnosed with AML ($p = 0.003$), patients treated with remission induction treatment ($p = 0.002$), patients with the resistant disease ($p = 0.01$), patients who underwent allo- or autologous transplantation ($p = 0.019$), patients who developed septic shock ($p < 0.001$) and those conditioned to mechanical ventilation ($p < 0.001$) had significantly higher mortality rates. No significant relation was detected between mortality and sex, antibiotic sensitivity, allotransplantation, disease status at the time of transplantation, use of meropenem during growth, use of levofloxacin, neutrophil count during infection, stay in the intensive care unit, transfer from another centre, invasive procedure, empirical treatment, bacteremia during former neutropenic fever, colonization during former neutropenic fever, development of IPA, rectal colonization at the time of transplantation, GVHD or use of immunosuppressive treatment ($p > 0.05$).

DISCUSSION

The present study aimed to identify the clinical characteristics, laboratory findings, antimicrobial sensitivities, disease development and mortality risk fac-

Table 4. CRKP antibiotic sensitivity.

Antibiotics	Sensitive frequency n (%)	Medium sensitive frequency n (%)	Resistant frequency n (%)
Gentamicin	12 (16.7)	0	60 (83.3)
Amikacin	17 (23.6)	0	55 (76.4)
Colistin	44 (61.1)	0	28 (38.9)
Tigecycline	19 (26.38)	28 (38.9)	25 (34.72)

tors of patients that developed CRKP among those with haematological patients who received chemotherapy or underwent allogeneic or autologous stem cell transplantation. The prevalence of CRKP varies depending on geography. The prevalence in China is around 10%, while it rises to 60% in India.[19] The prevalence of CRKP is increasing, given the studies on patients with haematological malignancy. In a review of 30 studies from 21 countries to determine the global prevalence of carbapenem-resistant infections, carbapenem resistance was 9% on average, ranging between 2-53%. On the other hand, CRKP strains have been identified at a higher rate in countries such as Italy, Greece and Israel, and these regions have been identified as endemic areas.[20]

In this study, gram-negative bacteria growth was detected in 1,519 (8.31%) of 19,179 blood-urine-sputum cultures taken from patients hospitalized in the Haematology and Bone Marrow Transplantation Unit of our hospital for more than four years between 2015-2019. Among them, 302 cases (1.57%) were CRKP. In the retrospective 5-year data of a single centre, published by Kara *et al*.[21], bloodstream infection was 14.5%. Gram-negative bacteria accounted for 2% of the CRKP growth. In a study by Trecarichi *et al*.10 involving thirteen Haematology centres in Italy, CRKP accounted for 161 (57.9%) of the 278 cases of Klebsiella pneumoniae growth, isolated between January 2010 and June 2014, 117 (42.1%) of them was meropenem-sensitive *Klebsiella pneumoniae* (MSKP); 84 out of 161 (52.2%) meropenem-resistant patients and 17 out of 117 (14.5%) patients with MSKP growth died in 21 days ($p < 0.001$). Septic shock, acute respiratory failure, inadequate initial antimicrobial treatment and carbapenem resistance were associated with mortality as an independent risk factor. In the present study, during the follow-up, 48 patients (66.7%) died in the first 30 days after CRKP growth, and 51 patients (70.83%) died in 60 days. A total of 13 patients (18.05%) died due to CRKP bacteremia, 35 patients (48.61%) were lost due to disease progression and CRKP infection, while three patients (4.16%) were lost due to GVHD and disease progression. In haematology patients, risk factors for the development of CRKP infection were found to include age > 50 years, especially male sex, AML patients, relapse or refractory leukaemia, long-term hospitalized patients, long-term neutropenia, rectal CRKP colonization, prior CRKP bacteremia, patients with a central catheter or urinary catheterization.[9,22]

Given the factors influencing survival after CRKP infection in our study, the mortality rates of patients diagnosed with AML ($p = 0.003$), patients treated with remission induction treatment ($p = 0.002$), patients with the resistant disease ($p = 0.01$), patients who underwent allo- or autologous transplantation ($p = 0.019$), patients who developed septic shock ($p < 0.001$) and those conditioned to mechanical ventilation ($p < 0.001$) had significantly higher mortality rates. No significant relation was detected between mortality and sex, antibiotic sensitivity, allotransplantation, disease studies at the time of transplantation, use of meropenem during growth, use of levofloxacin, neutrophil count during infection, stay in the intensive care unit, transfer from another centre, invasive procedure, empirical treatment, bacteremia during former neutropenic fever, colonization during former neutropenic fever, development of IPA, rectal colonization at the time of transplantation, GVHD or use of immunosuppressive treatment ($p > 0.05$). The high mortality rates of patients included in the study were associated with the high number of patients with relapsed refractory haematological malignancy and those diagnosed with AML. In a study in which we examined the infections developed by 199 patients who underwent allogeneic stem cell transplantation during 219 transplants from November 2012 to July 2018, 9 patients presented CRKP. One patient had MSKP growth in the catheter and peripheral blood cultures, seven had CRKP, and four had MSKP growth in urine cultures. Two patients had CRKP, and one had MSKP growth in sputum cultures. Five patients were lost due to CRKP sepsis during the follow-up of patients with MRKP growth. Three of them presented resistance to colistin and tigecycline. Colistin and tigecycline resistance were detected in 20% of the patients.[23]

A comparison of the data of the two studies revealed that colistin and tigecycline resistance increased over time, which indicates that colistin resistance rises over the years and shows a high rate of dispersion.

In haematological patients, the CRKP colonization rate is 3.8% in Italy, while in India, it increases up to 21%. It was observed that 14% of the colonized patients developed bloodstream infections with the same bacteria.[24,25] In a study conducted by Micozzi *et al*.[26] on haematological patients at Sapienza University of Rome, CRKP rectal colonization was detected in 22

out of 373 patients from January 2014 to September 2014, 12 (64%) of which developed bacteremia; while rectal colonization was detected in 14 out of 131 initial patients, those patients were then isolated, rectal culture was started to be taken every week, and colonization rate continuously decreased in subsequent hospitalizations. Rectal colonization was detected in 5 of the 242 patients hospitalized after the rectal culture started to be taken routinely ($p = 0.001$). 14 (58%) of the 22 patients with rectal colonization developed bacteremia, and all had AML ($p = 0.02$). Bacteremia grew in the neutropenic period in 86% of the patients. Ten of the 14 patients who developed bacteremia died in the follow-up, all of whom had AML. Initial adequate antibiotic therapy resulted in the only independent factor to protect against death ($p = 0.02$). The researchers claimed that starting initial antibiotics for patients with rectal CRKP during NPF based on CRKP culture antibiogram colonization would reduce mortality.[26] The present study included patients admitted to the Haematology service between January 1, 2015, and August 31, 2019. Rectal swabs were taken from patients during hospitalization as of January 2018. According to the hospitalization data of 72 patients with CRKP growth, it was observed that no rectal swab was taken from 37 patients, while rectal swabs were taken from 35 patients. Rectal colonization was detected in 12 patients, six patients with rectal colonization survived, but six patients were lost. In a study by Micozzi *et al*.26, the colistin sensitivity was 50% (12/22), and tigecycline sensitivity was 27% (6/22), while all patients were gentamicin resistant (0/22). After the documentation of CRKP infection, patients are usually administered combination treatments. Tigecycline/amikacin/colistin, colistin/tigecycline/gentamicin and colistin/tigecycline/meropenem combinations are used. However, some studies reported a synergic effect against carbapenem-resistant bacteria in in vitro environments[27-29], while other studies did not show such a synergic effect.[30]

The late start of the combination treatment is one of the key factors affecting mortality.27,28 In the present study, 41 patients (56.94%) were given a combination of meropenem, amikacin, colistin and tigecycline; 15 patients (20.83%) received meropenem, colistin, tigecycline, and eight patients (11.1%) had meropenem, gentamicin, colistin and tigecycline. Other patients received single or double antibiotics (11.13%). Combination therapy with three or four antibiotics is recommended in CRKP infections. 88.87% of our patients used triple or quadruple combination antibiotic therapy as recommended. Treatment was directed according to the antibiotic susceptibility obtained as a result of the cultures. Therefore, our study found no statistical significance between antibiotic susceptibility and mortality.

CONCLUSIONS

Currently, carbapenems are used empirically as part of the first line of treatment during neutropenic fever in patients with haematological malignancy. The widespread use of carbapenems is one of the critical factors in the increase of carbapenem-resistant strains. Today, early detection of CRKP colonization in high-risk haematological patients (e.g. patients with AML who receive remission-induction treatment, patients with relapsed refractory AML, or patients to undergo allogeneic or autologous bone marrow transplantation), taking rectal culture as a routine procedure during hospitalization, and if the patient presents rectal colonization of CRKP or had CRKP bacteremia during prior hospitalizations, early initiation of treatment with combined antibiotics acting against CRKP during NPF (meropenem, aminoglycoside, colistin and tigecycline) would significantly reduce mortality.

Conflict of Interest

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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Ethical Approval

The protocol of the study was approved by the Medical Ethics Committee of İzmir Katip Çelebi University, İzmir, Turkey. (Decision number: 0259, date: May 2021).

Authors' Contribution

Study Conception, Supervision, Critical Review: SK, SÇ, GE; Study Design, Fundings: GE, SK,; Data Collection and/or Processing: SK, SÇ,; Analysis and/or Interpretation: SK, SÇ,; Materials: GE,; Literature Review, Writer: SK, SÇ.

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Original Article

Subcutaneous and Intravenous Cladribine Treatment of Hairy Cell Leukemia Patients: Do We Still Need Intravenous Cladribine?

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ABSTRACT

Background Hairy cell leukaemia (HCL) is an uncommon neoplasm representing approximately 2% leukaemias and < 1% lymphoid neoplasms. Although HCL remains an incurable disease, first-line treatment with intravenous (IV) or subcutaneous (SC) cladribine (2-CdA) often leads to long-term remissions. Although long-term data are available for IV administration, similar comparable data for SC administration are lacking.

Material and Methods Demographic data, laboratory and clinical parameters of 20 patients with HCL, and IV and SC administrations of cladribine in primary treatment were analyzed.

Results All patients were administered 2-CdA as the first-line therapy. 2-CdA was administered intravenously to 11 patients and subcutaneously to 9 patients. The hospitalization times were shorter in the SC route, and the incidence of febrile neutropenia was less; therefore, statistical significance could not be determined. There was no difference between the route of administration and the treatment response. A correlation was recorded between the level of anaemia before treatment and the time to treatment response. In addition, a correlation was recorded between the level of anaemia before treatment and minimal residual disease status after treatment. The median overall survival (OS) was 43.5 months (confidence interval 95%: 1.5-79 months), and 2- and 5-year OS was 95%. There was no increase in the incidence of second primary cancer.

Conclusions The outcomes of HCL patients treated with SC 2-CdA are quite good, and, in most patients, one cycle of SC 2-CdA was adequate for long-term disease control. SC 2-CdA is an easily applicable option for outpatients, and their side effects are often easily manageable.

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INTRODUCTION

Hairy cell leukaemia (HCL) is an uncommon malignancy, representing approximately 2% of leukaemias and < 1% of lymphoid neoplasms.[1] The median age of onset is 50-55 years. The male-female ratio is approximately 4:1.[2,3] Most patients present with symptoms of splenomegaly or cytopenia, fatigue, infection, and hemorrhagic signs.[4,5] These signs are usually not accompanied by symptom B. Palpable splenomegaly is a classic feature of HCL. Pancytopenia is present in 60-80% of patients with HCL.[3-5] Circulating tumour cells specific to HCL are usually observed in the peripheral blood. Cytoplasmic slender protrusions give the cell a "hairy" appearance. Tartrate-resistant acid phosphate activity can be demonstrated in any patient, although the proportions of positive cells vary across patients. However, it is not a specific finding for HCL. Hairy cells strongly express pan-B cell antigens and typically express CD11c, CD25, CD103, CD123 (bright), and cyclin D1 (usually weak). Annexin A1 and BRAF V600E mutations are detected in approximately 75% of all HCL cases. Therefore, it should be remembered that HCL, as in other indolent lymphomas, cannot be cured. Asymptomatic patients are not treated. If patients present with signs of disease or a decrease in their haematological parameters, they should be treated. In general, the haematological parameters that indicate the need for treatment include at least one of the following: haemoglobin (Hgb) 11 g/dL, thrombocyte count 100,000/mL, or absolute neutrophil count 1000/mL.[6,7] Symptomatic splenomegaly may be an indication for treatment. Nucleoside analogues, such as pentostatin and cladribine, have been introduced recently and have emerged as effective therapeutic options with promising results.[8,9] The first choice, in this case, is 2-chlorodeoxyadenosine (cladribine, 2-CdA). 2-CdA is administered continuously through intravenous (IV) in most clinical trials, and it is still used in several centres as the first-line therapy for patients with HCL. However, although the plasma half-life is short, 2-CdA accumulates in leukemic cells and has a longer intracellular half-life, which makes it feasible to adopt subcutaneous (SC) applications.[10,11]

Although a cure is not achieved through this approach, complete remission (CR) is achieved with Hgb > 11 g/dL (without transfusion), thrombocyte >100x10^9/L, neutrophil > 1.5x10^9/L, morphologically undetectable HCL cells in the peripheral blood and bone marrow, absence of organomegaly in physical examination, and the absence of symptoms.[12] Piro *et al.*[13] reported 11 CR and one partial response (PR) for 12 patients with HCL who were treated with a single 7-day course of 2-CdA. Other studies have reported similar outcomes.[14-16] In 20-50% of the HCL patients who achieved CR with 2-CdA treatment, minimal residual disease (MRD) was subsequently demonstrated by immunohistochemical methods.[17,18] Disease recurrence has been associated with MRD in HCL.[19] If the response is inadequate or relapse occurs before 12 months, alternative purine analogues may be administered alone or in combination with rituximab, rituximab alone, interferon administration, or via referral to clinical trials.[20] Recently, vemurafenib, a BRAF inhibitor, was applied in treating resistant and relapsing HCL with significant outcomes.[21] The development of recombinant immunotoxins targeting CD25 or CD22 is ongoing.[22,23] In this study, we retrospectively analyzed the demographic data, treatments, length of hospital stay, treatment responses, complications, and survival rates of 20 patients with HCL who were diagnosed during 2015-2021.

MATERIAL AND METHODS

Twenty patients diagnosed with HCL and followed up at the Bursa Uludag University Hematology Department Clinic between 2015 and 2021 were included in the study. The inclusion criteria were pathological, flow cytometric, and morphologically confirmed HCL diagnosis, treatment for HCL diagnosis, and age > 18 years. Data on the clinical features of the patients, signs and symptoms at the time of presentation, laboratory values at the time of diagnosis, imaging and pathology reports, treatments applied, treatment modalities (IV and SC), treatment responses, the length of hospital stay, and the short- and long-term complications were retrospectively reviewed.

The standard protocol at our centre was IV administration. However, we used SC when there was no IV form. Then, we compared the outcomes with IV and SC applications. No difference was recorded between the groups regarding age, gender, spleen size, and laboratory results. Accordingly, we did not change the diagnosis, supportive treatment, or follow-up.

The treatment protocols in the studies included 0.14 mg/kg SC 2-CdA for five days or 0.1 mg/kg IV 2-CdA for seven days. In the study, the response evaluation was performed clinically and through the laboratory, every two weeks, and a bone marrow biopsy was performed approximately six months after the treatment. Response measures were made as suggested by Grever's consensus guidelines in 2017.[12]

Patients were classified as those with morpholog-

ical evidence of disease, those with MRD+, or those without any evidence of the disease. The morphological illness was defined as lymphoid infiltrates that could be identified on sections stained with hematoxylin and eosin. MRD required the absence of lymphoid infiltrates on the hematoxylin- and eosin-stained sections but the presence of HCL-specific B cells on flow cytometry or an HCL-specific lymphoid infiltrate on immunohistochemical staining alone. The samples were considered to have no residual HCL evidence if no lymphoid infiltrates were detected in the hematoxylin- and eosin-stained sections, < 5% CD20+ B lymphocytes, and no flow cytometric evidence of monoclonal B cells.

Statistical Analysis

Data were analyzed with IBM SPSS V23. Kaplan-Meier method was used for survival analysis. The conformity to the normal distribution was evaluated using the Shapiro-Wilk test. Categorical variables according to groups Chi-square and Fisher's Exact tests were used for comparison. An independent two-sample t-test was applied to compare normally distributed data according to paired groups. The Mann-Whitney U test was performed to compare non-normally distributed data. The data were presented as the mean ± SD for quantitative data. Categorical data as deviation and median (minimum: maximum) were presented as frequency (percentage). The significance level was set to $p < 0.05$.

Table 1. Demographic and clinical characteristics of twenty patients.

Gender (Female/Male)	4/16
Age (years)	44.5 (30:78)
Clinical Presentation	
Leukocytosis	3 (15)
Pancytopenia	14 (70)
Anemia, thrombocytopenia, bicytopenia	3 (15)
Blood values	
Haemoglobin (g/dL)	10.4 (5.6-15)
Platelets ($\times 10^9$/L)	61,000 (11,000-154,000)
Leukocytes ($\times 10^9$/L)	2,550 (1,200-97,000)
Neutrophil ($\times 10^9$/L)	755 (210-9,880)
Splenomegaly	14 (70)
Bone marrow reticulin fibrosis degree	
4+/4	10 (50)
3+/4	7 (35)
Not evaluated	3 (15)
Bone marrow cellularity	
Hypercellular	10 (50)
Hypocellular	1 (5)
Heterogeneous	9 (45)
Treatment	
IV cladribine	11 (55)
SC cladribine	9 (45)
Control bone marrow	
Normocellular	6 (30)
MRD cannot be excluded	7 (35)
Not done	7 (35)
Response status	
Complete response	18 (90)
Partial response	1 (5)
Could not be evaluated	1 (5)
Overall survival (months)	43.5 (1.5:79)
Final status	
Alive	19 (95)
Dead	1 (5)

Data were expressed n (%) or median (min:max). IV: intravenous, SC: subcutaneous, MRD: minimal residual disease.

RESULTS

The study population comprised 20 patients (4 women and 16 men). The most common complaint was fatigue. At the time of admission, 14 of the patients had pancytopenia. Fourteen of the patients had splenomegaly, and three of them were massive. The peripheral blood values included moderate anaemia (median Hgb 10.4 g/dL), thrombocytopenia (median 61×109/L), leukopenia (median 2.5×10^9/L), and neutropenia (median 0.755×10^9/L). In bone marrow examination, cellularity was hypercellular in 10 patients and reticulin fibrosis 4+/4 was detected. The demographic and clinical characteristics of the patients are summarized in Table 1. All patients were purine analogue naive at the time of inclusion. At study onset, all patients showed indications for treatment due to symptoms, peripheral cytopenia, and/or organomegaly. 2-CdA monotherapy was administered to all patients as the first-line therapy. Granulocyte-colony stimulating factor (G-CSF) was applied in the neutropenic period. 2-CdA was administered at the dose of 0.09 mg/kg in 11 patients via continuous infusion for seven days and, in 9 patients, at the dose of 0.14 mg/kg for five days via the SC route. A statistically significant difference was recorded between the median length of stay according to the cladribine groups (p = 0.003). While the median of the IV group was 22.0, the median of the SC group was 0.0. In SC application, the incidence of febrile neutropenia (FEN) was lower (22.2% vs 54.5%), albeit not statistically significant. FEN developed in 8 patients during cladribine treatment. While 6 of 8 patients who developed FEN received cladribine by the IV route, two received cladribine by the SC route. Culture positivity could be detected in 5 of 8 patients, and infection-related early mortality was observed in one patient. Reproductive characteristics of patients complicated with FEN were given in Table 2. In addition, no correlation was recorded between the level of neutropenia and FEN and between neutropenia and the length of stay. There was no difference between the route of administration and the treatment response. Time to response was longer in patients with Hgb levels < 11 g/dL relative to those with higher Hgb levels (median 30 vs 45 days) p = 0.014). There was no correlation among the spleen size, leukocyte, neutrophil, thrombocyte levels, and response time.

In the statistical analysis performed on 13 patients after excluding seven patients without a control bone marrow biopsy, a significant correlation was found between the Hgb level of < 11 g/dL and the group in which MRD could not be excluded pathologically (71% vs 28%) (p = 0.048). There was no correlation

Table 2. Culture characteristics of patients complicated with febrile neutropenia.

No	Route	Culture positivity	Culture site	Isolated pathogens	Antibiotherapy	Duration (days)	Early mortality
1	IV	Absent	-	-	Piperacillin tazobactam+metronidazole	10	Absent
2	IV	Present	Blood	*Salmonella enteritidis*	Cefepime+ciprofloxacin	14	Absent
3	IV	Absent	-	-	Piperacillin tazobactam+ciprofloxacin Meropenem	3 7	Absent
4	IV	Present	Blood	*Klebsiella pneumoniae*	Meropenem Vancomycin	36 48	Absent
5	IV	Present	Wound	*Staphylococcus aureus,* *Enterococcus faecalis*	Piperacillin tazobactam Teicoplanin Meropenem+daptomycin Colistin	15 20 7 16	Present
6	IV	Absent	-	-	Piperacillin tazobactam	10	Absent
7	SC	Present	Brain abscess	*Aspergillus fumigatus*	Liposomal Amphotericin B	30	Absent
8	SC	Present	Sputum	*Acinetobacter ursingii,* *Acinetobacter junii*	Piperacillin tazobactam Meropenem Teicoplanin	3 20 12	Absent

IV: intravenous, SC: subcutan.

Table 3. Comparative results of intravenous and subcutaneous administration.

	IV cladribine	SC cladribine	*P* value
Length of hospital stay (days)	22 (11-35)	0 (0-40)	0.003*
Febrile neutropenia	54.5%	22.2%	0.197**
Overall response rate	90.9%	100%	
Time to response (days)	45 (23-120)	30 (30-60)	1.000*

Data were expressed n (%) or median (min:max). IV: intravenous, SC: subcutaneous.
*Mann-Whitney U test, **Fisher's Exact test.

among the spleen size, leukocyte, neutrophil, thrombocyte levels, and MRD. The CR rate to treatment was 90%, and the overall response rate (ORR) was 95%. PR was detected in one patient who was administered IV 2-CdA. In one patient, response evaluation could not be performed due to early mortality after receiving IV 2-CdA; the patient died due to septic shock on the 30th day of treatment. Details about the application were given in Table 3. A statistically significant difference was found between the median overall survival (OS) of the cladribine groups ($p = 0.023$). While the median of the IV group was 51.9 months, the median of the SC group was 29.7 months. The median follow-up period in all patients was 44 months. While the median follow-up period of cladribine was 56.9 months in the IV group, it was 29.7 months in the SC group. The median OS was not reached (95% confidence interval [CI]: 1.5-79 months) (Figure 1). The median 2-year and 5-year OS was calculated as 95%. No secondary malignancy was detected during the follow-up period.

DISCUSSION

HCL is a rare indolent lymphoma. The results of cladribine treatment are quite successful for HCL. Especially the easy applicability of the SC form makes it preferable to patients and doctors. However, data on the SC form are scarce. In this study, we retrospectively evaluated the clinical features, treatments, treatment results, length of hospitalization, FEN frequency, presence of secondary malignancy, and survival of 20 patients diagnosed with HCL and being followed up between 2015 and 2021. Our study's male/female ratio was 4:1, similar to that in the literature, and the median age was 44.5 years. The median age of the patients was lower than in several studies.[24-27] At admission, 70% of the patients had pancytopenia and splenomegaly. The introduction of pentostatin and subsequent 2-CdA in the 1980s and 1990s completely changed the course of HCl with a CR of 80-90% and a long-term response rate of approximately 90% achieved long-term survival.[13,28,29] All the patients in our study were administered 2-CdA as the first-line therapy. 2-CdA was administered at the dose of 0.09 mg/kg in 11 patients via continuous infusion for seven days and, in 9 patients, at the dose of 0.14 mg/kg for five days via the SC route. G-CSF was administered to all patients during the neutropenic period.

Infections seen during cladribine treatment are among the common complications of treatment. A review by Maevis *et al.*30 reported the frequency of fever in patients treated with cladribine between 40-69%. Not all fevers may be associated with infection. In Klorshid *et al.*'s study[31], the fever frequency (related or not associated with infection) was 35%. In our study, the frequency of fever was 40%, which was compatible with the literature. Hospitalization times were significantly shorter, and the incidence of febrile neutropenia was lower with SC application than with IV application.

In Inbar *et al.*'s study[27], SC was administered to 32% of 203 HCL patients, and IV 2-CdA was administered to 68%. PFS and OS were not significantly

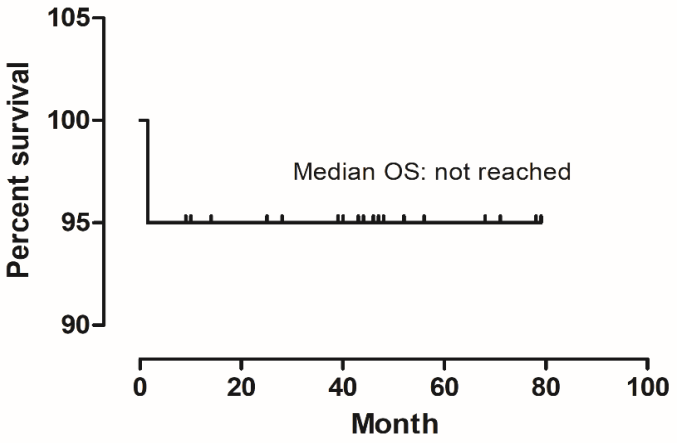

Figure 1. Survival of patients.

different in patients.[27] In our study, there was no difference between the administration route and treatment response. The complete response rate to treatment was 90%, and ORR was 95%. PR was detected in one patient who was administered IV 2-CdA. Response assessment could not be performed on a patient due to early mortality (died due to septic shock on the 30th day of treatment). The hospital stay was significantly longer in those treated with IV 2-CdA. We evaluated the treatment response by performing bone marrow control at six months. The median OS was 43.5 months (95% CI, range: 1.5-79 months), and the 2-year and 5-year OS was 95%, with a median 44-month follow-up.

Past studies have revealed a correlation between anaemia, thrombocytopenia, and disease-free survival (DFS).[28] A recent study also reported a correlation among anaemia, age, ECOG status, and survival, consistent with previous findings that link anaemia and thrombocytopenia with lower DFS. Anaemia appears to affect long-term survival and not early mortality.[29] Our study detected no significant correlation among age, spleen size, anaemia, leukocytosis, thrombocytopenia, LDH levels, bone marrow fibrosis degree, and OS. However, a statistically significant correlation was recorded among the Hgb level, treatment response, and MRD. In patients with Hgb > 11 g/dL, the response time to treatment was shorter than that in the group with a value < 11 g/dL. Moreover, while MRD was negative in the control bone marrow biopsies of patients with Hgb > 11 g/dL, MRD could not be excluded in the control bone marrow biopsies of patients with Hgb < 11 g/dL.

Other late complications, such as relapse and second neoplasms, have been described because of better control of the disease and more prolonged survival.[32] In a Swedish study, 18% of the patients had secondary primary cancer; the most significant associations were non-Hodgkin lymphoma and melanoma.[33] A survey that reported skin cancer incidence among 267 patients at the Memorial Sloan Kettering Cancer Center found a corresponding incidence of 11.3%.[34] Although it remains a controversial issue, purine analogues are believed to not contribute to the emergence of secondary malignancies.[35-38] In our study, no secondary malignancy was detected during the follow-up period. However, this result may be attributable to the small number of patients and the short follow-up period. The patients should be followed closely regarding the development of secondary malignancy in the long term.

In one of our patients, recurrence was detected in the first year of the control bone marrow biopsy, but it was followed for approximately 2.5 years without any treatment indication. The response was then obtained with the second course of cladribine. Unlike in other cases, MRD positivity was evident in this patient's 6-month control bone marrow biopsy. Recurrence may not have been observed due to our cases' relatively short follow-up period.

We did not observe any increase in the incidence of the second neoplasm in the patients in our study during the follow-up period. We believe this might be related to the small number of patients and the short follow-up period.

CONCLUSIONS

In summary, this study confirmed the efficacy of 2-CdA treatment in HCL, similar to that in the literature. In general, ease of application, good tolerance, and long-term safety data are the primary reasons for choosing 2-CdA. Moreover, we showed that the SC application is more practical than the IV application; hospitalization and febrile rates are significantly lower, and the response rates are at least as good as in the IV form. In most patients, a single course of SC 2-CdA is sufficient for long-term disease control. Although the frequency of infection is less in the SC form, severe infections may also occur in the SC form. Therefore the patients should be followed closely. We have demonstrated that the Hgb level can affect response time and MRD status; this finding deserves confirmation by further studies and more extensive series. Our response and survival data agree with the current literature, reaffirming purine analogues' role in HCL management.

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None

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

The protocol of the study was approved by the Medical Ethics Committee of Bursa Uludag University, Faculty of Medicine, Bursa, Turkey. (Decision number: 2022-4/9, date: Fabruary 2022).

Authors' Contribution

Study Conception: FÖ,; Study Design: FÖ, TE,; Supervision: VÖ, RA,; Literature Review: TE,; Critical Review: VÖ, TE,; Data Collection and/or Processing: SC, TDK,; Statistical Analysis and/or Data Interpretation: TE, İEP,; Manuscript preparing: TE.

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Original Article

Renal Involvement and Outcomes in Patients Hospitalized with COVID-19 Infection in A Tertiary Hospital

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ABSTRACT

Background Kidney involvement is seen frequently in COVID-19 patients and is essential to the prognosis. This study is undertaken to describe the clinical presentation of renal involvement in COVID-19 patients concerning acute kidney injury (AKI), chronic kidney disease (CKD) and urinary abnormalities and to correlate with the severity of COVID-19 illness and its outcome.

Material and Methods A retrospective cross-sectional study reviewed the medical records of patients admitted with COVID-19 infection who had pre-existing renal conditions or renal manifestations in the form of deranged renal function tests or abnormal urinary findings. All the relevant clinical and laboratory parameters, including the treatment details and outcome, were noted, and statistical analysis was done.

Results A total of 72 out of 1,544 patients satisfied the inclusion criteria. Hypertension (72%) and Diabetes (62%) were the commonest co-morbidities noted. CKD was seen in 51 (70%) patients, and 21 patients (29%) were on maintenance dialysis. 39 (76%) patients with CKD were diagnosed with severe COVID-19, 25 (49%) of the patients developed acute worsening of CKD, and 45% had mortality. AKI was seen in 19 patients (26%). Urinary abnormality was seen in 34 (47%) patients, out of which 27 (37%) had proteinuria of more than 1+. Haematuria was seen in 27 (37.5%) patients, of which 12(17%) had gross haematuria. Dialysis was required in 24 patients (33%) additionally. Mechanical ventilation was required in 32(44%) patients, and inotropes in 41(56%). 21 (29%) patients developed acute respiratory distress syndrome, 39 (54%) had sepsis, with six patients developing multiorgan dysfunction syndrome. 62.5% of patients had mortality. The presence of other comorbid conditions, thrombocytopenia, coagulopathy, abnormality in arterial blood gases and usage of inotropes were found to be significantly associated with adverse outcomes.

Conclusions Most cases had severe renal system involvement, with an AKI prevalence rate of 1.2% and a case-specific mortality rate of 62.5%.

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INTRODUCTION

Coronavirus disease 19 (COVID-19), caused by severe acute respiratory syndrome-coronavirus (SARS-CoV-2), a beta coronavirus, mainly involves the respiratory tract and other systems like the cardiovascular, nervous system, haemopoietic, digestive system and renal system. Kidney involvement is seen frequently and plays an important role in the prognosis of severely affected patients, especially the development of acute kidney injury (AKI).[1] The overall incidence of AKI varies between 5% to 15%[2], and it is an independent risk factor for mortality. Other renal manifestations noted are the presence of proteinuria and haematuria, proteinuria being more common.[3] COVID-19 patients suffering from chronic kidney disease (CKD) and dialysis-dependent patients are reported to be at higher risk of a severe form of the disease. They are advised to take extra preventive measures to avoid exposure to SARS-CoV-2. Kidney involvement is more likely in the presence of additional comorbidities.[4]

The coronavirus or a cytokine storm could directly attack renal impairment due to abnormal immunity.[5] Hypotension or dehydration, hypoxemia, sepsis, and nephrotoxic drugs could also be involved in developing AKI. ACE2 is considered a functional receptor of SARS-CoV-2. It is expressed in lung tissue and can also be detected in the kidneys, mainly in proximal tubules, afferent arterioles and collecting ducts. In addition, viral nucleic acid could also be found in the urine, suggesting that the kidneys might be the target of this novel coronavirus. Tubular epithelial cell necrosis, degeneration, interstitial hyperemia, microthrombus, or focal fibrosis were the main pathological features, while glomerular lesions are uncommon.

The management strategies for COVID-19-related kidney issues are largely supportive.[6] Renal replacement therapy (RRT) may be required in many critically ill patients with AKI. However, in a developing country like India with limited resources and health infrastructure, it may be challenging to combat the situation of patients with multiorgan failure. The impact of COVID-19 on patients' long-term kidney function is unclear. SARS-CoV-2's effects on the kidney and in patients with underlying kidney disease are not well-characterized. Therefore, this study aimed to describe the clinical presentation of kidney involvement in COVID-19 patients, examine the laboratory profile and treatment modality of kidney disorders, correlate the severity of COVID-19 disease with renal system involvement and know the outcome of pre-existing kidney disease.

MATERIAL AND METHODS

This retrospective study was conducted on patients admitted to a tertiary care hospital from coastal Karnataka after obtaining ethical approval from an institutional committee. All patients, including children with pre-existing kidney disease, impaired renal function tests (RFT), or abnormal urine findings at admission, diagnosed with COVID-19 by RT-PCR or rapid antigen testing between March 2020 and October 2020, were included in the study. Case files that did not have urinalysis and kidney function evaluation reports were excluded from the study.

Data regarding history, particularly the presence of the chronic renal condition and other comorbid conditions, present clinical manifestations, laboratory investigations including blood and urine examination and management of the patients, and the dialysis requirement during their hospital stay were noted. KDIGO definitions were used to define AKI based on serum creatinine and urine output. CKD was defined as eGFR <60 mL/min per 1.73 m2 or proteinuria at urinalysis within 180 days before hospital admission. Proteinuria was defined by the presence of ≥1+ protein in urine analysis. Hematuria was defined as more than five red blood cells per high-power field on centrifuged urine sample. A correlation between the severity and outcome of COVID-19 and pre-existing renal diseases, along with other comorbid conditions, was done. Development of AKI, factors leading to developing AKI and outcome with respect to the disease's mortality was noted.

Statistical Analysis

Descriptive statistics were performed using SPSS software 27.0. A chi-square test was performed to correlate the severity of COVID-19 disease with kidney symptoms. Logistic regression was used to analyse the outcome with various confounding factors.

RESULTS

A total of 1,544 patients were admitted with COVID-19 infection during the study period at our hospital. Out of these, 72 patients had renal manifestations or were known cases of renal disorders diagnosed with COVID-19 infection. The mean age in adults was 52.2 years. 2 were paediatric patients aged 11 and 15 years, respectively. Of 72, 46 (63%) were

Table 1. The frequency of symptoms and signs.

Variables	n (%)
Symptoms	
Cough	26 (36.1%)
Fever	35 (48.6%)
Reduced urine output	7 (9.7%)
Haematuria	2 (2.8%)
Breathlessness	40 (55.6%)
Edema	12 (16.7%)
Co-morbidities	67 (93.1%)
Diabetes mellitus	45 (62.5%)
Hypertension	52 (72.2%)
Ischemic heart disease	20 (27.8%)
Neurological disease	5 (6.9%)
Chronic kidney disease	50 (69%)
Other renal disease	2 (2.8%)
Maintenance haemodialysis	22 (30.6%)
Signs	
Impaired consciousness	44 (61.1%)
Tachypnea	52 (72.2%)
Tachycardia	45 (62.5%)
Reduced SPO_2	36 (50.0%)
Respiratory distress	57 (79.2%)
CVS (congestive cardiac failure)	12 (16.7%)
Per abdomen (ascites)	6 (8.3%)
Central nervous system (encephalopathy)	17 (23.6%)
High blood pressure on admission	11 (15.3%)
Shock	22 (30.6%)

male patients, and 26 (36%) were females. Out of 72 patients, four were mild, eight were moderate, and 60 (83%) were of severe category. Details of their presenting symptoms and signs and the common co-morbid condition were given in Table 1. Most of the patients presented with cough, fever and breathlessness, but common symptoms related to the renal system were oedema, reduced urine output and haematuria. Other symptoms like abdominal pain, vomiting, loose stools, headache, myalgia, generalised weakness, seizures, and altered sensorium were noted in 36 (50.0%) patients. Hypertension was noticed in 72% (52) of the patients. In contrast, diabetes was seen in 62% (45), ischemic heart disease (IHD) in 20 (27%) and neurological conditions in 5 (6.9%) of the patients. Other co-morbid conditions noted in 23 (31.9%) patients were hypothyroidism, anaemia, connective tissue disorders, chronic obstructive pulmonary disorders (COPD), asthma, obesity, chronic liver disorders, pancreatitis, peripheral vascular diseases, arrhythmias, malignancies.

Table 2. Laboratory parameters.

Variables	Mean value
Haemoglobin	9.6 g/dL
Total leukocyte count	11,531.92 cells/µL
Neutrophil/lymphocyte ratio	5:1
Platelet count	222,333.35/µl
Erythrocyte sedimentation rate	64.57 mm/hour
C-reactive protein	72.029 mg/L
Urea	140.79 mg/dL
Creatinine	6.992 mg/dL
Uric acid	8.418 mg/dL
Lactate dehydrogenase	1005.96 U/L
D-dimer	4149.14 ng/mL
Ferritin	741.19 µg/L

AKI was seen in 19 patients (26%). Twelve patients had stage 3 AKI, 5 had stage 2 AKI, and 2 had stage 1 AKI. Of 72 patients, 51 (70%) were already diagnosed with chronic kidney disease, and 21 (29%) were on maintenance dialysis. 25 (49%) of the patients developed acute worsening of chronic kidney disease. Out of 51 cases of chronic kidney disease, 39 (76%) patients were diagnosed with severe COVID-19. Out of the two paediatric patients, one patient had AKI, and the other was a CKD case on maintenance dialysis. Other renal diseases noted were renal calculi, bilateral hydroureteronephrosis, polycystic kidney disease, single kidney, pyelonephritis, IgA nephropathy and atypical haemolytic uraemic syndrome. One patient was the recipient of a kidney transplant.

Details of the investigation report were given in Table 2. Abnormal serum electrolyte levels were seen in 61 patients (84%).14 (19%) of them had sodium abnormality, hyponatremia being more common. 40 (55%) patients had hyperkalemia, and 6 had hypokalemia. Arterial blood gas (ABG) was abnormal in 45 patients, out of which 21 (29%) had metabolic acidosis, whereas 13 patients had both hypoxia and metabolic acidosis (18%), and only 11 patients had isolated hypoxia (6.9%). 24 (33%) patients had abnormal liver function tests, and 12 (16%) had coagulopathy. Urinary abnormalities were observed in 34 (47%) patients, and 27 (37%) had proteinuria greater than +1. 27 (37.5%) patients had hematuria, and 12 (17%) had gross hematuria. On the statistical analysis of laboratory parameters with the risk of development of AKI, none of the parameters was found to be associated with the development of AKI (Table 3).

Remdesevir was used in 9 (12.5%) patients. Anti-inflammatory agents were used in 41(56) % of patients. Anticoagulants were used in 40 (55%) patients, with regular heparin (unfractionated) commonly used. Dialysis was required in 24 patients (33%), apart

Table 3. The risk of acute kidney injury with the laboratory parameters.

Acute kidney injury	N	Mean ± SD	95% CI for mean		Median (IQR)	*P* value
			Lower bound	Upper bound		
Neutrophil	6	69.5000±22.49222	45.8959	93.1041	80.00 (73.25, 86.00)	0.513
(cells/µL)	11	74.9391±26.61532	57.0587	92.8195	83.00 (70.00, 93.00)	
Lymphocyte	6	20.1000±19.89321	-.7766	40.9766	11.00 (8.425, 18.250)	0.339
(cells/µL)	11	9.9264±8.46863	4.2371	15.6157	9.00 (1.700, 15.00)	
CRP (mg/L)	6	50.300±37.9401	10.484	90.116	90.00 (61.675, 90.00)	0.218
	11	76.727±19.6728	63.511	89.944	81.00 (60.00, 90.00)	
LDH (U/L)	6	518.17±271.806	232.92	803.41	465.50 (302.75, 681.75)	0.421
	11	1047.36±1607.228	-32.39	2127.11	632.00 (345.00, 900.00)	
D-dimer	6	3062.67±1789.710	1184.48	4940.85	3460.00 (1559.25, 5521.75)	0.087
(ng/mL)	11	5779.18±3433.313	3472.65	8085.71	4703.00 (2000.00, 9732.00)	
Ferritin	6	463.00±446.152	-5.21	931.21	1000.00 (594.00, 1000.00)	0.137
(µg/L)	11	783.64±339.815	555.35	1011.93	1000.00 (648.00, 1000.00)	

CI: confidence interval, CRP: C-reactive protein, LDH: lactate dehydrogenase.

from those on maintenance dialysis, including both 6 AKI patients and the other 18 patients who had acute worsening of a pre-existing CKD. In another ten patients, dialysis was indicated, but it could not be done due to very poor general condition (3 AKI patients). Oxygen was required in all patients except mild cases, venturi mask being used commonly to deliver oxygen. A high-flow nasal cannula (HFNC) was used in 8 patients, and non-invasive ventilation was used in 11 patients. Mechanical ventilation was required in 32 (44%) patients. Inotropes were used in 41 (56%) patients. 54 (75%) patients had bilateral pneumonia, out of which 21 (29%) patients developed acute respiratory distress syndrome (ARDS), 39 (54%) had sepsis, with six patients developing multiorgan dysfunction syndrome (MODS). 62.5% of patients had mortality. Among AKI patients, 57.8% had mortality, and 45% had mortality among CKD patients.

The results of logistic regression used to examine clinical signs and symptoms, laboratory parameters, and treatment of kidney disorders are shown in Table 3. All variables were entered into the model. The presence of other comorbid conditions, thrombocytopenia, coagulopathy, ABG abnormality, and use of inotropes was significantly associated with adverse outcomes (Table 4).

DISCUSSION

There were 72 patients in our hospital, including both paediatric and adult patients who had, for the first-time renal manifestations due to COVID-19 or were known cases of renal disorders diagnosed with COVID-19 infection. Our study's mean age at presentation was 52 years, with male predominance. A similar finding was noticed by Allemailem *et al.*[7], where most patients were males older than 50. Breathlessness (55.6%) and fever (48.6%) were the common presenting symptoms similar to the study mentioned above[7], as the majority of the cases were severe to the moderate category in both the study groups. Underlying CKD also might have contributed to the increased prevalence of breathlessness among the study group. More than 80% were classified as having severe disease and had renal involvement, either pre-existing or newly developed renal manifestations. But its significance is not measured statistically as we had not taken the COVID-19 cases without renal involvement. Apart from a kidney involvement, the most common associated co-morbidities were hypertension and diabetes mellitus, followed by ischemic heart disease. None of the above factors was independently associated with an increased risk of mortality which is in contrast to other studies.8 Still, it was noticed that additional other co-morbid conditions increased the chances of morality, like malignancy and COPD ($p = 0.02$).

In a systematic review done by Chen *et al.*[9], the incidence of COVID-19 in haemodialysis patients was 7.7%, and the overall mortality rate was 22.4% in these patients with COVID-19. Similarly, our study's mortality rate was 28% with patients on haemodialysis. So both the incidence and mortality of COVID-19 infection were higher in haemodialysis patients. Among CKD patients without dialysis, 76% had severe disease, and 45% had mortality. There was a decline in the number of patients who underwent kidney transplantation initially when the COVID-19 pandemic began. A study done by Akalin *et al.*[10] noted a very high mortality rate in kidney transplant patients, 28% at the end of 3 weeks. We had one patient with kidney

Table 4. The logistic regression studying the clinical symptoms and signs, laboratory parameters along with the mode of treatment for renal conditions with the outcome (Significance was decided at 5%.).

Variables	B	S.E.	Wald	Df	Significance	Exp (B)
Symptoms and co-morbid conditions						
Decreased urine output	-1.728	1.210	2.042	1	0.153	0.178
Haematuria	0.397	1.807	0.048	1	0.826	1.487
Chronic kidney disease	-0.090	0.537	0.028	1	0.867	0.914
Co-morbid conditions	0.739	1.258	0.345	1	0.557	2.093
Hypertension	-0.201	0.542	0.137	1	0.711	0.818
Diabetes mellitus	-0.795	0.507	2.454	1	0.117	0.452
Neurological disease	1.552	1.182	1.726	1	0.189	4.723
Ischemic heart disease	-0.961	0.729	1.739	1	0.187	0.382
Maintenance haemodialysis	0.363	0.665	0.298	1	0.585	1.438
Other renal diseases	-0.902	1.339	0.454	1	0.501	0.406
Other co-morbidities	-2.645	1.149	5.295	1	0.021	0.071
Signs						
Conscious level	-0.194	0.873	0.049	1	0.824	0.824
Blood pressure	-0.274	0.480	0.325	1	0.569	0.760
SPO_2	-0.367	0.820	0.200	1	0.655	0.693
Respiratory system	-1.939	1.100	3.109	1	0.078	0.144
Per abdomen	2.113	1.639	1.663	1	0.197	8.276
Cardiovasculur system	-1.311	1.074	1.489	1	0.222	0.270
Central nervous system	-2.019	1.081	3.487	1	0.062	0.133
Laboratory investigations						
Haemoglobin	-0.003	0.165	0.000	1	0.984	0.997
Total count	0.000	0.000	1.256	1	0.262	1.000
Erythrocyte sedimentation rate	-0.004	0.015	0.064	1	0.800	0.996
Urea	0.001	0.006	0.020	1	0.887	1.001
Creatinine	-0.107	0.087	1.506	1	0.220	0.898
Liver function test	-0.310	0.801	0.150	1	0.699	0.733
C-reactive protein	-0.027	0.014	3.809	1	0.051	0.973
Coagulopathy	-2.574	1.309	3.865	1	0.049	0.076
Urine abnormality	2.158	2.603	0.687	1	0.407	8.652
Proteinuria	-1.457	0.825	3.119	1	0.077	0.233
Haematuria	-0.368	1.363	0.073	1	0.787	0.692
Lactate dehydrogenase	0.000	0.000	0.257	1	0.612	1.000
D-dimer	0.000	0.000	2.625	1	0.105	1.000
Arterial blood gas analysis	-0.510	0.255	4.002	1	0.045	0.601
Acute kidney injury	0.548	0.793	0.478	1	0.489	1.730
Ferritin	-0.003	0.002	3.079	1	0.079	0.997
Sodium	0.050	0.040	1.543	1	0.214	1.051
Potassium	0.061	0.118	0.270	1	0.604	1.063
Thrombocytopenia	0.051	0.017	9.215	1	0.002	1.052
Treatments						
Anti-inflammatory	-1.474	1.344	1.202	1	0.273	0.229
Anticogulants	0.991	1.310	0.573	1	0.449	2.694
Inotropes	-3.459	1.037	11.122	1	0.001	0.031
Severity	-19.946	8589.258	0.000	1	0.998	0.000
Dialysis	-1.061	0.551	3.709	1	0.054	0.346
Ventilation	-0.349	0.238	2.159	1	0.142	0.705

transplantation who had expired.

In our study, 27 (37%) patients had proteinuria of more than 1+ and haematuria was seen in 27 (37.5%) patients. Still, in a study by Vasist *et al*.[11], proteinuria was positive in 75 patients (17.6%) and hematuria in 39 patients (9.15%), which is lower than our study. They also noticed that patients with proteinuria and/or hematuria were more likely to have severe COVID-19 illness. More than 80% of cases were severe COVID-19 cases in our study, which may explain the higher incidence of proteinuria and haematuria.

AKI was seen in 19 patients, which accounts for 1.2% of the total admissions with COVID-19 during the same period, which is lower compared to 39.9% of AKI in COVID-19 hospitalised patients as observed by Jia *et al*.[12]. They also noticed that among AKI cases which required dialysis, 79.3% patients expired with 30.6% of survivors remaining on dialysis at the time of discharge. In contrast, in our study, it was around 57% of mortality, which is slightly lower. Smarz *et al*.[13] observed that elevated leukocytes with neutrophil predominance and elevated D-dimers were associated with an increased risk of AKI, which correlates with the severity of COVID-19. However, in our study, none of the parameters was significantly associated with AKI. Remdesivir was used in a low percentage of cases due to renal impairment, which limits its use. Similarly, regular heparin was used as fractioned heparin usage is avoided with renal dysfunction. Głowacka *et al*.[14] reviewed articles on AKI and COVID-19 and noticed that 64% of critically ill COVID-19 patients required dialysis who had AKI. Still, in our study, additional dialysis was required in 24% of patients, including AKI and acute on CKD.

We observed increased mortality with thrombocytopenia and coagulation abnormalities; similar findings were noticed in other studies.1[5,16] Metabolic acidosis (29%) is the predominant ABG finding noted in our research, contributed by kidney disease. In a study by Alfano *et al*.[17], metabolic acidosis was noticed in 2.8% of all COVID-19 patients, which is much lower than our study. Still, all of them had mortality, and the authors noted that they had high SOFA scores with kidney impairment.

In a study done by Sindhu *et al*.[18], it was noticed that inotropes usage and mechanical ventilation were associated with an increased risk of poor outcomes. In contrast, as in our study, only inotropes were noted to be significant on logistic regression. Though the statistical presence of AKI, CKD or mechanical ventilation has not come significant on logistic regression analysis due to the interrelation of confounding factors, and the majority of cases were of severe category, which might have led to an inconclusive picture in the end, it gives the scope for further exploration, as their clinical significance can not be undetermined. Also, long-term effects, particularly on kidney function and persistence of proteinuria or haematuria, should be investigated further. In case of persistent proteinuria or haematuria at intensive care unit discharge, COVID-19 patients should be advised for follow-up by a nephrologist, as the course of the long-term kidney involvement is still unclear.

CONCLUSIONS

Most of the cases were in a severe category, with 62.5% case-specific renal system involvement and mortality. The development of AKI and the presence of CKD were responsible for increased mortality and morbidity.

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Ethical Approval

The protocol of the study was approved by the Yenepoya Ethics Committe-1, University Road, Deralakatte, Mangalore-575018 India, (Decision number: YEC-1/2020/071, date: Novamber 2020).

Authors' Contribution

Study Conception: SKS, SPBH,; Study Design: SKS, RG,; Supervision: SKS, SPBH,; Literature Review: SKS, RG,; Critical Review: SKS, SPBH,; Data Collection and/or Processing: SKS, RG,; Statistical Analysis and/or Data Interpretation: SKS, SPBH,; Manuscript preparing: SKS, SPBH, RG.

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Original Article

Factors Affecting Kidney Functions in One-year Follow-up After COVID-19 in Kidney Transplant Patients

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ABSTRACT

Background Coronavirus disease (COVID-19) is more severe, and mortality is higher in kidney transplantation (KTx) patients; it is still unclear how renal functions progress and the conditions affecting renal functions in the post-COVID-19 period. We aimed to investigate the changes in kidney functions and the factors affecting this change after COVID-19.

Material and Methods Forty-one kidney transplantation patients who were hospitalised for COVID-19 were included in this retrospective study. The patient's personal information, examination, and treatment information regarding their hospitalisation and follow-ups were obtained from the hospital system.

Results Patients with elevated serum creatinine in the first year post-COVID had higher baseline proteinuria and systemic immune inflammation index (SII). Proteinuria increased more in patients with a long transplantation period, hypertension, high basal creatinine, and SII. Also, proteinuria was higher in patients who developed AKI during the COVID period. In addition, baseline SII was an independent predictor of the change in serum creatinine and proteinuria.

Conclusions We found that patients with signs of increased inflammation, such as high SII were more fragile regarding renal functions. Therefore, the post-COVID-19 follow-up process of KTx patients with COVID-19 should be individualised.

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INTRODUCTION

Although the primary affected system of the coronavirus disease - 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the respiratory system, the condition also frequently affects other organ systems. Renal involvement is commonly reported in patients hospitalised with COVID-19 and may be detected from isolated hematuria and proteinuria to acute kidney injury (AKI) requiring renal replacement therapy.[1] Many mechanisms thought to cause renal involvement have emerged in COVID-19. Some of these mechanisms are direct viral cytopathic effect on the kidney, dehydration, tissue hypoxia, systemic inflammation (cytokine storm), immune complex deposition, and diffuse intravascular coagulation. Tubular, glomerular, or vascular damage may occur in the kidney. In histopathology studies, acute tubular necrosis is the most common type of injury. Tubular and vascular damage occurs mainly in the form of AKI, and glomerular damage occurs in the form of proteinuria, while co-occurrence of these findings is a typical and expected situation.[2-4]

Considering the multiple comorbidities and immunosuppressive treatments of kidney transplant patients (KTx), a more severe disease course is inevitable during COVID-19. KTx patients are more likely to develop AKI and need haemodialysis (HD) than the average population.[5] However, it is thought that the immunosuppressive state in KTx patients may also have protective effects against the systemic inflammatory response responsible for the severe disease in the course of COVID-19.[6]

Derived inflammation markers such as neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are more sensitive inflammatory markers than leukocytes, neutrophils or lymphocytes.[7] While the systemic immune inflammation index (SII) was a significant prognostic indicator used only in malignant patients initially, it has become an essential indicator of inflammation in different patient groups, recently in Ktx patients and COVID-19.[8,9] SII is an independent predictor of albuminuria in the general population.[10]

Although it is well known that the course of COVID-19 is more severe and mortality is higher in KTx patients, it is still not clear how kidney function progresses in the post-COVID-19 period and the conditions that affect its course. Immune dysregulation and increased proinflammatory cytokine levels occurring during the response to SARS-CoV-2 are considered the primary cause of tissue damage. Considering the negative effect of increased inflammation on graft functions, the impact of the hyperinflammatory state in the COVID period on long-term renal functions is also a matter of interest. Based on this idea, we aimed to investigate the changes in renal functions and the factors affecting this change one year after COVID-19.

MATERIAL AND METHODS

Ethics committee approval was obtained from our institution for this retrospective study. The data of 75 kidney transplant patients hospitalised with the diagnosis of COVID-19 between January 1 and March 31, 2021, were scanned retrospectively from our hospital system. Twenty patients with negative COVID-19 PCR results, five who died during hospitalisation, and nine whose data for the first year could not be reached after the COVID-19 diagnosis date were excluded from the study. Forty-one patients whose data could be entirely determined were the study group. A review of medical records (including information on age, sex, weight, medications, duration of the disease, and laboratory results) was done. Inclusion criteria were: 1) Being a KTx patient aged 18-60. 2) Having a positive COVID-19 PCR result. 3) Having hospital records in the 1st year after the diagnosis of COVID-19. Exclusion criteria were: 1) being under 18 and over 60, 2) Negative COVID-19 PCR result, 3) Presence of pregnancy, 4) history of acute rejection in the last three months or positive donor-specific antibody test (DSA), 5) presence of previously detected proteinuria over 500 mg/day. The patients included in the study were divided into two groups according to their creatinine levels and proteinuria levels based on the 1st year post-COVID-19 data. In these groups, those whose creatinine level increased and non-increased; were divided into those whose proteinuria level increased and non-increased.

Obtaining Baseline Data and Post-COVID First Year Data

Basal values were accepted on the day when the COVID-19 PCR test of the patients was positive or on the day of treatment was started by hospitalisation. The control performed at least one year after the diagnosis of COVID-19 was accepted as the first year of control examinations. The treatments applied to the patients during the hospitalisation, and the changes made in the immunosuppressive therapies were obtained by scanning the hospital registry system. All patients included in the study had negative DSA tests

before and after COVID-19. DSA test positivity was taken as an exclusion criterion as it would cause suspicion of graft rejection.

Biochemical Analyses

Venous blood samples were drawn after an overnight fast and stored at -80 °C for biochemical analyses in patients. All biochemical studies were undertaken in the Central Biochemistry Laboratory of our hospital. Serum creatinine was measured with Jaffe Method. Using an automated clinical chemistry analyser, serum C-reactive protein (CRP) levels were measured with an immunoturbidimetric assay (Diasis Diagnostic System). Serum levels of calcium, phosphate, and intact parathyroid hormone (iPTH) were measured. iPTH was measured using the Elecsys PTH assay.

24-hour Urinary Proteinuria

The 24-hour urinary proteinuria levels detected within the first three days of hospitalisation were recorded as initial proteinuria. Total protein concentration levels were measured by a turbidometric assay using benzethonium chloride. The results were expressed as mg/L.

Calculation of the Systemic Immune Inflammation Index

The SII value was calculated according to the basal blood values of the patients. The NLR x platelet count formula was used to calculate SII.11

Statistical Analyses

The data obtained were evaluated using the Statistical Package for Social Sciences for Windows 21.0 (SPSS Inc. Chicago, Illinois, USA) statistical program. Descriptive statistics were determined for each variable. Data were expressed as mean±standard deviation or median and interquartile range (IQR). The $\chi 2$ test for categorical variables determined a statistically significant difference between the groups. Non-parametric statistics (Mann-Whitney U test) and parametric statistics (independent sample t-test) were all used for continuous variables. Associations between the variables were explored using Spearman's rho test. Binary logistic regression analysis was performed to determine independent predictors for increased proteinuria and serum creatinine levels. Factors with a *p* - value of < 0.2 were included in the univariate analysis in the regression test, while those significant in the univariate analysis were included in the multivariable evaluation. A statistically significant difference was considered when the *p* - value <0.05.

RESULTS

Demographic Characteristics of Patients and Pre-COVID-19 Data

A total of 41 kidney transplantation patients, 14 (34%) female and 27 (66%) male, with a mean age of 45.96 ± 9.46 years, who met all inclusion and exclusion criteria, were included in the study. The most common comorbidity was hypertension (n: 26, 61.9%). Of 41 patients, 9 (22%) were transplanted from deceased donors and 32 (78%) from living donors. Before COVID-19, mean creatinine values were 1.45 mg/dL, and 24-hour urinary proteinuria averages were 285 mg/L. The demographic characteristics of the patients and the initial data for the diagnosis of COVID-19 and pre-hospitalization were in Table 1.

Characteristics of Patients during Treatment of COVID-19

All patients whose diagnosis was confirmed by the COVID-19 PCR test were included in the study.

Table 1. Demographic, clinical characteristics, and biochemical parameters of 41 kidney transplant patients with COVID-19

Parameters	Values
Age (years)	45.96 ± 9.46
Gender (Female/Male)	14/27
Body mass index (kg/m^2)	25.26 ± 3.76
Posttransplant period (years)	7.5 (7)
History of diabetes mellitus	7 (16.7%)
History of hypertension	26 (61.9%)
History of coronary artery disease	6 (14.3%)
COVID-19 associated AKI	14 (34.1%)
Sistolic blood pressure (mmHg)	128.41 ± 11.96
Diastolic blood pressure (mmHg)	76.46 ± 10.2
Creatinine (mg/dL)	1.45 (0.55)
White blood count (10^3/uL)	7.37 (4.13)
Haemoglobin (g/dL)	12.75 ± 2.07
Platelet count (10^3/mm^3)	220.27 ± 77
Albumin (g/L)	4.17 ± 0.52
Alanine aminotransferase (U/L)	15.5 (11.3)
Proteinuria (mg/L)	285 (431)
Lactate dehydrogenase (U/L)	147 (74)
Ferritin (mg/dL)	220 (898.09)
C-reactive protein (mg/L)	10.79 (34.42)
SII	684.5 (683.19)

Data were expressed as mean±SD, median (IQR) or frequency. AKI: acute kidney injury, SII: systemic immune inflammation index.

Table 2. Changes in immunosuppressive therapies during COVID-19.

Medications	Continued	Stopped or reduced	Total
Glucocorticoids	41 (100%)	0	41 (100%)
Tacrolimus	24 (75%)	8 (25%)	34 (82%)
Cyclosporin	2 (50%)	2 (50%)	4 (10%)
Mycophenolic acid analogue	7 (19%)	29 (81%)	36 (87%)
m-TOR inhibitor	0	4 (100%)	4 (10%)
Azathioprine	0	2 (100%)	2 (5%)

Data were expressed as n (%). m-TOR: mammalian target of rapamycin.

When evaluated with thorax computed tomography, 11 (26%), patients did not have any involvement in the lung, while 30 (72%) patients had different degrees of involvement. All of the patients were receiving various immunosuppressive treatments before COVID-19, and multiple changes were made in their treatments during the disease period. Treatment changes of all patients were shown in Table 2. Of the 41 patients included in the study, 26 (63%) were receiving angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) treatments. Treatments of 16 (61%) patients, including 14 patients who developed AKI, were stopped during their hospitalisation. ACEi/ARB treatment was started again in all patients at least one month after discharge. The mean hospital stay of the patients was 11.09 days, and 9 (22%) patients required follow-up in the intensive care unit for varying periods. The treatments for COVID-19 during their hospitalisation were also evaluated. Hydroxychloroquine in 16 (39%) patients, favipiravir in 39 (95%) patients, pulse steroid in 27 (66%) patients, tocilizumab in 11 (27%) patients, and plasma infusion in 13 (32%) patients were used.

Characteristics of Patients Developing Acute Kidney Injury during COVID-19

During the hospitalisation period, acute kidney injury (AKI) developed in 14 (34.1%) 41 patients in our

Table 3. Comparison of demographic, clinical characteristics and biochemical parameters of patients according to increase in serum creatinine value.

Parameters	Increased creatinine (n: 29)	Stable creatinine (n: 12)	P value
Age (years)	44.93 ± 9.3	48.5 ± 9.75	0.277
Gender (Female/Male)	12/17	2/10	0.129
Body mass index (kg/m^2)	25.16 ± 4.09	25.52 ± 2.96	0.787
Post-transplant period (year)	7.5 (5)	8 (8)	0.667
History of diabetes mellitus	6 (20.6%)	1 (8.3%)	0.339
History of hypertension	18 (62%)	8 (66.6%)	0.781
History of coronary artery disease	5 (17.2%)	1 (8.3%)	0.463
COVID-19 associated AKI	11 (38%)	3 (25%)	0.427
Sistolic blood pressure (mmHg)	129.31 ± 12	126.25 ± 12.08	0.463
Diastolic blood pressure (mmHg)	77.07 ± 11.14	75 ± 7.68	0.561
Creatinine (mg/dL)	1.34 (0.67)	1.6 (0.69)	0.339
White blood count (10^3/uL)	7.37 (4.04)	5.97 (4.6)	0.623
Haemoglobin (g/dL)	12.45 ± 2	13.53 ± 2.1	0.125
Platelet count (10^3/mm3)	229.31 ± 80.6	198.42 ± 65.48	0.247
Albumin (g/L)	4.07 ± 0.55	4.4 ± 0.36	0.065
Alanine aminotransferase (U/L)	16.85 (12.8)	14.7 (9.7)	0.501
Proteinuria (mg/L)	320 (601.8)	161.5 (282.8)	0.046
Lactate dehydrogenase (U/L)	147 (74)	142 (119.8)	0.311
Ferritin (mg/dL)	230.5 (533.25)	145 (1339.6)	0.423
C-reactive protein (mg/L)	3.11 (34.32)	10.8 (50.43)	0.770
SII	823.14 (730.91)	412.39 (352.86)	0.013

Data were expressed as mean ± SD, median (IQR) or frequency. AKI: acute kidney injury, SII: systemic immune inflammation index.

Table 4. Comparison of demographic, clinical characteristics and biochemical parameters of patients according to increase in amount of proteinuria.

Parameters	Increased proteinuria (n: 25)	Stable proteinuria (n: 16)	*P* value
Age (years)	46.68 ± 10	44.87 ± 8.62	0.558
Gender (Female/Male)	8/17	6/10	0.717
Body mass index (kg/m^2)	25.19±3.68	25.37 ± 4.02	0.883
Post-transplant period (year)	9 (5)	4 (7)	0.001
History of diabetes mellitus	5 (20%)	2 (12.5%)	0.534
History of hypertension	20 (80%)	6 (37.5%)	0.006
History of coronary artery disease	5 (20%)	1 (6.25%)	0.224
COVID-19 associated AKI	13 (52%)	1 (6.25%)	0.003
Sistolic blood pressure (mmHg)	131.6 ± 10.17	123.44 ± 13.13	0.031
Diastolic blood pressure (mmHg)	79.2 ± 8.74	72.19 ± 11.1	0.03
Creatinine (mg/dL)	1.67 (1.88)	1.17 (0.58)	0.001
White blood count (10^3/uL)	7.8 (16.23)	5.18 (2.91)	0.024
Haemoglobin (g/dL)	12.49 ± 2.1	13.16 ± 2.01	0.317
Platelet count (10^3/mm3)	235.72 ± 83.75	196.13 ± 59.74	0.109
Albumin (g/L)	4.07 ± 0.58	4.32 ± 0.38	0.110
Alanine aminotransferase (U/L)	23 (19.5)	13.1 (5)	0.058
Proteinuria (mg/L)	311 (545)	280 (233)	0.066
Lactate dehydrogenase (U/L)	147 (58.5)	145 (109)	0.135
Ferritin (mg/dL)	291 (969)	189 (421)	0.658
C-reactive protein (mg/L)	13.2 (63.09)	7 (17.17)	0.046
SII	868.44 (829.46)	529.96 (448.93)	0.050

Data were expressed as mean±SD, median (IQR) or frequency. AKI: acute kidney injury, SII: systemic immune inflammation index.

study. According to the KDIGO AKI classification, 4 (28%) patients were evaluated as stage-1, 3 (21%) patients as stage-2, and 7 (50%) patients as stage-3 AKI. 7 (50%) of the 14 patients who developed AKI needed HD. The mean HD session duration was 3.77 sessions. None of the patients who developed AKI needed HD at discharge.

First-Year Data after COVID-19

In the post-COVID first year, 29 (70.7%) patients with increased serum creatinine compared to the pre-COVID period and 12 (29.3%) patients with a stable course were detected. The two groups had no significant difference regarding age, gender, body mass index, time spent post-transplant, diabetes mellitus, hypertension, history of coronary artery disease, and blood pressure (Table 3).

Interestingly, no significant difference was observed between the two groups regarding basal creatinine levels and AKI development during the COVID-19 process. AKI developed during the period of COVID-19 in 11 (38%) patients in the patient

Table 5. Binary logistic regression analysis with other parameters of the increase in amount of proteinuria in kidney transplant patients with COVID-19.

Parameters	Univariable analysis		Multivariable analysis	
	OR (95% CI)	P value	OR (95% CI)	*P* value
Post-transplant period (years)	1.469 (1.14-1.89)	0.003	1.204 (0.86-1.67)	0.269
History of hypertension	6.667 (1.63-27.27)	0.008	1.562 (0.19-12.76)	0.677
COVID-19 associated AKI	16.250 (1.85-142.46)	0.012	6.244 (0.53-73.19)	0.145
Sistolic blood pressure (mmHg)	1.066 (1.003-1.133)	0.039	1.036 (0.91-1.17)	0.590
Diastolic blood pressure (mmHg)	1.094 (1.004-1.192)	0.040	0.996 (0.86-1.15)	0.959
White blood count (103/uL)	1.00 (1.00-1.00)	0.059	-	-
Creatinine (mg/dL)	16.940 (1.56-183.06)	0.020	3.789 (0.28-49.68)	0.310
C-reactive protein (mg/L)	1.023 (0.99-1.05)	0.087	-	-
SII	1.002 (1.00-1.003)	0.012	1.001 (1.00-1.002)	0.048

CI: confidence interval, AKI: acute kidney injury, SII: systemic immune inflammation index.

Table 6. Binary logistic regression analysis with other parameters of the increase in serum creatinine values in kidney transplant patients with COVID-19.

Parameters	Univariable analysis		Multivariable analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Gender (Male/Female)	0.785 (0.257-0.890)	0.043	0.283 (0.052-1.53)	0.143
Haemoglobin (g/dL)	0.764 (0.54-1.08)	0.129	-	-
Albumin (g/L)	0.225 (0.04-1.16)	0.075	-	-
Proteinuria (mg/L)	1.002 (0.999-1.004)	0.180	-	-
SII	1.004 (1.001-1.007)	0.028	1.002 (1.00-1.005)	0.039

CI: confidence interval, SII: systemic immune inflammation index.

group with increased serum creatinine and 3 (25%) patients in the other group, but it was not statistically significant ($p = 0.427$). While the mean of basal creatinine in the group with stable serum creatinine was 1.6 mg/dL, it was 1.34 mg/dL in the other group ($p = 0.339$). The mean basal proteinuria in the group with increased serum creatinine was 320 mg/L, while the other group had 161.5 mg/L ($p = 0.046$). While there was no significant difference in the peripheral blood variables (haemoglobin, white blood count, platelet count) of the two groups, the SII value was found to be significantly higher in the group with increased creatinine level (823.14 vs 412.39, $p = 0.013$) (Table 3).

Patients were divided into two groups according to the increase and stability of proteinuria in the first year of follow-up after COVID. There were 25 (60.9%) patients in the group with increased proteinuria and 16 (39.1%) patients in the other group. The two groups had no significant difference regarding age, gender, body mass index, and basal proteinuria. In the group with increased proteinuria, the time elapsed after KTx was longer ($p = 0.001$), the history of hypertension was more common ($p = 0.006$), and systolic and diastolic blood pressures were higher ($p = 0.031$ and 0.03, respectively). While the mean basal creatinine was 1.67 mg/dL in the increased proteinuria group, it was 1.17 mg/dl in the other group ($p = 0.001$). In addition, 13 (52%) patients in the increased proteinuria group and 1 (6.25%) patient in the other group developed AKI during the COVID-19 period ($p = 0.003$). When inflammatory markers were evaluated, both CRP (13.2 vs 7 mg/L) and SII (868.44 vs 529.96) were found to be higher in the increased proteinuria group ($p = 0.046$ and $p = 0.05$, respectively) (Table 4).

Evaluation of Factors Associated with Increase in Proteinuria and Serum Creatinine

Parameters associated with an increase in proteinuria at one-year post-COVID were evaluated. In the univariable analysis, the time elapsed post-KTx, history of hypertension, AKI developed during COVID-19, systolic and diastolic blood pressure elevations, serum creatinine, and SII, were associated with post-COVID increased proteinuria. However, only SII was associated with increased proteinuria in the multivariable analysis ($p = 0.048$) (Table 5). Also, associated factors with increased serum creatinine were evaluated in the multivariable analysis. SII alone was an independent predictor of the change in serum creatinine value ($p = 0.039$) (Table 6).

DISCUSSION

The study investigated the changes in renal functions and the factors affecting this change in the one-year follow-up of KTx patients diagnosed with COVID-19. Patients with elevated serum creatinine in first-year post-COVID had higher baseline proteinuria and SII. We found that patients with long transplantation time, hypertension and high basal creatinine and SII values had higher proteinuria increases in the follow-up. The development of AKI during the COVID period increased the risk of proteinuria in the long term. Finally, we showed that baseline SII was an independent predictor of the change in serum creatinine and proteinuria.

In COVID-19, renal damage can be seen in a wide range, from hematuria and proteinuria to developing severe AKI that may require RRT. While the prevalence of acute kidney injury (AKI) is approximately 17% in all populations during the COVID-19 period[12], publications are reporting that it is very high, such as 45-83% in KTx patients.[13-15] AKI developed during hospitalisation in 34.1% of our patient group, and HD need in 50% of these patients. In COVID-19, the risk of developing AKI and the need for HD are higher in KTx patients than in the normal population.[5] The frequency of proteinuria in patients followed up for

COVID-19 has been reported as 10-45%, and the severity of proteinuria is directly related to mortality.[13,16] In our study, the rate of patients with newly developed proteinuria was 60%. This difference between studies is due to the severity of COVID-19. While severe disease, predominantly with tubular damage, may cause more moderate proteinuria and accompanying higher creatinine levels, higher proteinuria levels may be detected in patients with milder disease.[17] In our study group, it was impossible to differentiate tubular or glomerular damage due to the failure of any patient to undergo a kidney biopsy.

Knowing the mechanisms that cause renal damage in COVID-19 is essential for what we should pay attention to in the follow-up of renal functions. While systemic inflammation (cytokine storm) and immune dysregulation are the primary factors causing organ damage, acute tubular injury is the most common histopathological finding.[2-4] The association of inflammatory markers with COVID-19 has always been of interest, as systemic inflammation and immune dysregulation are significant causes of renal injury during COVID-19. Inflammatory markers have been used to diagnose the disease or predict its prognosis, but there is no study on how they can predict damage in any organ system in the long term. SII is a stronger predictor of inflammatory status than traditional inflammatory markers such as NLR, PLR, and monocyte/lymphocyte ratio (MLR).[11,18,19] In COVID-19, it has been determined that the prognostic value of SII is stronger than inflammation indicators such as NLR, derived NLR (d-NLR), MLR, and PLR.[20,21] The usefulness of SII in identifying COVID-19 patients at higher risk of death is attributed to the different roles played by lymphocytes, neutrophils, and platelets during the disease response.[22]

Our study is the first to show that SII, an important indicator of systemic inflammation, is associated with increased creatinine and proteinuria in the long term after COVID-19. Kidney injury during COVID-19 results from increased local and systemic inflammation and microthrombotic events on both tubules and glomeruli.[3,4] The relationships between tubular injury markers with increased interleukin 6 (IL-6) level, collapsing glomerulopathy and inflammatory cytokine production, and systemic inflammation markers such as NLR and CRP with the severity of renal injury are findings that draw attention to the role of inflammation in renal injury in COVID-19. Considering all these findings, SII, one of the strong indicators of systemic inflammation, may also be an important predictor in the relationship between COVID-renal dysfunction.[23,24] Patients with high SII values may be exposed to more renal inflammation and have worse renal functions in the long term. Many studies are showing that, in COVID-19, SII is a useful indicator to predict the development of AKI, that patients with AKI have higher white blood cell and CRP levels than patients without AKI, and patients with evidence of renal involvement, have higher white blood cell counts and lower lymphocyte count. All these studies support our hypothesis.[18,25,26]

Neutrophil-related endothelial damage is the other mechanism thought to cause renal injury in COVID-19 patients.1[2,25,27,28] Tumor necrosis factor (TNF) overactivation increases the oxidative stress of the glomeruli in addition to direct renal endothelial damage. Cytokines such as interferons (IFN: alpha and beta) also cause podocyte dysfunction and glomerulosclerosis.[29] The increased neutrophil activity also causes platelet activation, increasing inflammatory cytokine production and microthrombus formation.[30] Evidence of extensive endothelial inflammation, inflammatory cell deposits, and endotheliitis in post-mortem autopsies supports this theory.[30] Proteinuria is a consequence of this hyperinflammatory state.[31] Over time, the increased expression of all these cytokines and the role of local tissue inflammation in renal cells have become more evident in COVID-19.[32] Since high SII is an indicator of increased neutrophil and platelet counts and indirectly increased activity of these two cells, it can be considered an indirect indicator of endothelial damage.

SII is helpful with other inflammatory markers to predict prognosis after kidney transplantation.[33] In addition, when studies reporting the relationship between delayed graft function and the development of acute rejection and inflammatory markers[34] and the relationship between high NLR-PLR and the development of immunological damage are evaluated, SII may be an important indicator of both renal and overall survival in KTx patients. The role of SII in predicting renal survival in KTx patients includes neutrophilia and thrombocytosis that occur during ischemia-reperfusion injury, which contributes to the formation of immunogenic microthrombi in renal vascular structures.3[5,36]

Regardless of allograft function and underlying renal allograft histology, the level of proteinuria at any time after transplantation is one of the main deter-

minants of graft survival.[37] Bajpai *et al*.[13] found that basal proteinuria level is an independent predictor of renal survival in the follow-up of renal functions after COVID-19. Our study found that creatinine increases were higher in the first year in patients with higher initial proteinuria. In addition, it was determined that transient proteinuria due to post-COVID febrile disease resolved within three weeks. It is also known that the increase in proteinuria in the follow-up indicates increased long-term mortality in KTx patients.[16] Therefore, proteinuria detected in the COVID-19 period should be followed up, and transient proteinuria should be differentiated from long-term proteinuria in the early period.

We found that patients with increased proteinuria during long-term follow-up developed more AKI during COVID-19. AKI, proteinuria, and hematuria developing during COVID-19 are independently associated with a higher risk of death.[26,38] This can be explained by the increase in the severity of the disease and the increase in organ dysfunctions. The demonstration that patients who develop proteinuria during hospitalisation have a worse prognosis can also be interpreted as the renal reflection of the systemic inflammatory state. Although publications report that 35-60% of patients who develop AKI during COVID-19 have permanent deterioration in renal function during follow-up[13,39], there are also studies showing all patients who develop AKI return to their initial kidney functions.[15] This difference is because the severity of the disease differs between studies.

As in our study group, hypertension is the most common co-morbidity in COVID-19 patients and is a risk factor for severe illness and death. Impaired hypertension regulation is also an independent predictor of the development of AKI.40 We found that creatinine and proteinuria increases were higher in patients with a history of hypertension during follow-up. The relationship between hypertension and COVID-19 is explained by the interaction of the virus with ACE2, the gateway to hosting cells[41], and endothelial dysfunction.[42] Tubular and glomerular visceral epithelial cells of the kidney are the main targets of SARS-CoV-2. The resulting inflammatory state increases endothelial damage while the endothelium is not expected to be directly infected with SARS-CoV-2.[28] Our patient group also found that blood pressures were higher than basal levels at the end of follow-up in groups with both proteinuria and creatinine increases. Other reasons for the deterioration in blood pressure regulation in our patients may be the discontinuation of RAS blocker drugs in the early stages of the epidemic, with the belief that ACEi or ARB drugs adversely affect the course of the disease or the fact

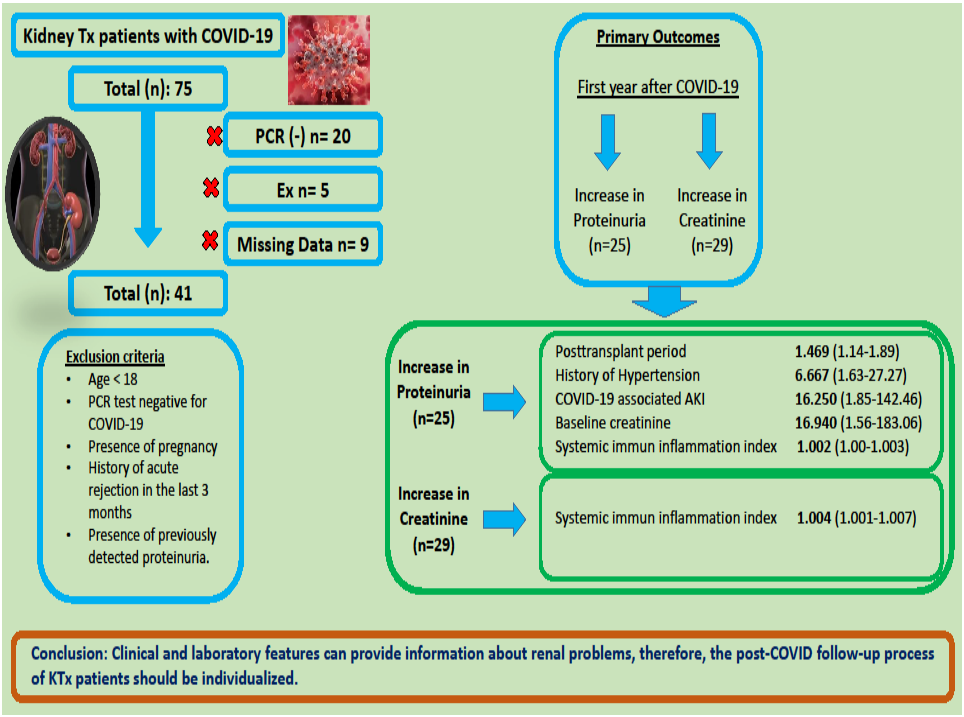

Figure 1. Study brief.

that the virus may change the homeostasis of the renin-angiotensin-aldosterone system (RAAS) with the ACE2 interaction.[43]

Longer transplantation time is associated with higher mortality and more frequent AKI during COVID-19.[5] We found that patients with a long transplantation period have more proteinuria after COVID-19. This is due to the advanced age of the graft being more prone to renal injury.

In the long term, one of the critical factors affecting renal functions is the immunosuppressive treatments reduced during COVID-19. All of our patients' immunosuppressive therapies were returned to the basal levels within two weeks at the latest after COVID. In a study evaluating the effects of reduced immunosuppressive treatments on renal functions in the early post-COVID period (within the first three weeks), it was observed that patients with reduced immunosuppression had better graft function in the early period, which was attributed to a temporary glomerular filtration rate elevation as a result of reduction of calcineurin inhibitors (CNI).[44] Immune dysregulation due to COVID-19 may trigger disease activation or acute T-cell-mediated graft rejection in patients with the underlying immune disease.[4] Studies, including histopathological evaluations, show that rejection frequency is very low in the early period, and reduced immunosuppression is well tolerated.[3,4,13-1]5 However, rejection should be considered in the differential diagnosis of patients with proteinuria or increased creatinine in long-term follow-up. Since a kidney biopsy was not performed on any patient in our study, we can't comment on this situation.

Our study has some limitations. One of the most important limitations of our study is the absence of a control group. The limited number of patients is another limitation. The fact that no biopsy was performed in any patient with proteinuria or increased creatinine prevents us from commenting on the type of renal dysfunction that developed. Although DSA test positivity was taken as an exclusion criterion since it would lead to suspicion of graft rejection, the most important limitation of this study is that kidney biopsy could not be performed in patients with increased creatinine or worsening proteinuria.

CONCLUSIONS

As a result, we have shown that clinical and laboratory features such as high SII, increased proteinuria, hypertension, development of AKI, and long post-transplant time can provide information about renal problems we may encounter in the long term (Figure 1). Therefore, the post-COVID follow-up process of KTx patients should be individualised.

Highlights

- Patients with high SII were more fragile regarding renal functions after COVID-19.
- SII was an independent predictor of the change in serum creatinine and proteinuria after COVID-19.
- Post-COVID renal dysfunction may be more severe in patients with high initial proteinuria and creatinine level.
- Patients with a long transplantation period and hypertensive had higher renal dysfunction risk in the long term after COVID-19.

Conflicts of Interest

All authors declare that there is no conflict of interest in this study.

Ethical Approval

The protocol of the study was approved by the Medical Ethics Committee of Necmettin ERBKAN University, Faculty of Medicine, Meram, Konya, Turkey. (Decision number: 2022-3845, date: June 2022).

Authors' Contribution

Study Conception: HÖ, KZ,; Study Design: HÖ, HZT,; Supervision: HÖ, İB, FY,; Literature Review: HÖ, İB, KT,; Critical Review: HZT, KT NYS,; Data Collection and/or Processing: YÖ, FY, İB,; Statistical Analysis and/or Data Interpretation: İB, NYS,; Manuscript preparing: HÖ, İB.

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Original Article

Retrospective Analysis of Follow-up and Results of Patients with High D-Dimer Value and Discharged without Emergency Pathology

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ABSTRACT

Background This study aimed to retrospectively examine the morbidity and mortality rates after discharge of patients who applied to the emergency department with high D-dimer values but had no pathology upon evaluation.

Material and Methods Patients over the age of 18 who applied to Bursa Uludağ University Faculty of Medicine Emergency Department with preliminary diagnosis of pulmonary embolism in a two-year period between January 2018 and December 2019 were included in the study. The patient group consisted of cases with high D-dimer levels while the control group inluded patients with negative D-dimer and no pathology on discharge.

Results A total of 594 cases; 297 D-dimer positive (+) and 297 D-dimer negative (-), were included in the study. A significant difference existed between the percentage of patients developing illness post-discharge in the D-dimer (+) 18.86% (n=56) and D-dimer (-) 1.68% (n=5) groups, respectively. The most common illness identified in the dimer (+) group after discharge up was pneumonia (n=11), followed by Coronary Artery Disease (n=5). Death rate was 1.68% (n=5) in the D-dimer (-) group and 11.78% (n=35) in the D-dimer (+) group; a statistically significant difference (p=0.001).

Conclusions In conclusion, both morbidity and mortality rates were found to be significantly higher in the D-dimer positive group.

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Introduction

D-dimer is a fibrin degradation product.[1] Blood D-dimer level increases in various conditions related to coagulation.[2] D-dimer; a fast, simple and cheap test, is performed to rule out deep vein thrombosis (DVT) and pulmonary embolism (PE).[3] Normal plasma D-dimer level is usually less than 500 micrograms per liter (µg/L).[4] In many studies, the formula; Agex10=ng/mL, is proposed for the threshold value in patients over 50 years of age.[5] Blood D-dimer levels may be high in old patients, patients with cancer or systemic infection, pregnancy, recent surgery or trauma.[4] D-dimer may also increase in infectious diseases such as endocarditis and mycoplasma pneumonia.[6,7] D-dimer has high sensitivity and low specificity in the diagnosis of acute aortic dissection (AAD).[8]

D-dimer is not only a diagnostic tool, but also a useful biomarker in predicting prognosis.[9] D-dimer levels are found to be high in patients with community-acquired pneumonia and may help in determining the risk of mortality.[10] Rodelo et al.[11] Reported higher mortality rates in septic patients with elevated D-dimer levels. High D-dimer values, especially in lung malignancies, is associated with poor prognosis.[12] Our objective was to retrospectively examine the morbidity and mortality rates after discharge of patients who applied to the emergency department with high D-dimer values but had no pathology upon evaluation.

Material and Methods

This study, a specialization thesis, was carried out retrospectively with the approval of Uludag University Faculty of Medicine Clinical Research Ethics Committee dated 16 June 2021 number 2021-8/25. Patients over the age of 18 who applied to Bursa Uludag University Faculty of Medicine Emergency Department with preliminary diagnosis of pulmonary embolism in a two-year period between January 2018 and December 2019 were included in the study. Data was obtained through Mia-Med Hospital Information and Management System and E-pulse electronic system. The patient group consisted of cases with high D-dimer levels while the control group included patients with negative D-dimer without any pathology upon discharge.

The upper limit for plasma D-dimer concentration was accepted as 500 ng/mL in young patients, whereas the corrected formula (Agex10=ng/mL) was used in patients over 50 years of age. D-dimer above these values were accepted as positive (+) while D-dimer below these values were considered negative (−). Patients were followed up through Mia-Med and E-pulse electronic system until January 2022. In cases where sufficient data could not be found, patients or their relatives were reached to using the contact information registered in our system.

Statistical Analysis

In calculating the sample size of the study, Power was 0.80 for each variable, Effect size was 0.2, and Type-1 error (a) was 0.05. Descriptive statistics for continuous variables were; Mean/median, standard deviation, minimum and maximum whereas categorical variables were expressed as number (n) and percentage (%). Continuous measurements' distribution was examined by Kolmogorov-Smirnov (n>50) and Skewness-Kurtosis tests. In the case of normal (parametric) distribution of continuous variables, comparisons according to categorical factors were made using the independent T-test (Single-test).

Results

A total of 594 cases; 297 D-dimer (+) and 297 D-dimer (−), were included in the study. Table 1 showed the distribution of cases according to age, D-dimer, period after discharge and gender. As seen in Table 1, the mean age of D-Dimer (+) patients was significantly higher ($p<0.05$). The period (days) between discharge and control dates had no significant difference according to the D-Dimer groups ($p>0.05$). Distribution of patients according to D-dimer groups and the outcomes were shown in Table 2 and Figure 1. As seen in Table 2, morbility and mortality rate was significantly higher in the D-dimer (+) group ($p<0.001$). Diseases detected in both groups are shown in Table 3. As seen in Table 3, the most common disease identified in the dimer (+) group was pneumonia (n: 11), followed by coronary artery disease (n: 5), cholecystitis (n: 4), and pyelonephritis (n: 4), respectively.

Table 1. Comparison between the distribution of patients' age, D-dimer, period after discharge and gender according to D-dimer groups.

	D-dimer negative	D-dimer positive	P value
Age (years)	43.26±15.21	50.03±18.99	0.001*
Gender (male/female) n (%)	184 (61.95)/113 (38.05)	143 (48.15)/154 (51.85)	0.001**
D-dimer (ng/mL)	325.12±116.44	1691.95±1656.63	0.001*
Period (days)	1201.98±78.99	1250.48±692.85	0.231*

*Student's t test, ¶ Fisher's exact test, † Pearson Chi-Square test, ϕ Mann-Whitney U test.
ER: estrogen receptor, PR: progesterone receptor, DCIS: ductal carcinoma in situ, LVI: lymphovascular invasion, LN: lymph node metastasis.

Table 2. Distribution of patients according to D-dimer groups and the outcomes.

	D-dimer negative	D-dimer positive	P value*
Healthy n (%)	287 (96.63)	206 (69.36)	0.001
Ill n (%)	5 (1.68)	56 (18.86)	0.001
Exitus n (%)	5 (1.68)	35 (11.78)	0.001
Total n	297	297	0.231

*significance levels according to two-ratio Z-test results.

Discussion

D-dimer is a fast and cheap test included in PE diagnostic algorithms.[3,13] However, blood D-dimer values can increase in many diseases other than PE.[4,6,7] D-dimer is also a useful biomarker in predicting prognosis.[8] In our study, we followed up patients with high D-dimer levels that were discharged after evaluation in the ED without any pathology. Blood D-dimer level is generally higher in the elderly.[4] For this reason, the corrected formula (Agex10=ng/mL) is recommended for the threshold value in patients over 50 years old.[5] In our study, the mean age of D-Dimer (+) patients was found to be significantly higher.

High D-dimer levels in pneumonia is directly proportional to severity.[12] In our study, the most common disease in the dimer (+) group during follow-up was found to be pneumonia (n:11). Studies have revealed high D-dimer levels in patients with coronary arter disease.[14] Another study has shown that high D-dimer levels are associated with coronary

Table 3. Distribution of illnesses according to D-dimer groups.

	Illness	n (%)
D-dimer negative	Factor 5 Leiden mutation	1 (20)
	Hepatitis B infection	1 (20)
	Myocardial infarction	1 (20)
	Stomach carcinoma	1 (20)
	Papillary thyroid carcinoma	1 (20)
	Total	5 (100)
D-dimer positive	Pneumonia	11 (19.6)
	Coronary artery disease	5 (8.9)
	Cholecystitis	4 (7.1)
	Pyelonephritis	4 (7.1)
	COVID-19	3 (5.4)
	Malignancy	3 (5.4)
	Pulmonary embolism	3 (5.4)
	Peripheral artery disease	3 (5.4)
	Sepsis	2 (3.6)
	Others	18 (32.1)
	Total	56 (100)

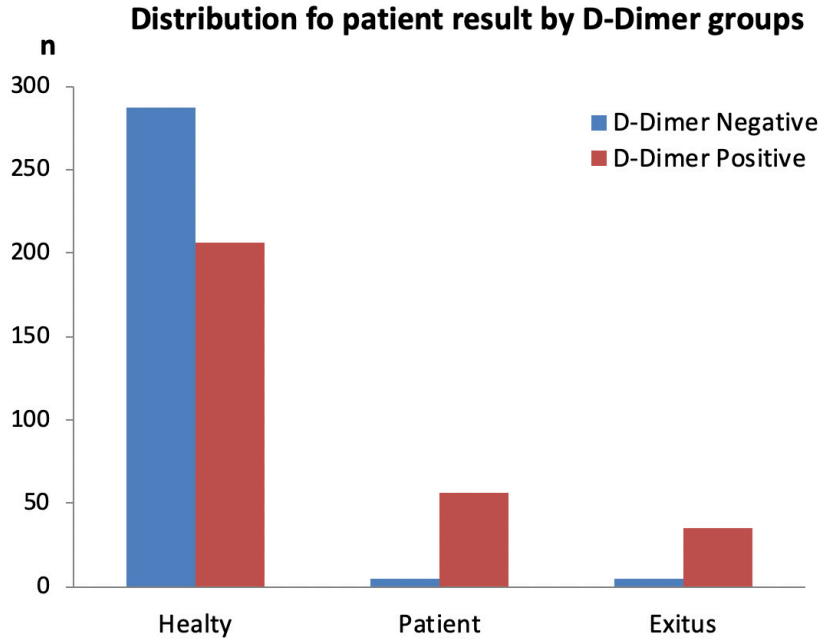

Figure 1. Distribution of patient outcomes according to D-dimer groups.

arter disease occurring under the age of 45.[15] In our study, we also encountered coronary arter disease in the D-dimer (+) group. A study has shown that D-dimer predicts the presence of bacteremia in septic patients and is correlated with the severity of sepsis.[16] In another study, high D-dimer levels were associated with severe organ dysfunction and high mortality.[17] Sepsis was another disease identified in the dimer (+) group during our follow-up. Peripheral artery disease is also related to high D-dimer levels.[18] In our study, peripheral arterial disease was detected in some cases among the dimer (+) group.

D-dimer is a test with high sensitivity but low specificity in the diagnosis of PE.[3] We identified only 3 (5.4%) PE patients in the dimer (+) group during our follow up period. In cases of malignancy, intravascular stasis, released cytokines and coagulation factors, and vascular injuries caused by chemotherapy lead to increased D-dimer values.[19] High blood D-dimer concentration during pre-treatment period in small cell lung cancer may be a reliable factor to predict prognosis.[11] Another disease group encountered in the dimer (+) group during our follow-up was malignancy.

Elevated D-dimer is frequently detected in patients with COVID-19 and is significantly associated with high mortality risk.[20] In our study, we came across some COVID-19 cases in the dimer (+) group.

D-dimer is not only a diagnostic tool, but also a useful biomarker in predicting prognosis.[9] High D-dimer is frequently encountered in critically ill patients and is inversely proportional to survival.[21] D-dimer levels may be helpful in determining the risk of mortality in patients with community-acquired pneumonia.[10] Elevated D-dimer in COVID pneumonia are associated with severity and increased risk of mortality.[22] D-dimer has been found to correlate with the severity of sepsis.[16] D-dimer correlate with the prognosis in cancer patients.[23] High D-dimer, especially in lung malignancies, is associated with poor prognosis.[12]

Conclusions

As a result, both morbidity and mortality rates were found to be significantly higher in the D-dimer (+) group during follow-up. A closer follow-up of D-dimer positive cases will be beneficial in cases discharged without acute pathologies during ED evaluation and adding D-dimer to the screening program is likely to positively affect prognosis.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

Local ethics committee approved the study protocol.

Authors' Contribution

Study Conception: KFB; Study Design: CHI, DVA; Supervision: KFB; Literature Review: KFB, KB; Critical Review: AE; Data Collection and/or Processing: KB, KFB; Statistical Analysis and/or Data Interpretation: KFB, OIM; Manuscript preparing: KFB, OIM.

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Case Report

Acute Arterial Thrombosis in Anticoagulated Patient for Acute Pulmonary Thromboembolism

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ABSTRACT

Acute limb ischemia is a rare condition in patients with venous thromboembolism (VTE), who already receive anticoagulation treatment. Inflammation is a risk factor for thrombus formation. Patients with active ulcerative colitis, especially at time of exacerbation, are more prone to thromboembolism, both venous and arterial. Risk for thrombosis is 18% higher risk, with also higher risk of bleeding. Up to date, there is no contraindication to any anticoagulant drug in patients with ulcerative colitis. We represent a case of a 73 year - old woman with ulcerative colitis (UC) exacerbation, hospitalized initially for pulmonary thromboembolism, that developed acute arterial thrombosis when switched on novel oral anticoagulant (NOAC).

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INTRODUCTION

Ulcerative colitis (UC) is a highly inflammatory disease with various intestinal and extraintestinal manifestations. The elevated risk of thromboembolism in patients with UC, especially in acute exacerbation, is well documented. Acute arterial thrombosis is a rare condition in patients already on anticoagulation therapy due to venous thromboembolism (VTE).[1] We presented a rare case of acute limb ischemia in a patient treated with rivaroxaban for pulmonary thromboembolism occurring during acute exacerbation of UC. .

CASE REPORT

A 73-year-old woman was transferred from the clinic of gastroenterohepatology. She was initially hospitalised for moderate UC exacerbation because of computer tomography pulmonary angiography (CTPA) finding of segmental pulmonary thromboembolism (PTE) with thrombus on the branching of the left pulmonary artery. Her previous medical history revealed hypertension, except for UC, diagnosed 40 years ago. Before hospitalisation, the patient was treated with mesalazine for UC. Additionally, she received carvedilol, spironolactone, pantoprazole, perindopril and furosemide. During her stay in the gastroenterohepatology clinic, she received additional metronidazole, methotrexate and corticosteroid treatments. On admission, the patient was hemodynamically stable with a blood pressure of 140/80 mmHg, ECG showing sinus rhythm with 90 bpm and an S1Q3T3 pattern. Her oxygen saturation was 88% on ambient air. The initial laboratory showed mild microcytic anaemia with haemoglobin levels 10 g/L, elevated troponin levels (984 ng/L) and D-dimers (1,548 mmol/L), as well as high inflammatory markers (C-reactive protein [CRP] 95 mg/L). Liver and kidney laboratory parameters and other values were in their referential range. Bedside echocardiography showed dilated right ventricle (50 mm), pulmonary artery hypertension with systolic pulmonary artery pressure (sPAP) 60 mmHg, moderate tricuspid regurgitation and collapsible vena cava inferior with a diameter of 21 mm. The right ventricle was with preserved function (TAPSE 18, S' 9). There were no structural or functional abnormalities of the left ventricle. Venous Doppler ultrasound of the lower extremities also did not show any abnormalities. Treatment was started with low molecular weight heparin (LMWH) with a dose of 1 mg/kg/12 h. Additionally, she received parenteral antibiotics, beta-blockers (BB), angiotensin-converting enzyme inhibitors (ACEi), thiazide diuretics and proton pump inhibitors (PPI). On the seventh day of her hospitalisation, she was switched from LMWH to apixaban 5

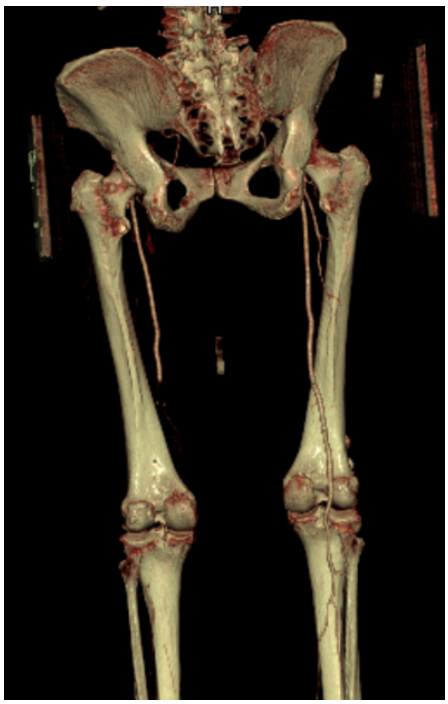

Figure 1. Anteroposterior view of peripheral angiography demonstrating thrombus occluding the distal part of the superficial femoral artery.

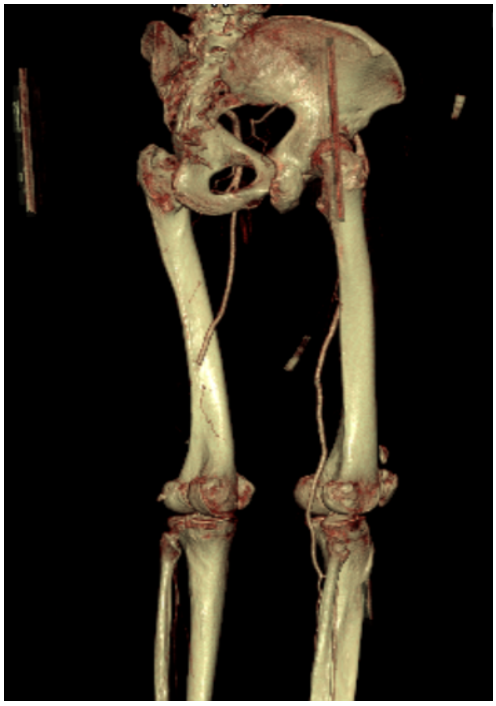

Figure 2. Lateral view of peripheral angiography demonstrating occlusion of the distal part of the superficial femoral artery.

mg twice daily.[2]

Two days later, she complained of pain and paresthesia on the left foot. The examination showed a pale, cold left foot with no pulsation on the dorsal pedal artery. Doppler ultrasound was made with absent signals on the superficial femoral artery, popliteal and posterior tibial artery. Computed tomography angiography of the lower extremities showed an acute thrombus with a length of 22 cm occluding the distal part of the left femoral artery continuing up to the exit site of the anterior and posterior tibial artery.[3] (Figure 1 and 2).

The patient was again switched on LMWH 1 mg/kg/12 h. Dual antiplatelet therapy and a high dose of statins were immediately started. No abnormality was observed in thrombophilia examinations. After consulting a vascular surgeon, an indication for emergency surgery was made. The patient was transferred to the University Clinic of Cardiac Surgery, where a thrombectomy was made immediately after admission. The Doppler ultrasound postoperatively showed normal signals on the dorsal pedal artery. There was no loss in motorial and sensory function of the foot, but there was necrosis starting from the toe and tip of the second and third finger due to reperfusion ischemia. After a few days, the demarcation line developed in the necrotic tissue and reached the first three fingers. She was transferred to a plastic surgeon for finger amputation. The patient was then discharged home. On her ambulatory control after one month, the patient was in good condition, and the wound was healing well. Unfortunately, she was suffering from depression that developed due to her amputation.

DISCUSSION

Patients with active UC, especially during an exacerbation, are more prone to venous and arterial thromboembolism, with an 18% higher risk for VTE. The arteries and veins in the upper and lower limbs, digits, cerebral and retinal vasculature, pulmonary, portal, hepatic, retinal, mesenteric and cardiac systems have all been reported to be involved with thromboembolic events.[3,4]

On the other hand, the risk of bleeding in this patient population is also higher, which should be considered when choosing a suitable anticoagulant. The current practice in treating VTE for patients with inflammatory bowel disease (IBD) is similar to patients with non-IBD.[2,5] Anticoagulation with unfractionated heparin or low-molecular-weight heparin is recommended in the acute setting, with the eventual transition to oral anticoagulation. For novel anticoagulants (NOACs), new evidence from studies suggests that they have comparable efficacy to that of vitamin K antagonists (VKAs) with a more favourable safety profile.[6]

Although NOACs are not officially approved for treating arterial thrombosis, ongoing studies show promising results. Rivaroxaban is the only NOAC approved for secondary prevention of arterial thrombosis, together with aspirin.[7] Nevertheless, developing arterial thrombosis while on NOACs is a rare condition. Compared to VKAs such as warfarin, NOACs have more predictable pharmacokinetics and do not need to be routinely monitored in the laboratory for their plasma level. However, due to the variation in the plasma drug level between the individuals, some patients may be at increased risk of treatment failure or bleeding events.

CONCLUSIONS

Acute arterial limb thrombosis in patients already on NOAC because of VTE is rare but possible in extraordinary conditions like UC. To date, the concentration of anti-Factor Xa is the only way to evaluate the treatment's efficacy indirectly.[8] It should be routinely taken in patients undergoing therapy with NOACs. There are still no guidelines that can stratify patients with UC as low or high risk for thromboembolism. More research is needed on the efficacy of anticoagulation drugs in this specific patient group.[5]

Conflicts of Interest

All authors declare that there is no conflict of interest in this study.

Authors' Contribution

Study Conception: AC, LP,EG,; Study Design: LP,AC,; Supervision: LP, OB, ZZ,; Literature Review: ZZ, EG,; Critical Review: LP, OB,; Data Collection and/or Processing: AC, EK,; Statistical Analysis and/or Data Interpretation: AC, ZZ,; Manuscript preparing: AC, EK.

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Case Report

Methicillin-Resistant Staphylococcus aureus Co-infection in a Pakistani Patient with COVID-19: A Case Report

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ABSTRACT

Bacterial co-infections in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia are not very common as the prevalence of co-infections with other respiratory viruses. The rate of bacterial co-infection in hospitalised patients infected with influenza is higher than 30%, whereas it is lower than 4% in hospitalised patients with SARS-CoV-2. Respiratory viral infections associated with bacterial co-infection have higher mortality and morbidity rates. The literature shows that most SARS-CoV-2 patients admitted to the hospital do not necessarily screen for bacterial infections and antimicrobial susceptibility. Therefore, clinicians' misdiagnosis of these co-infections can pose a significant risk to the lives of vulnerable patients with COVID-19. In that light, we presented a complicated case of methicillin-resistant *Staphylococcus aureus*.

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INTRODUCTION

Coronavirus disease-19 (COVID-19) was first reported in December 2019 in China's Wuhan city and then spread worldwide. Later on March 11, 2020, the COVID-19 pandemic was declared after being reported in many countries. Since the first COVID-19 pandemic, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 370 million people, including over 5 million deaths. Both viral and bacterial respiratory infections substantially contribute to the burden of mortality and morbidity worldwide.[1] Bacterial co-infection is a life-threatening complication in many respiratory viral infections, contributing to the severity of the disease in the form of respiratory failure, shock, prolonged stay in the intensive care unit (ICU), and even death.[1,2] SARS-CoV-2 causes pneumonia and multi-organ failure, including myocarditis and thrombosis, as well as hypoxic-type respiratory failure.[3] SARS-CoV-2 infection makes the patients vulnerable to bacterial co-infections. However, the mechanisms of respiratory co-infection and the likelihood of bacteraemia favouring the respiratory tract epithelium are still unknown.[3]

The 1918 influenza pandemic data showed that nearly all deaths were caused by bacterial co-infection, including the most prevalent pathogens as *Streptococcus pneumoniae, Staphylococcus aureus (S. aureus), Beta-hemolytic streptococci,* and *Haemophilus influenza*.[4] Similarly, in the 2009 influenza pandemic, around 55% of the patient's autopsy samples were positive for bacterial co-infections.[5] The SARS-CoV-1 and Middle East Respiratory Syndrome (MERS-CoV) coronaviruses pandemic reported 20% and 30% of cases with bacterial co-infections.[6] However, research on the current pandemic has shown that patients with SARS-CoV-2 infection have lower than 4% of bacterial co-infections. Since bacterial co-infections with SARS-CoV-2 pneumonia have a known clinical impact, the disease might be missed diagnosed due to the low yield of diagnostic tests.[3]

For the benefit of public health, it is essential to assess the various uncertainties surrounding the effects of bacterial co-infections during the pandemic, particularly in intensive care settings. Here, we described a case of bacterial co-infection in a COVID-19 patient who was severely infected by methicillin-resistant *S. aureus* co-infection, along with their presentation, radiographic observation, and the outcomes.

CASE REPORT

A 90-year-old male patient who had previously been diagnosed with ischemic heart disease and hypertension was presented to the emergency room with several health complications, including shortness of breath, angina, fever, and a severe dry cough that had been going on for the previous six days. He was hospitalised in the intensive care unit (ICU) after being diagnosed with hypoxemic respiratory failure caused by COVID-19 pneumonia with oxygen saturating of 82% on room air. On admission to the emergency room, his widespread weakness, loss of appetite, taste, and smell were also noticed. He was breathing at a rate of 20 breaths per minute (bpm) with a heart

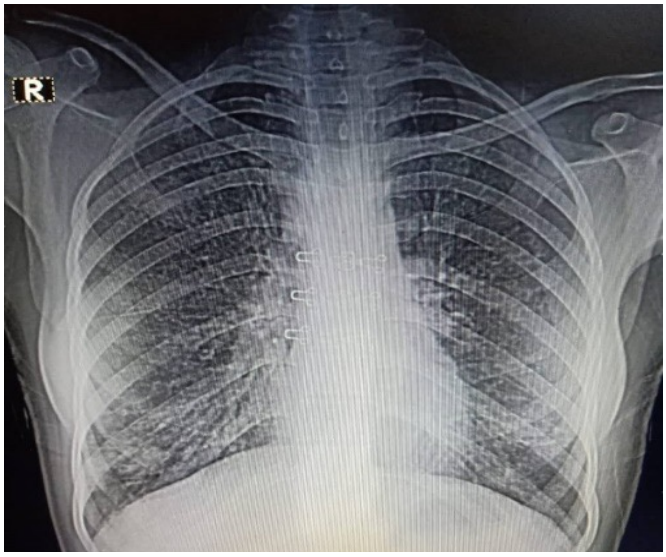

Figure 1. Chest X-ray showing bilateral infiltrates.

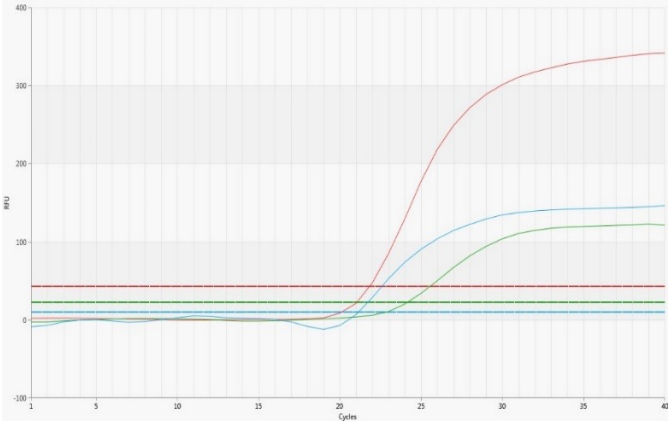

Figure 2. The graph illustrates reverse transcription PCR gene detection; red represents nucleocapsid, blue represents RNA-dependent RNA polymerase gene, and green represents internal control.

rate of 82 bpm and a temperature of 38.3 °C. He was found to have leukopenia with a leucocyte count of 5,700 mcL and a low neutrophil count of 88 mcL. The erythrocyte sedimentation rate (ESR) was elevated to 52 mm/h, with elevated interleukin-6 (IL-6) 12.7 pg/mL, C-reactive protein 3.2 mg/L, and procalcitonin (PCT) 0.4 µg/L, respectively. The chest radiograph showed multifocal pneumonia (Figure 1).

A rapid COVID-19 antigen test detected him as positive for COVID-19. Therefore, a nasopharyngeal sample was collected for further testing. Nasal cultures were collected from the patient using sterile cotton, plated on mannitol salt agar (MSA; Becton Dickinson Microbiology Systems), and incubated at 35 °C for 48 hours. We detected golden colour colonies on the MSA plate and subcultured on a trypticase soy agar supplemented with 5% sheep blood plates (Becton Dickinson Microbiology Systems) and incubated it at 35°C overnight. *S. aureus* was identified through colony morphology, a latex agglutination assay (Remel, Lenexa, KS), and a tube coagulase test using ethylenediaminetetraacetic acid (Becton Dickinson Microbiology Systems). *S. aureus* isolates were screened for methicillin resistance using the National Clinical and Laboratory Standards Institute disk diffusion method. Disk diffusion was conducted using a 1 µg oxacillin disk with Mueller-Hinton agar. The agar plates were incubated overnight at 35°C and measured the zone diameters.

He received both enteral and intravenous drugs with supplementary oxygen treatment. His PCR test showed positive results for the RNA-dependent RNA genes of SARS-CoV-2 on the second day of admission (Figure 2). He was treated primarily with remdesivir and hydrocortisone along with normal saline for the COVID-19 infection. Despite the high-flow nasal cannula's assistance (15 L/min), he was tachycardic and tachypneic. Therefore, he was intubated for further support on the third day of admission to the ICU. However, his repeated tests revealed an increase in the level of IL-6 of 33 pg/mL, PCT 0.6 µg/L, lactate dehydrogenase (LDH) 452 U/L, random blood sugar 141 mg/dL, total leukocyte count (TLC) 9,070/µL and platelets 32.7× 10^3/µL. Low levels of albumin and electrolytes were observed. However, alanine aminotransferase (ALT) and blood urea nitrogen (BUN) levels were raised to 55 U/L and 52 mg/dL, respectively. On culturing, it was revealed that he had bacteraemia and *S. aureus* co-infection. The culture of *S. aureus* was grown on differential media to confirm the isolates, and through Kirby Baur disk diffusion assay, the isolates showed resistance to oxacillin antibiotics (Figures 3 and 4). His therapy, antivirals, and supportive medication were further adjusted with piperacillin/tazobactam antibiotics for bacterial infections. Unfortunately, his health condition was severely exacerbated due to bacterial co-infection and finally expired on day seven of his post-hospital admission.

DISCUSSION

The patient presented to the ICU had a history of ischemic heart disease and hypertension. The patient had a productive dry cough with sputum production. A

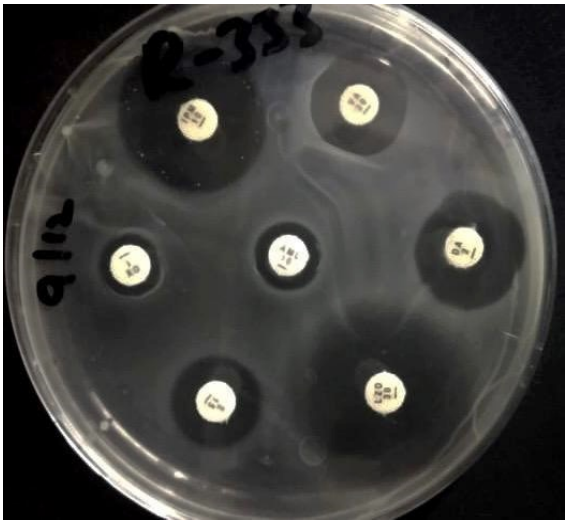

Figure 3. Antibiotic susceptibility pattern of methicillin-resistant S. aureus.

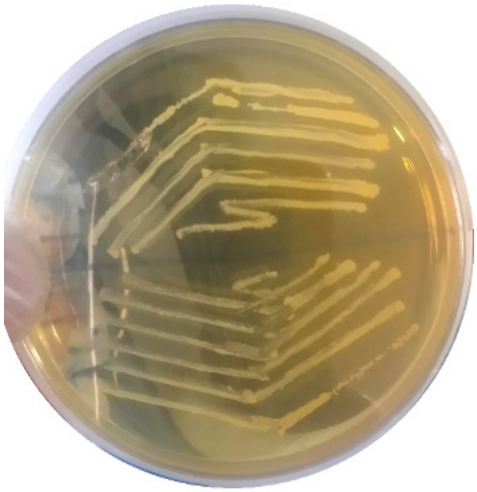

Figure 4. Growth of methicillin-resistant S. aureus on mannitol salt agar with characteristics of golden yellow colonies.

productive cough is not a unique finding for bacterial pneumonia because, according to a Zhou *et al*.[7] study, 50% of patients with SARS-CoV-2 infection had a similar presentation. The chest radiograph of the patient showed pneumonia with bilateral infiltrates. His rapid SARS-CoV-2 antigen test and PCR both were reported positive for COVID-19. Positive growth for *S. aureus* was seen in the sputum and nasopharyngeal swab cultures. The isolates showed resistance to oxacillin, one of the markers for methicillin-resistant *S. aureus* (MRSA) identification. According to the lab results, the patient had leukopenia and high ferritin, IL-6 and PCT levels.

Khilnani *et al*.[8] reported that immunocompromised, critically ill, and ICU patients were more susceptible to bacterial co-infections with increased usage of preventive or therapeutic antibiotics than non-invasive patients. Older people who live in nursing homes for long-term care also have a high risk of caring for *S. aureus*. Liu *et al*.[9] reported that the prevalence of MRSA in upper respiratory tract-infected patients was associated with high mortality ranging from 18.[9] to 30.0%. Co-infections can increase the severity of the disease and even mortality rates. According to Pacheco *et al*.[10], the overall co-infection rates in respiratory diseases can reach up to 68% of hospitalised patients co-infections could be explained by a possible dysregulation of the host immune response in case of infection with one pathogen, making the subsequent infection with the other pathogen more accessible. In previous viral pandemics, *S. aureus* was the leading cause of secondary bacterial infections, considerably increasing patient mortality rates. For influenza virus infection, mainly, *S. aureus* co-infection and bacteraemia were associated with mortality rates of nearly 50%, in contrast to the 1.4% mortality rates observed in influenza-infected patients alone.[10] A more similar phenomenon with reinfection and co-infection during the recent COVID-19 pandemic was recorded, which severely affected the hospitalised patients.[11]

Bacterial co-infections in respiratory viral infections are the leading cause of morbidity and mortality. According to Zhou *et al*.[7] and Lai *et al*., 12 studies on the 1918 influenza pandemic, between 20 and 60 million deaths were caused by bacterial co-infection rather than the virus itself. Although the rate of bacterial co-infection in COVID-19 is unclear, several studies reported that the rate is significantly lower than in prior pandemics.[7,12] Due to the lack of resources and the heavy burden on the healthcare system during a pandemic, COVID-19 patients are not being thoroughly diagnosed with co-infections.[7] In recent research, individuals with COVID-19 who also had *S. aureus* co-infection had the greatest fatality rate, at 61.7%.[12] The primary *S. aureus* co-infection diagnosis in individuals admitted to a medical facility suggests that the community serves as a favourable environment for the spread of pathogenic infections.[13] The mortality rate in *S. aureus* bacteraemia is associated with the severity of the disease, age, acute renal failure, length of mechanical ventilation, and antimicrobial resistance.[14] The patient's laboratory examination showed low albumin and electrolyte levels and increased ESR. He was given plenty of intravenous fluids, antivirals, antibiotics, anti-allergic, and hydrocortisone. Due to severe bacteraemia and COVID-19 complications, he expired on the seventh day of his post-hospital admission.

CONCLUSIONS

S. aureus is an opportunistic pathogen for humans and animals amongst the staphylococcal species with low guanine-cytosine content. It is also known as a commensal coloniser of the skin, nares and nasal cavity. It can enter the body through openings in the skin and cause various infections, from minor skin to life-threatening blood infections. The empirical anti-MRSA therapy methods were included with vancomycin hydrochloride or linezolid plus guideline-recommended standard antibiotics such as β-lactam, macrolide, or tetracycline hydrochloride, or extended-spectrum of quinolone.15 We propose that despite the low level of bacterial co-infection associated with COVID-19, it is important to investigate a severely ill patient for a possible co-infection. The role of evaluating empirically covering co-infections is very significant. Proper and timely evaluation of bacterial co-infection in high-risk patients may overcome the rate of morbidity and mortality.

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Conflict of Interests

The authors declare no conflict of interest.

Authors' Contribution

All authors participated in the literature and critical review for the case report preparation.

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