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Original Article

Bacteriological and Clinical Evaluation of 32 Cases of Diabetic Foot

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ABSTRACT

Objective: The primary objective of the present study was to investigate clinical and laboratory characteristics of patients diagnosed with diabetic foot (DF) in order to aid in selection of antibiotic treatment and clinical follow-up. Potential relationship between DF, renal complications, and the mechanism of action of diseases were examined.

Methods: Thirty-two patients diagnosed with DF in Department of Internal Medicine between June 2014 and June 2015 were enrolled in the study. Retrospective screening of medical data was conducted and patient lipid and microalbuminuria levels, microalbumin/creatinine ratio, creatinine clearance (formulated using Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation), and glycated hemoglobin (HbA1c) level were recorded.

Results: Of the 32 patients diagnosed with DF, 13 were female (40.6%) and 19 were male (59.4%). Age range was 32 to 88 years, and mean age was 59.03±10.3 years. Duration of disease of the patients was 5 to 40 years at time of study, and mean was 15.5±7.06 years. Mean HbA1c level was 9.01±2.26% (range: 5.4–14.7%). Mean CKD-EPI level was 75±27.34 mL/min/1.73 m2 (range: 11–130 mL/min/1.73 m^2). Bacterial growth was observed in 22 cases (68.8%), and was not detected in 10 cases (31.3%). Two cases (6.3%) presented with growth of multiple microorganisms.

Conclusion: Evaluation of causative microorganisms in terms of patient age and gender revealed main bacterial species found were Gram-positive cocci bacteria. There was no statistically significant difference based on CKD-EPI level in terms of mean duration (p=0.001; p<0.01). Staphylococcus aureus was the most common bacteria present among cases having CKD-EPI level of 60-89 mL/dk/m^2.

INTRODUCTION

Diabetes mellitus (DM) has both microvascular (e.g., retinopathy, nephropathy, and neuropathy), and macrovascular (e.g., coronary heart disease, peripheral vascular diseases, and cerebrovascular diseases) complications. Diabetic nephropathy (DN) predominates as critical health problem, as it leads to end-stage renal failure.[1] Microalbuminuria is defined as urinary excretion of albumin of 30–300 mg/24 hr or 20–200 µg/min.[2] Microalbuminuria is important as an indicator of diabetic microangiopathy.[3] Therefore, aim of present study was to determine relationship between microalbuminuria and DN in the development of diabetic (DF), and to identify causative microorganism of DF for selection of appropriate empirical antibiotic therapy.

PATIENTS AND METHODS

A total of 32 cases diagnosed with DF at internal medicine outpatient clinics between 2014 and 2015 were included in the study. Patient data were retrieved from their medical files and retrospectively analyzed with the approval of the ethics committee. Patients without urinary tract infection, pregnancy, or diabetic renal disease were included and data related to 24-hour urinary albumin excretion rate and

creatinine clearance, other biochemical test results, and medical examination findings were obtained from routine follow-up records.

Statistical evaluation of the study data was performed using SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA) software package. Normality of distribution was evaluated using Shapiro-Wilks test. In addition to descriptive statistical methods (mean, SD, frequency), for intergroup comparisons of quantitative data with normal distribution, one-way analysis of variance test was used. To determine which group was different, Tukey Honest Significant Difference test and Tamhane's T2 test were used. For intergroup comparisons of parameters without normal distribution, Kruskal-Wallis test was used. Student's t-test was applied to compare parameters with normal distribution, as was Mann-Whitney U test, accordingly. Chi-square test and Fisher's exact test were used for qualitative data. Statistical significance was evaluated at level of $p<0.05$.

RESULTS

The study was conducted between 2014 and 2015 with total of 32 cases (female: n=13, 40.6%; male: n=19, 59.4%; median age: 58.5 years; range: 32-88 years) who were diagnosed and treated for DF at outpatient clinics of internal medicine (Table 1).

Mean duration of diabetes was 15.5±7.06 years (range: 5–40 years). Mean HbA1C value was 9.01±2.26%

Table 1. Distribution of general characteristics of the patients (n=32)

	%	Mean±SD	Median (min.-max.)
Age (years)			58.5 (32–88)
Duration of diabetes (years)		15.5±7.06	
Fasting blood glucose		207.03±78.81	
Glycated hemoglobin		9.01±2.26	
Cholesterol		183.22±41.22	
Creatinine			1.0 (0.6–4.24)
Albuminuria (n=20)			115.5 (9–2785)
Albuminuria/Creatinine (n=20)			97.3 (11.7–2486)
CKD-EPI (mL/min/1.73 m^2)		75±27.34	
Gender			
Female	40.6		
Male	59.4		
Age groups			
<65 years	71.9		
≥65 years	28.1		
Albuminuria analysis	62.5		
CKD-EPI group (mL/min/1.73 m^2)			
<15	3.1		
15–29	3.1		
30–59	21.9		
60–89	40.6		
≥90	31.3		
Growth of microorganism			
Present	68.8		
Absent	31.3		
Patients whose antibiogram revealed growth of more than 1 microorganism	6.3		

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration equation; SD: Standard deviation; Min.: Minimum; Max.: Maximum.

Table 2. Distribution of microorganisms found based on gender and age groups

Gender	Age group	Microorganism	n	%
Female	<65 years	*Candida glabrata*	1	25
		Corynebacterium striatum	1	25
		Serratia marcecens	1	25
		Staphylococcus aureus	1	25
	≥65 years	*Citrobacter freundii*	1	12.5
		Klebsialle pneumonia	1	12.5
		Morganella morgani	1	12.5
		Psudomonas aeruginosa	1	12.5
		Extended-spectrum beta-lactamase	1	12.5
		Staphylococcus aureus	3	37.5
Male	<65 years	*Acinetobacter baumanni* complex	1	9.1
		Eikenella corrodens	1	9.1
		Enterococcus faecalis	1	9.1
		Morganella morgani	2	18.2
		Proteus mirabilis	1	9.1
		Psudomonas aeruginosa	1	9.1
		Staphylococcus aureus	1	9.1
		Streptococcus dysgalactiae sp. *equisimilis*	1	9.1
		Streptococcus mitis	1	9.1
	≥65 years	*Staphylococcus aureus*	1	33.3
		Staphylococcus haemoliticus	1	33.3
		Streptococcus agalactia	1	33.3

(range: 5.4–14.7%). Median creatinine value was 1.00 mg/dL. Urinary albumin level was measured in 62.5% of cases, and median value was 115.5 mg/dL (range: 9–2785 mg/dL). Mean CKD-EPI level was 75±27.34 mL/min/1.73 m^2 (range: 11–130 mL/min/1.73 m^2). CKD-EPI levels were <15 mL/min/1.73 m^2 in 1 patient (3.1%), 15–29 mL/min/1.73 m^2 in 1 patient (3.1%), 30–59 mL/min/1.73 m^2 in 7 patients (21.9%), 60–89 mL/min/1.73 m^2 in 13 patients (40.6%), and ≥10 mL/min/1.73 m^2 in 10 patients (31.3%). Bacterial growth was detected in wound cultures of 22 cases (68.8%) and not present in 10 (31.3%). Growth of multiple microorganisms was observed in wound cultures of 2 cases (Table 2).

In bacterial cultures of female patients under age of 65 years, growth of Candida glabrata (n=1; 25%), Corynebacterium striatum (n=1; 25%), Serratia marcescens (n=1; 25%), and Staphylococcus aureus (n=1; 25%) were found. Staphylococcus aureus was also observed in 3 female patients (37.5%) older than 65 years of age, as well as Citrobacter freundii (n=1;12.5%), Klebsialle pneumonia (n=1; 12.5%), Morganella morgani (n=1; 12.5%), Psudomonas aeruginosa (n=1; 12.5%) and Extended-spectrum beta-lactamase (n=1; 12.5%).

In male patients younger than 65 years of age, growth of Acinetobacter baumanni complex (n=1; 9.1%), Eikenella corrodens (n=1; 9.1%), Enterococcus faecalis (n=1; 9.1%), Morganella morgagnii (n=2; 18.2%), Proteus mirabilis (n=1; 9.1%), Psudomonas aeruginosa (n=1; 9.1%), Staphylococcus aureus (n=1; 9.1%), Streptococcus dysgalactiae sp. equisimilis (n=1; 9.1%), and Streptococcus mitis (n=1; 9.1%) were observed on bacterial culture media. In males patients 65 years of age or older, Staphylococcus aureus (n=1; 33.3%), Staphylococcus haemoliticus (n=1; 33.3%), and Streptococcus agalactia (n=1; 33.3%) were found (Table 2).

A statistically significant difference was not found in growth rates of microorganisms based on mean age or distribution of genders (p>0.05) (Table 3).

Groupings based on CKD-EPI level revealed statistically significant differences in mean age (p=0.028;

Table 3. Evaluation of parameters based on bacterial growth

	Growth of microorganism						p
	Present			Absent			
	n	%	Mean±SD (Median)	n	%	Mean±SD (Median)	
[1]Age (years)			59.73±9.31			57.5±12.62	0.579
[2]Gender							
Female	8	61.5		5	38.5		0.699
Male	14	73.7		5	26.3		
[3]C-reactive protein			6.91±6.39 (4.5)			1.3±1.01 (%1.3)	0.018*

[1]Student's t-test; [2]Fisher's exact test; [3]Mann-Whitney U test; *p<0.05. SD: Standard deviation.

Table 4. Evaluation of parameters based on CKD-EPI groups

	CKD-EPI Group			p
	≤59	60–89	≥90	
[1]Age (years), (mean±SD)	64.56±10.26	60.23±7.76	52.5±10.55	0.028*
[2]Gender, n (%)				
Female	4 (30.8)	5 (38.5)	4 (30.8)	1.00
Male	5 (26.3)	8 (42.1)	6 (31.6)	
[1]Duration of diabetes (years), (Mean±SD)	21.78±8.26	14.62±5.33	11±3.2	0.001**
[1]Glycated hemoglobin (Mean±SD)	7.67±2.874	9.26±1.83	9.9±1.9	0.083
[3]Albuminuria (n=20), Median (min.-max.)	234 (19–1124)	126 (9–2785)	57 (33–258)	0.389
[3]Albuminuria/Creatinine (n=20), Median (min.-max.)	86.6 (17.1–1021.8)	119.2 (11.7–2486)	81.4 (39.3–385)	0.459

[1]One-way analysis of variance; [2]Fisher's exact test; [3]Kruskall-Wallis test; *p<0.05; **p<0.01. CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration equation; SD: Standard deviation; Min.: Minimum; Max: Maximum.

p<0.05). In pairwise comparisons performed to find the group responsible for the statistically significant intergroup difference, mean age of patients with CKD-EPI level of ≤59 mL/min/1.73 m^2 was found to be statistically significantly higher than those with CKD-EPI level of ≥90 mL/min/1.73 m^2 (p=0.024; p<0.05). No statistically significant difference was seen between other CKD-EPI groups on basis of mean age or distribution of male and female patients (p>0.05).

Mean duration of diabetes differed statistically significantly between CKD-EPI groups (p=0.001; p<0.01). In pairwise comparisons performed to determine the group responsible for the statistically significant intergroup difference, mean duration in patients with CKD-EPI levels of ≤59 mL/min/1.73 m^2 was found to be statistically significantly higher than those with CKD-EPI levels of ≥90 mL/min/1.73 m^2 (p=0.012; p<0.05).

No statistically significant difference was detected between other CKD-EPI groups with regard to mean duration, HbA1C levels, urine albumin levels, urine albumin/creatinine ratios (p>0.05) (Table 4).

Growth of various microorganisms was observed in patients according to CKD-EPI level as follows: <15 mL/min/1.73 m^2: Candida glabrata (n=1; 100%); 15–29 mL/min/1.73 m^2: Enterococcus faecalis (n=1; 100%); 30–59 mL/min/1.73 m^2: Acinetobacter baumanii (n=1; 20%), Corynebacterium striatum (n=1; 20%), Serratia marcescens (n=1; 20%), Staphylococcus aureus, (n=1; 20%), Staphylococcus haemolyticus (n=1; 20%); ≥90 mL/min/1.73 m^2: Eikenella corrodens (n=1; 20%),

Table 5. Distribution of microorganisms found according to CKD-EPI group

CKD-EPI group	Microorganism	n	%
<15 (mL/min/1.73 m²)	*Candida glabrata*	1	100
15–29 (mL/min/1.73 m²)	*Enterococcus faecalis*	1	100
30–59 (mL/min/1.73 m²)	*Acinetobacter baumanni* complex	1	20
	Corynebacterium striatum	1	20
	Serratia marcecens	1	20
	Staphylococcus aureus	1	20
	Staphylococcus haemoliticus	1	20
60–89 (mL/min/1.73 m²)	*Citrobacter freundii*	1	7.1
	Klebsialle pneumonia	1	7.1
	Morganella morgani	2	14.3
	Psudomonas aeruginosa	2	14.3
	Proteus mirabilis	1	7.1
	Extended-spectrum beta-lactamase	1	7.1
	Serratia marcecens	1	7.1
	Staphylococcus aureus	4	7.1
	Streptococcus dysgalactiae sp *equisimilis*	1	28.6
≥90 (mL/min/1.73 m²)	*Eikenella corrodens*	1	20
	Morganella morgani	1	20
	Staphylococcus aureus	1	20
	Streptococcus agalactia	1	20
	Streptococcus mitis	1	20

Table 6. Evaluation of bacterial growth according to CKD-EPI group

	CKD-EPI Group						p
	≤59		60–89		>90		
	n	%	n	%	n	%	
Growth							
Present	6	66.7	11	84.6	5	50	0.204
Absent	3	33.3	2	15.4	5	50	

Fisher's exact test.

Morganella morgagnii (n=1; 20%), Staphylococcus aureus (n=1; 20%), Streptococcus agalactiae (n=1; 20%), Streptococcus mitis, (n=1;20%) (Table 5, 6).

A statistically significant difference was not found between CKD-EPI groups with respect to bacterial growth (p>0.05).

DISCUSSION

According to national population estimates, prevalence of DM in 2010 was 285 million patients worldwide aged between 20–79 years, and this number is expected to rise to estimated 439 million by 2030.[4] Several long-term complications may may occur, including DN, the most lethal, and increased urine albumin is an alarming sign of renal dysfunction or renal nephropathy. Renal dysfunction develops in 20–40% of all diabetic patients.[5]

Microalbuminuria is defined as creatinine levels of ≥30 mg, 20 µg/min or ≥30 µ/mg creatinine. Prevalence of microalbuminuria increases in direct correlation with

duration of DM, age, glycemic level, cardiovascular risk factors (e.g., hypertension, smoking, hyperlipidemia, and male gender), ethnic origin (black race), and renal disease.[6] Microalbuminuria is associated with nephropathy, retinopathy, and cardiovascular disease. There is thought to be strong relationship between years since diagnosis, smoking status, and microalbuminuria in development of DF ulcer. Therefore, microalbuminuria has been accepted as an important indicator of risk for development of DF.[7]

In the present study, 20 of 32 cases were evaluated as for the presence of albuminuria. Consistent with the literature, urinary albumin levels in cases with DF were between 9 and 2785 mg/dL (median: 115.5 mg/dL). Still in accordance with literature findings, statistically significant difference was found in mean age between CKD-EPI groups ($p=0.028$; $p<0.05$). Pairwise studies revealed mean age of cases with CKD-EPI level of ≤59 mL/min/1.73 m^2 was statistically significantly higher than that of cases with CKD-EPI level of ≥90 mL/min/1.73 m^2 ($p=0.024$; $p<0.05$). Mean duration of diabetes of patients with CKD-EPI level of ≤59 mL/min/1.73 m^2 was statistically significantly higher than that of the cases with CKD-EPI level of ≥90 mL/min/1.73 m^2 ($p=0.012$; $p<0.05$).

Diabetic foot ulcer is seen in 15% of diabetic patients. As a reflection of interest, scientific papers on DF have increased from 0.7% in the 1980–88 period to more than 2.7% between 1998 and 2004.[8]

Classical triad of DF ulcer consists of infection, neuropathy, and ischemia. Impaired metabolic mechanisms, infection, decrease in response to cellular and growth factors, decreased peripheral blood flow, and angiogenesis impair wound healing. Deformation of peripheral nerves, ulcerations, and, eventually, gangrene develop. Hyperglycemia, increase in aldose reductase, sorbitol dehydrogenase, with ensuing accumulation of sorbitol, and increase in fructose in blood lead to decrease in inositol in nerve cells. Result is slowing of nerve conduction velocity, neuropathic changes, and increase in proinflammatory cytokines. Consequently, these processes affect chemotactic and intracellular apoptotic functions of nuclear leucocytes and have role in immunopathy and vasculopathy by means of inducing endothelial cell dysfunction.[9–11]

Cases of DF constitute large number of amputations performed for non-traumatic etiology in the United States of America, and it has been reported that 22% to 42% of these amputated patients underwent a second amputation within 2 or 3 years. However, nearly 85% of amputations can be prevented with early and appropriate treatment.[12–14]

In a multidisciplinary study conducted in Turkey between 2011 and 2013 of 455 cases of patients with diabetic foot infections, Gram-negative microorganisms were isolated most frequently. Pseudomonas aeruginosa was most common, followed by Escherichia coli. Among Gram-positive bacteria found, methicillin-sensitive Staphylococcus aureus was isolated.[15] In present study, Staphylococcus aureus was most common bacteria detected among all participants. We think that multidisciplinary design of study and number of cases may be responsible for this difference. The most frequently isolated microorganism in patients with CKD-EPI level between 60–89 mL/dk./m^2 was Staphylococcus aureus, followed by Morganella morganii, and Pseudomanos aeruginosa, in order of decreasing frequency. In other CKD-EPI groups, microorganisms were distributed equally.

DF infections should be treated using a multidisciplinary approach, and narrow-spectrum antibiotics effective against most frequently encountered pathogens should be selected for empirical antibiotherapy. Severity of infection, presence of vascular disease, and microorganisms resistant to antibiotics should be taken into consideration. Generally, for superficial infections, broad-spectrum antibiotics effective against aerobic and Gram-positive cocci are first preference; in cases of serious infection, broad-spectrum antibiotics effective against Gram-negative and anaerobic microorganisms should be selected.[16,17]

In conclusion, DM can become a fatal disease because of its microvascular and macrovascular complications. While monitoring the disease, microalbuminuria warns the physician of development of microvascular complications, and these complications should be investigated during routine follow-up visits. Early diagnosis of neuropathy, which plays a role in the development of DF, and consequently the selection of appropriate treatment and shoes, is important issue. Great variety of microorganisms that may be found is one of the factors that complicate treatment success. Initiation of treatment at an early stage with appropriately selected empirical antibiotherapy is very effective in prevention of complications and healing of the wound site.

Therefore, it is critically important to raise aware-

ness of patients about need for compliance with routine treatment and follow-up schedule in order to prevent complications and preserve quality of life.

Conflict of interest

None declared.

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Diyabetik Ayak Tanılı 32 Olgunun Bakteriyolojik ve Klinik Değerlendirilmesi

Amaç: Bu çalışmanın temel amacı diyabetik ayak (DA) tanısı alan hastaların klinik ve laboratuvar özelliklerini araştırarak antibiyotik tedavisi ve klinik takip seçimine yardımcı olmaktır. Diyabetik ayak, böbrek komplikasyonları ve hastalıkların etki mekanizması arasındaki potansiyel ilişki incelendi.

Gereç ve Yöntem: Çalışmaya 2014–2015 tarihleri arasında dahiliye polikliniğinde DA nedeni ile izlenen toplam 32 olgu alındı. Hastaların tıbbi kayıtlarından incelenerek geriye dönük olarak lipid düzeyleri, mikroalbüminüri, mikroalbümin/kreatinin oranı, kreatinin klirensi (Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] ile formülize edilerek), HbA1c düzeyi kaydedildi.

Bulgular: Çalışma 13'ü kadın (%40.6), 19'u erkek (%59.4) toplam 32 olgu ile yapıldı. Olguların yaşları 32 ile 88 yıl arasında değişmekte olup, median değeri 58.5 yıldı. Olguların diyabet yaşları beş ile kırk yıl arasında değişmekte olup, ortalaması 15.5±7.06 yıl, HbA1c düzeyleri 5.4 ile 14.7 arasında değişmekte olup, ortalaması 9.01±2.26 idi. Olguların CKD-EPI düzeyleri 11 ile 130 arasında değişmekte olup, ortalaması 75±27.34'tü. Olguların 22'sinde (%68.8) mikroorganizma üremesi görülürken, 10'unda (%31.3) görülmedi. Olguların ikisinde (%6.3) birden fazla mikroorganizma üremişti.

Sonuç: Yaş ve cinsiyete göre etken mikroorganizma incelendiğinde en çok Gram (+) kok grubu bakterilerin ürediği görüldü. CKD-EPI grupları arasında diyabet yaşı ortalamaları açısından anlamlı farklılık saptanmadı ($p=0.001$; $p<0.01$). CKD-EPI'ye göre üreyen mikroorganizmalara bakıldığında ise; 60–89 mL/dk/m^2 arasında olan olgularda en çok üreyen mikroorganizma Staphylococcus aureus olarak görülmektedir.

Anahtar Sözcükler: Bakteri; CKD-EPI; diyabetik ayak; mikroalbüminüri.