ASSOCIATION OF COMPLEMENT C3 AND INTERLEUKIN-1 WITH FOOT INFECTIONS IN DIABETIC PATIENTS

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The study of the bacteriological profile, the association of complement C3, interleukin-1 β , and zinc therapy of diabetic foot ulcers (type two) was investigated. Twenty diabetics without foot ulcers (group I), 50 diabetics with foot ulcers (group II), and 10 matched normal controls (group III) were enrolled in this study. Diabetic foot ulcers were mostly of grade 2. The most frequent organisms were *Clostridium* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, respectively. Vancomycin, Imipenem, and Meropenem were the most effective against Gram-positive and Gram-negative aerobes, while Imipenem, Meropenem and Chloramphenicol for Gram-positive anaerobes. Group II had abnormal levels of C3 (72%). A significant higher concentration of C₃ was found in group II. Group II had abnormal levels of IL-1 β (60%). A significant higher concentration of IL-1 β was found in group II. Zinc therapy (25 mg/day/oral) induced a highly significant decrease in the frequency of Gram-positive anaerobes and levels of IL-1 β . Significantly increases all mineral concentrations in serum level except Mn⁺². The study highlights the prevalence of antibiotic multi-drug resistant bacteria causing foot infections in diabetics which require combined antimicrobial therapy. Altered levels of serum complement C3 and IL-1 β might be responsible for depressed immune response which might be causes for delayed wound healing and repeated infections. Zinc supplementation may help in healing the wounds by enhancing the immune response.

Keywords: diabetes foot ulcers, bacterial profile, complement C3, interleukin-1 β

Introduction

Type 2 diabetes mellitus (DM) is a serious public health problem and remains an important cause of morbidity and mortality worldwide [1]. Its prevalence is expected to increase exponentially around the world particularly in developing countries [2, 3]. Major advances have been conducted in understanding the mechanisms that are more involved in the pathogenesis of the disease which is mainly based on a decrease in insulin-stimulated glucose uptake [4, 5].

Foot ulcers are a frequent complication in patients with DM, accounting for up to 20% of all hospital admissions [6]. Combined ischemia, neuropathy, and infection in those patients induce tissue necrosis and ulceration which account approximately 15% of all persons with diabetes during their lifetimes [7, 8]. Ulcer infection is certainly a significant underlying risk factor for lower extremity amputation and has an adverse effect on the outcome of patients [9]. Various poorly characterized immunologic disturbances may also increase the risk and severity of foot infection [10].

Wagner [11] classified the severity and depths of tissue injury into five grades. Grades 1 and 2 ulcers are superfi-

cial tissue injury and do not threaten the limb but Wagner grades 3–5 are deeper and limb-threatening ulcers where hyperglycemia is a common sign of infection [12].

Polymicrobial involvement is the rule in severe diabetic foot infections, whereas mild infections are often monomicrobial. Gram-positive cocci predominate in most infections, but Gram-negative rods and anaerobic bacteria are also frequently isolated from deeper or limb threaten infections [13].

Early recognition of lesions and prompt initiation of appropriate antibiotic therapy as well as aggressive surgical debridement of necrotic tissues and modification of host factors, such as hyperglycemia, concomitant arterial insufficiency, are all equally important [14]. The choice of empiric antimicrobial treatment is influenced by various factors such as the location/type of infection, severity of the illness, the likely causative pathogens, and the coexisting complications. In addition, the meticulous wound care regimens to choose the appropriate antiseptics and to restore the body's own bacterial barriers will often prevent amputation [15].

Diabetes affects zinc homeostasis in many ways, although it is most probably the hyperglycemia, rather than any primary lesions related to diabetes, which is respon-

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sible for the increased urinary loss and decrease in total body zinc. Zinc also plays an important role in the synthesis, storage, and secretion of insulin. Decreased zinc levels may affect the ability of islet cells of pancreas to produce and secrete insulin [16].

Pathogenesis of type 2 diabetes includes low-grade inflammation due to activation of the innate immune system manifested by elevated concentrations of various inflammatory markers in the circulation such as interleukin-6, interleukin-1 β , TNF- α , soluble TNF receptors, C-reactive protein, and the complement system that controls the inflammatory reactions [17, 18]. Various poorly characterized immunologic disturbances may increase the risk and severity of foot complications and overwhelming bacterial infections [19].

The objectives of the present study were to evaluate the relative frequency of causative pathogens, the extent of tissue lesions, *in vitro* antimicrobial susceptibility pattern of the isolated pathogens, the effects of zinc therapy, and serum levels of complement C3 and interleukin-1 β in association with the infected foot ulcers of diabetic patients.

Patients, materials and methods

Subjects

The study was carried out on 70 male and female diabetic patients, their age ranged between 15 and 55 years, selected from Al-Doaa and Ain Shams University Hospitals in Egypt. Clinical history regarding age, sex, duration of DM, metabolic control, cardiovascular risk factors, and any associated diseases or complications, such as hypertension, ischemic heart disease, cerebrovascular accident, neuropathy, were reported. The duration of foot ulcers, their localization, and history of amputation were also recorded. Ulcers were graded according to Wagner classification method [11]. Patients with DM were divided into 2 groups: 20 diabetics without foot ulcer complication (group I) and 50 diabetics with foot ulcer complication (group II); they were inpatients and received antibiotic treatment. All patients were on insulin treatment. Ten age- and sex-matched control subjects (group III) were included for determination of the base line of laboratory investigations.

Sample collection

The materials used for microbiological evaluation of infected foot ulcers were curettage of the base of the ulcer after debridement or by applying a sterile cotton swab to the wound. The clinical specimens were transported to the laboratory and processed for aerobic bacteria and anaerobic in thioglycolate liquid medium. Serum was also collected from each blood sample and preserved at -80°C until used for the measurement of minerals levels, complement C3, and interleukin-1 β concentrations.

Macroscopic and microscopic examinations

The clinical specimens were examined macroscopically for any distinguishing characteristics of the organisms present and then a direct smear stained with Gram stain was prepared and examined microscopically.

Culture and identification

The specimens were aerobically cultured on nutrient agar, 5% blood agar, MacConkey's agar and incubated at 37°C for 24 h. Anaerobic cultures performed on thioglycolate liquid, cooked meat media and solid agar were incubated at 37°C for 48 h in an anaerobic jar. The isolated bacteria were identified to the species level using standard methods [20].

Antimicrobial susceptibility test

Antibiotic susceptibility of the isolated bacteria was determined according to the National Committee for Clinical Laboratory Standards [21] using the disc diffusion method (Kibry Bauer). Different antibiotic discs were applied onto the inoculated plate surface with bacterial suspension diluted to 0.5 McFarland turbidity standard {Penicillin (10 $\mu\text{g/ml}$), Ampicillin (10 $\mu\text{g/ml}$), Amoxicillin (25 $\mu\text{g/ml}$), Methicillin (5 $\mu\text{g/ml}$), Cefotaxime (30 $\mu\text{g/ml}$), Cefuroxime (30 $\mu\text{g/ml}$), Amikacin (30 $\mu\text{g/ml}$), Garamycin (10 $\mu\text{g/ml}$), Tetracycline (30 $\mu\text{g/ml}$), Meropenem (10 $\mu\text{g/ml}$), Imipenem (10 $\mu\text{g/ml}$), Chloramphenicol (30 $\mu\text{g/ml}$), Vancomycin (30 $\mu\text{g/ml}$), Ciprofloxacin (5 $\mu\text{g/ml}$), Novobiocin (30 $\mu\text{g/ml}$), Erythromycin (15 $\mu\text{g/ml}$), Amoxicillin/Clavulanic (30 $\mu\text{g/ml}$), Piperacillin/Tazobactam (110 $\mu\text{g/ml}$), Ampicillin/Subactam (20 $\mu\text{g/ml}$)}. After 24 h of incubation at 37°C , the diameter of the inhibition zone was recorded, and the results were interpreted as sensitive or resistant according to NCCLS recommendations.

Measurement of complement C3 and interleukin-1 β (IL-1 β) concentration in serum

Assay Max Human complement C3 and IL-1 β ELISA Kits (EC2101-1 and E12200-1), respectively, were used. They employ a quantitative competitive sandwich enzyme immunoassay in which the intensity of the color development measured by a microplate reader at a wavelength of 450 nm is proportional with the concentration of complement C3 or IL-1 β in sera.

Effect of zinc therapy

To investigate the effect of zinc therapy on the frequency of bacteria-causing foot infection, diabetic patients with

Table 1. Demographic data of diabetic patients and healthy control

Patient's feature	Group I Diabetic without complication <i>n</i> =20	Group II Diabetic with foot ulcer complication <i>n</i> =50	Group III Healthy control <i>n</i> =10	<i>p</i> Value
Age (year); mean±SD	46.2±13.81	57.48±9.69	31.6±13.3	<0.01
Gender (%)				
Male	6 (30%)	34 (68%)	8 (80%)	<0.01
Female	14 (70%)	16 (32%)	2 (20%)	
Duration of diabetes (year)	9.4±5.23	11.38±5.28	–	>0.05
Associated other disease				
*Hypertension	–	36 (72%)	–	
*Ischemic heart disease	–	21 (42%)	–	
*Peripheral vascular disease	–	7 (14%)	–	
*Cerebrovascular	–	3 (6%)	–	
*Neuropathy	–	5 (10%)	–	
*Diabetic retinopathy	–	1 (2%)	–	

infected foot ulcers were further classified into: 23 patients treated with zinc (25 mg/day/oral) for one month and 27 patients not treated with zinc.

Measurement of minerals level in serum

Mineral levels were measured using Inductively Coupled Argon Plasma (ICAP 6500 Duo), Thermo Scientific, England and 1000 mg/l multi-element certified standard solutions; Merck, Germany [22].

Statistical analysis

Data were analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 16. Parametric data were expressed as mean±SD, and non-parametric data were expressed as number and percentage of the total. Comparing more than two groups was performed using the analysis of variance (ANOVA) [23].

Results

Demographic data of diabetic patients

The mean age of 20 diabetic patients without foot ulcer complication (group I) and 50 patients with foot ulcer (group II) were 46.2±13.81 and 57.48±9.69, respectively. Patients with infected foot ulcers had a significantly old age ($p<0.01$) and longer disease duration, they were mainly males (68%), 72% of them had hypertension, 42% had ischemic heart disease, 14% had peripheral vascular disease, and 10% had neuropathy (Table 1).

Clinical features of foot ulcers

Out of the 50 diabetic patients with foot ulcer (group II), 42 (84%) had foot ulcer for more than 1 year while 16% had ulcers less than one year duration. The localization of ulcers was 68%, 20%, 12% in the sole, the big toe and on other parts of the foot, respectively. The grades of ulcers were 40%, 24%, 20%, and 16% Wagner grade 2 ulcer, 5, 4, and grade 3, respectively. Thirty-six percent of ulcers were neuropathic, 24% were vascular while 40% were mixed ulcers. All patients of this group were previously hospitalized, under antibiotic therapy and local antiseptic treatment (Table 2).

Microbiology of foot ulcer infection

Foot ulcers of the 50 diabetic patients were examined for the presence of bacteria (Table 3). Ulcers of 2 patients gave negative cultures. Bacteria isolated from the remaining 48 patients were 22% Gram-positive aerobes, 32% Gram-negative aerobes and 32% Gram-positive anaerobes. *Clostridium* spp. (32%), *Staphylococcus aureus* (14%), *Escherichia coli*, and *Pseudomonas aeruginosa* (10%) for each were the bacterial species most commonly isolated from infected foot ulcers. Five (10%) of ulcers revealed polymicrobial involvement; *S. aureus*/*E. coli* in two patients, *Pseudomonas*/*E. coli* in two patients while *Corynebacterium kutscheri*/*E. coli* in one patient.

The antimicrobial susceptibility pattern of Gram-positive cocci is shown in Table 4a, the most effective antimicrobial agents against *S. aureus* and *C. kutscheri* were Garamycin, Imipenem, Meropenem, Cefprofloxacin, Piperacillin/Tazobactam, and Ampicillin/Subactam. One hundred percent of *S. aureus* were resistant to Cefotaxime, while *C. kutscheri*

Table 2. Clinical features of foot ulcers of diabetic patients

Feature	Number of patients (%) (n=50)
Duration of foot ulcer	
< 1 year	8 (16%)
>1 year	42 (84%)
Repeated ulcer (previous hospitalization)	50 (100%)
Localization of ulcer	
Toes	10 (20%)
Sole	34 (68%)
Other part of foot	6 (12%)
Etiology of ulcer	
Neuropathic ulcer	18 (36%)
Vascular ulcer	12 (24%)
Mixed ulcer	20 (40%)
Grade of ulcer (Wagner classification)	
grade 2	20 (40%)
grade 3	8 (16%)
grade 4	10 (20%)
grade 5	12 (24%)
Antibiotic treatment	50 (100%)
Local antiseptic	50 (100%)

Table 3. Bacteria isolated from the infected foot ulcers of 50 diabetic patients

Bacterial isolates	Number	%*
Gram-positive aerobes (total N°)	11	22
<i>S. aureus</i>	7	14
<i>Corynebacterium kutscheri</i>	4	8
Gram-negative aerobes (total N°)	16	32
<i>E. coli</i>	5	10
<i>Pseudomonas aeruginosa</i>	5	10
<i>Citrobacter freundii</i>	2	4
<i>Proteus vulgaris</i>	1	2
<i>Edwardsiella tarda</i>	1	4
<i>Citrobacter diversus</i>	1	2
<i>Aeromonas</i> sp.	1	2
Gram-positive anaerobes (total N°)	16	32
<i>Clostridium</i> spp.	16	32
Mixed cultures (total N°)	5	10
<i>S. aureus</i> and <i>E. coli</i>	2	4
<i>P. aeruginosa</i> and <i>E. coli</i>	2	4
<i>E. coli</i> and <i>C. kutscheri</i>	1	2
No growth	2	4

*Percentage of the bacteria to the number of patients

were resistant 100% to groups of Penicillins, Tetracycline, Chloramphenicol, Glycopeptides, and Macrolides.

Table 4b demonstrates the susceptibility pattern of Gram-negative aerobes, Imipenem, Meropenem, and Piperacillin/Tazobactam were the most effective antimicrobial agents against all the bacterial species. All the enterobacteria except *Citrobacter freundii* and *Aero-*

monas spp. were resistant 100% to groups of Penicillins. *Pseudomonas aeruginosa* were resistant to almost all the agents studied while *E. coli* were resistant to Tetracycline, Vancomycin, Novobiocin, Erythromycin, Amoxicillin/Clavulanic, and Ampicillin/Subactam.

Among Gram-positive bacilli (16 *Clostridia* spp.), the most effective antimicrobial agents were groups of Carboxypenems and Chloramphenicol and the highest rate of resistance was found with Aminoglycoside group (Table 4c).

Fig. 1. (a) Frequency of patients with abnormal levels of complement component C3. Sixty-five of group I had abnormal levels of C3; 20 had low levels and 45 had high levels. Among group II, 72 had abnormal levels of C3; 12 had low levels and 60 had high levels. Ten of controls had low level of C3 whereas 20 had high levels. Significant differences $p < 0.01$ between patient groups I and II and controls group III. (b) Frequency of patients with abnormal levels of interleukin-1. Sixty of group I had abnormal levels of IL-1 β ; 10 had low levels and 50 had high levels. Among group II, 60 of the patients had abnormal levels of IL-1 β ; 12 had low levels and 48 had high levels. Ten of the controls have low level of IL-1 β and 30 have high levels. Significant differences $p < 0.01$ between patient groups I and II and group III.

Table 4a. Antimicrobial resistance pattern of Gram-positive cocci

Groups	Antibiotics	<i>Staphylococcus aureus</i>		<i>Corynebacterium kutscheri</i>	
		<i>n</i> =7	%	<i>n</i> =4	%
Penicillin	Penicillin	0	0.00	4	100
	Methicillin	2	28.6	4	100
	Amoxicillin	1	14.3	4	100
	Ampicillin	0	0.00	4	100
Cephalosporin	Cefotaxime	7	100	0	0.00
	Cefuroxim	3	42.8	4	100
Aminoglycosides	Amikacin	1	14.3	0	0.00
	Garamycin	0	0.00	0	0.00
Tetracycline	Tetracycline	3	42.8	4	100
Carboxypenems	Imipenem	0	0.00	0	0.00
	Meropenem	0	0.00	0	0.00
Chloramphenicol	Chloramphenicol	3	42.8	4	100
Glycopeptides	Vancomycin	0	0.00	4	100
Quinolones	Ciprofloxacin	0	0.00	0	0.00
	Novobiocin	0	0.00	3	75
Macrolides	Erythromycin	4	57.1	4	100
Combinations	Amoxicillin/Clavulanic	0	0.00	4	100
	Piperacillin/Tazobactam	0	0.00	0	0.00
	Ampicillin/Subactam	0	0.00	0	0.00

Table 4b. Antimicrobial resistance pattern of Gram-negative aerobes

Groups	Antibiotics	Enterobacteria					Other bacteria	
		<i>E. coli</i>	<i>Proteus vulgaris</i>	<i>Edward-siella tarda</i>	<i>Citro-bacter freundii</i>	<i>Citro-bacter diversus</i>	<i>Aero-monas spp.</i>	<i>P. aerugin-osa</i>
		<i>n</i> =5 %	<i>n</i> =1 %	<i>n</i> =1 %	<i>n</i> =2 %	<i>n</i> =1 %	<i>n</i> =1 %	<i>n</i> =5 %
Penicillin	Penicillin	5	1	1	1	1	1	5
		100	100	100	50	100	100	100
	Methicillin	5	1	1	1	1	1	5
		100	100	100	50	100	100	100
	Amoxicillin	5	1	1	1	1	1	5
		100	100	100	50	100	100	100
Cephalosporin	Ampicillin	5	1	1	1	1	1	5
		100	100	100	50	100	100	100
	Cefotaxime	0	0	1	0	0	0	5
		0	0	100	0	0	0	100
	Cefuroxim	0	0	0	1	1	1	5
		0	0	0	50	100	100	100
Amino glycosides	Amikacin	0	0	1	0	0	0	0
		0	0	100	0	0	0	0
	Garamycin	0	1	0	0	0	0	5
		0	100	0	0	0	0	100

Table 4b. *Continued*

Groups	Antibiotics	Enterobacteria					Other bacteria	
		<i>E. coli</i>	<i>Proteus vulgaris</i>	<i>Edward-siella tarda</i>	<i>Citro-bacter freundii</i>	<i>Citro-bacter diversus</i>	<i>Aero-monas spp.</i>	<i>P. aerugin-osa</i>
		<i>n</i> =5 %	<i>n</i> =1 %	<i>n</i> =1 %	<i>n</i> =2 %	<i>n</i> =1 %	<i>n</i> =1 %	<i>n</i> =5 %
Tetracycline	Tetracycline	5 100	1 100	1 100	0 0	0 0	1 100	5 100
	Imipenem	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Carboxypenems	Meropenem	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	Chloramphenicol	1 20	1 100	1 100	0 0	0 0	0 0	5 100
Glycopeptides	Vancomycin	5 100	1 100	1 100	1 50	1 100	0 0	5 100
	Ciprofloxacin	0 0	1 100	0 0	0 0	0 0	0 0	0 0
Quinolones	Novobiocin	5 100	1 100	1 100	2 100	1 100	1 100	5 100
	Erythromycin	5 100	0 0	1 100	2 100	1 100	1 100	5 100
Macrolides	Amoxicillin/ Clavulanic	5 100	0 0	1 100	1 50	1 100	1 100	5 100
	Piperacillin/ Tazobactam	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Combinations	Ampicillin/ Subactam	5 100	0 0	1 100	1 50	1 100	1 100	5 100

Complement C3 concentration of the studied population

Among the 20 patients of group I, 13 (65%) had abnormal levels of C3; 4 (20%) had low levels; and 9 (45%) had high levels. Out of the 50 patients of group II, 36 (72%) had abnormal levels of C3; 6 (12%) had low levels, and 30 (60%) had high levels. One (10%) of healthy controls had low level of C3 whereas 2 (20%) had high levels. Significant differences ($p<0.01$) were observed between patient groups (I and II) and controls (group III) (Fig. 1a).

The mean concentration of C3 (ng/ml) of groups I, II, and III were (13.49 ± 8.04), (18.48 ± 8.64), and (12.63 ± 7.12), respectively. A significant higher concentration of C3 was found in group II compared to group I ($p<0.05$) and controls ($p<0.05$) (Fig. 2a).

Interleukin-1 β concentration of the studied population

Among group I, 12 (60%) of the patients had abnormal levels of IL-1 β ; 2 (10%) had low levels; and 10 (50%) had high levels. Among group II, 30 (60%) of the patients had abnormal levels of IL-1 β ; 6 (12%) had low levels; and 24 (48%) had high levels. Among group III, 1 (10%) of the healthy control has low level of IL-1 β and 3 (30%) have high levels. Significant differences ($p<0.01$) were observed between patient groups (I and II) and group III (Fig. 1b). The mean concentration of IL-1 β (pg/ml) of groups I, II, and III were (7.19 ± 4.4), (10.68 ± 6.59) and (6.2 ± 4.59),

respectively. A significant higher concentration of IL-1 β was found in group II ($p<0.05$) as compared to group I ($p<0.05$) and controls ($p<0.01$) (Fig. 2b).

Effect of zinc therapy on the frequency of isolated bacteria, laboratory investigations, and serum levels of complement C3 and IL-1 β and minerals in diabetic foot infection

Zinc therapy induced a highly significant decrease ($p<0.01$) only in the frequency of Gram-positive anaerobes, while it had no effect on Gram-positive and -negative aerobes and polymicrobial isolation rates. Zinc therapy also significantly decreased fasting blood sugar ($p<0.01$), WBCs ($p<0.05$), CRP ($p<0.01$), and IL-1 β concentration in zinc-treated group as compared to the non-treated group, while it had no effects on C3 and other parameters. On the other hand, zinc therapy induced significant increase in all mineral concentrations ($p<0.01$) and ($p<0.05$) except Mn⁺² (Table 5).

Discussion

The present study was undertaken to investigate the infected foot ulcers in diabetic patients regarding the frequency of causative pathogens, the extent of tissue lesions, *in vitro* antimicrobial susceptibility pattern of the isolated pathogens, the effects of zinc therapy, and serum levels of complement C3 and interleukin-1 β .

In this study, 84% of patients have foot ulcers for more than one year, mainly affect the sole (68%) and most of them (40%) are of grade 2 according to Wagner's classification followed by grade 5 (24%). In India, Shankar et al. [24] found the localization of the ulcers mainly in the big toe (31%) followed by the sole (27%). Among those patients, 90% were presenting with ulcers of Wagner's grade 2 or more which could be due to the use of self-medication or local herbs for treatment in this environment thereby tampering with their ulcers before presenting to hospital. However, Ozer et al. [25] and Candel et al. [26] found that ulcers are mostly of grades 4 and 3. This may also explain in part the multidrug-resistant nature of the bacterial isolates observed in those studies.

The present study demonstrated that the aetiology of the ulcer is neuropathy, vascular, or mixed with both in 36%, 24% and 40%, respectively. Neuropathy was found to be the most common aetiology (74.4%), (48%) in the study of Candel et al. [26] and Hena and Growther [27]. They explained that peripheral neuropathy that affects the

foot is the most common serious complication of diabetes. Loss of sensation results in failure to perceive damage caused by mechanical trauma. In addition, Lipsky et al. [13] suggested that detection of neuropathy before its complications develop is the best way to prevent diabetic foot infection. They complained that foot ulcers in those patients are caused by friction from bad fitting shoes and can be infected easily. Categorization helps to determine the degree of risk to the patient and methods of management.

The bacteriological analysis of foot ulcers in the current work revealed that the most frequent isolated pathogens are Gram-negative aerobes (32%) mainly *E. coli* and *P. aeruginosa* as a monomicrobial infection and as polymicrobial infection. Ozer et al. [25] revealed an overt predominant involvement of Gram-negative aerobes and Dhanasekaran et al. [28] documented that 84% of diabetic foot ulcers are frequently monomicrobial. Ozer et al. [25] and Ozkara et al. [29] reported that severe infections are usually polymicrobial, whereas mild infections are monomicrobial and may often be due to previous treatment. This result

Table 4c. Antimicrobial resistance pattern of Gram-positive bacilli

Groups	Antibiotics	<i>Clostridia</i> spp.	
		n=16	%
Penicillin	Penicillin	11	68.75
	Methicillin	10	62.50
	Amoxicillin	11	68.75
	Ampicillin	10	62.50
Cephalosporin	Cefotaxime	11	68.75
	Cefuroxim	14	87.50
Aminoglycosides	Amikacin	16	100
	Garamycin	16	100
Tetracycline	Tetracycline	12	75.00
Carboxypenems	Imipenem	0	00.00
	Meropenem	0	00.00
Chloramphenicol	Chloramphenicol	0	00.00
Glycopeptides	Vancomycin	8	50.00
	Ciprofloxacin	2	12.50
Quinolones	Novobiocin	4	25.00
Macrolides	Erythromycin	8	50.00
	Amoxicillin/ Clavulanic	11	68.75
Combinations	Piperacillin/ Tazobactam	0	00.00
	Ampicillin/ Subactam	8	50.00

Fig. 2. (a) Mean concentration of complement component C3 in the studied groups. The mean concentration of C3 ng/ml of groups I, II, and III were 13.49 ± 8.04 , 18.48 ± 8.64 , and 12.63 ± 7.12 , respectively. A significant higher concentration of C3 in group II compared to group I ($p < 0.05$) and controls ($p < 0.05$). **(b)** Mean concentration of Interleukine-1 beta IL-1 β in the studied groups. The mean concentration of IL-1 β in groups I, II, and III were 7.19 ± 4.4 , 10.68 ± 6.59 , and 6.24 ± 4.59 , respectively. A significant higher concentration of IL-1 β in group II as compared to group I ($p < 0.05$) and controls ($p < 0.01$).

Table 5. Effect of zinc therapy on laboratory investigations and serum levels of C3, IL-1 β and minerals of diabetic patients with foot infection and controls

Parameter (mean $\pm$ SD)	Diabetic with infected foot ulcer (Group II)		Group III	p Value
	Not treated with zinc n=27	Treated with zinc n=23	Healthy control n=10	
Fasting blood sugar (mg/dl)	273.74 $\pm$ 113.17	172.65 $\pm$ 83.93	80.8 $\pm$ 10.6	<0.01
WBCs $\times$ (10 ³ /UL)	13.33 $\pm$ 6.79	9.85 $\pm$ 5.519	7.51 $\pm$ 2.14	<0.05
RBCs $\times$ (10 ⁶ /UL)	4.17 $\pm$ 0.55	4.42 $\pm$ 0.77	4.79 $\pm$ 0.39	>0.05
Haemoglobin (g/dl)	10.77 $\pm$ 1.67	11.93 $\pm$ 2.43	13.07 $\pm$ 2.43	>0.05
Platelets $\times$ (10 ³ /UL)	274.22 $\pm$ 136.32	288.41 $\pm$ 108.73	227.8 $\pm$ 60.61	>0.05
CRP (mg/dl)	26.19 $\pm$ 14.94	15.30 $\pm$ 4.14	8.2 $\pm$ 2.29	<0.01
ComplementC3 conc. (ng/ml)	18.89 $\pm$ 6.99	18.06 $\pm$ 10.29	12.63 $\pm$ 7.12	p<0.05
				p ₁ <0.05
				p ₂ >0.05
Interleukin-1 β conc. (pg/ml)	12.44 $\pm$ 6.98	8.92 $\pm$ 6.20	6.92 $\pm$ 4.59	p>0.05
				p ₁ >0.05
				p ₂ <0.05
Mineral conc. (μ g/dl)				
Zn ⁺²	30 $\pm$ 18	35 $\pm$ 32	24 $\pm$ 22	<0.01
Ca ⁺²	472 $\pm$ 67	615 $\pm$ 105	70 $\pm$ 16	<0.01
Mg ⁺²	106 $\pm$ 14	139 $\pm$ 29	69 $\pm$ 6	<0.01
Fe ⁺²	117 $\pm$ 122	149 $\pm$ 125	119 $\pm$ 101	<0.05
Cu ⁺²	18 $\pm$ 16	13 $\pm$ 11	8 $\pm$ 11	<0.01
Mn ⁺²	1 $\pm$ 0.4	1 $\pm$ 0.4	1 $\pm$ 0.2	>0.05

P: between group I vs. group III, P₁: between group II vs. group III, P₂: between group I vs. group II.

could explain the low rate of polymicrobial infections in our study since the majority of ulcers are of grade 2, and all patients received treatment for diabetes and foot infections.

The finding that the isolation rate of Gram-positive aerobes was 22% and *S. aureus* constitutes the most frequent pathogen (14%) as monomicrobial and as polymicrobial infection with *E. coli* is in accordance with that of Abdulrazak et al. [30] who isolated *S. aureus* from 20% of foot lesions and El-Tahawy [31] who detected *S. aureus* in 19% of foot infections followed by *Pseudomonas* (18%) and *Proteus mirabilis* (10%). The low isolation rate of *S. aureus* may be due to the low grade of ulcers and tissue damage. Lipsky et al. [13] documented that most cases with mild foot infections are caused by aerobic Gram-positive cocci such as *S. aureus*.

It is interesting that anaerobic bacteria particularly *Clostridia* spp. was isolated from (32%) of foot ulcers of diabetics. This finding is inconsistent with the reports of Shankar et al. [24] and Abdulrazak et al. [30], who identified anaerobes in (2.5%) and (10.5%) of patients, respectively. They added that anaerobic infections usually develop in ulcers of high grades, and the use of aminoglycosides may predispose to anaerobic superinfections because they are inactive against these organisms. This is also true for monotherapy with antibiotics that has minimal activity against anaerobes.

Understanding the bacteriology of diabetic foot infections is important in guiding antibiotic selection and correlating culture results with appropriate definitive therapy in order to eliminate or contain the infectious agents. However, the choice of empiric antimicrobial treatment before microbiological results is necessary to avoid the spread of an infection. The antibiogram results in this study suggest that most Gram-positive and Gram-negative pathogens remain sensitive to a number of agents such as aminoglycosides (Amikacin and Garamycin), Carboxypenemes (Imipenem), and Quinolones (Ciprofloxacin). Vancomycin and Chloramphenicol were found to be the most effective drugs overall against Gram-positive organisms. These findings are consistent with a previous study of Abdulrazak et al. [30], who reported that no single antimicrobial agent can cover all of the possible organisms isolated from diabetic foot infections.

Similar to our results Abdulrazak et al. [30], Gadepalli et al. [32] and Raja [1] revealed that Imipenem, Meropenem, and Vancomycin were the most effective agents against most bacteria isolated from diabetic foot infections. Abdulrazak et al. [30] added that the fourth generation Cephalosporins, Cefepime, and Fluroquinolone lack adequate activity against MRSA, enterococci, and anaerobes, and they should not be used empirically as single agents. Prolonged or broad-spectrum antibiotic therapy

may predispose to infections with antibiotic-resistant organisms like MRSA or Vancomycin-resistant enterococci (VRE). This study also highlights the prevalence of the antibiotic multi-resistant bacterial isolates among diabetic foot ulcers and *Clostridium* spp. were found to be resistant to Methicillin, Ampicillin, Cephalosporins. The prevalence of multi-resistant bacterial strains also portends the possibility of longer period of hospitalization for patients as healing may be compromised when bacteria are highly resistant to antimicrobials [33].

Patients with diabetes have an increased risk of infections, but information on their immune response is incomplete and contradictory. It has been suggested that chronic low-grade inflammation may be involved as pathophysiological mechanisms for the chronic activation of the innate immune system including the complement proteins and proinflammatory cytokines [34]. The complications of diabetes contribute to compromised delivery of nutrients to surrounding tissues, poor elimination of metabolic waste products, impaired wound healing, immunosuppression, and susceptibility to bacterial infection [35].

The present study demonstrated that 72% of diabetics with foot infections had abnormal levels of C3, 12% had low levels, and (60%) had high levels with a significant higher concentration compared to diabetics without foot infections ($p < 0.05$) and controls ($p < 0.05$). Under physiological conditions, complement promotes the clearance of immune complexes, an important way of eliminating antibody-coated bacteria. If, however, immune complexes cannot be eliminated, complement becomes chronically activated leading to increased consumption of the components. This might be the reason for lower level of complement C3 that could affect the formation of membrane-attack complex and lower bactericidal activity and the repeated infections seen in those patients [36]. In addition, Islam et al. [37] found that the levels of complement component C3 in 83.7% of type 2 diabetes mellitus patients were within the normal range, whereas 12.7% had elevated, and 3.6% had lower levels, and the concentration of C3 was slightly lowered in the DM patients. They also found that the serum C3 of the DM patients exhibited significantly lower bactericidal activity than those of the healthy controls. The reasons for depressed bactericidal activity might be due to lower activation of the classical pathway complement as evidenced by significantly elevated levels of C4 arising from either low consumption or because of defects in other complement components. Hyperglycemia associated with DM might have affected complement functions by non-enzymatic glycosylation. Moreover, Oncul et al. [38] reported that metabolic disorders impair organs and systems, including the immune system and glycosylation of immunoglobulin G with glucose leads to covalent incorporation of the sugar into the protein that causes marked decrease in biological activity including the bactericidal ability of PMN leukocytes and complement. The deficiency of complement protein C3 has been found to result in overwhelming bacterial infections. Because of repeated infection episodes, the func-

tions of serum complement in killing intracellular bacteria could be impaired in the diabetic patients.

Among our patients with foot infections, 60% had abnormal levels of IL-1 β ; 12% had low levels and 48% had high levels with a significant higher concentration compared to patients without foot infections and controls. The reduced levels of IL-1 could be explained by Zykova et al. [39] who proved that, in response to inflammatory stimuli, local macrophages and mast cells release several early response cytokines such as IL-1 which is correlated with the regulation of wound healing. In non-healing wounds, there is neutrophil impairment and reduction in IL-1 production.

Oncul et al. [38] reported that polymorphonuclear leukocytes functions and IL-1 regulation deteriorated in patients with diabetic foot infection, and that such deteriorations might indicate inefficient therapeutic responses. They claimed that PMN remove microorganisms by phagocytosis and intracellular killing that requires glucose, and their antimicrobial function is inhibited by hyperglycemia. However, insulin treatment may modulate PMN functions [40, 41]. This previous finding may explain the increased levels of IL-1 in the current study despite the defect in neutrophil function since most of our patients were under metabolic control of insulin. Reed and Pai [42] and Top et al. [43] observed that there is an increase in phagocytic activity in patients with acute bacterial infections which was normalized after the cure of infection, and defects in neutrophil functions were reversible with insulin treatment.

Zinc therapy (25 mg/day/oral) induced a highly significant ($p < 0.01$) decrease only in the frequency of Gram-positive anaerobes and significantly decreases the fasting blood sugar ($p < 0.01$), white blood cells ($p < 0.05$), and C-reactive protein ($p < 0.01$). In addition, the finding that zinc therapy decreased and normalized the level of IL-1 concentration in serum is explained by the results of Gürer et al. [44] who studied the effect of some antibiotics and zinc therapy on polymorphonuclear functions. They found that ciprofloxacin significantly increase PMN phagocytosis activity after zinc supplementation since it has an immunostimulatory effect on PMN function. It has been also documented that antibiotics with the potential properties of biological response modifier should not depress the natural defense factors such as phagocyte or lymphocyte function; antibiotics should increase the bactericidal effect of cells or humoral factors. This author recommended the treatment of elderly patients with a moderate dose of antibiotics and supplementation with zinc so that their immune system can be restored. This could be true since all our patients received antibiotics. Boulton [45] and Brandao-Neto et al. [16] reported that zinc supplementation has potential beneficial effects on glucose homeostasis in chronic diabetes. Vitamins and minerals play diverse roles in the human body, not only in preventing deficiency diseases but also in regulating metabolism, gene expression, and influencing the development and progression of many chronic diseases.

Our data showed that zinc supplementation not only increases serum zinc level but also significantly increases almost all mineral concentrations. This finding was also reported in the studies of Maret and Sandstead [46] and Heidarian and Amini [47]. It is generally agreed that disturbed body distribution of Zn, Cr, and Mg are often found in patients with diabetes mellitus. Chronic diseases in the elderly patients are associated with the decrease of zinc levels in serum which plays an important role for immune system cells and many enzymes and the decreases of humoral and cell-mediated immune system. Plasma zinc concentrations are reduced by many non-specific factors such as infections and trauma [44].

Conclusion

Clostridia spp., *S. aureus*, *E. coli*, and *P. aeruginosa*, were the most common causes of diabetic foot infections. The study highlights the prevalence of antibiotic multi-drug resistant bacteria among diabetic foot infections which requires combined antimicrobial therapy for initial patient management. Our results also indicate that the altered levels of serum complement and IL-1 might be responsible for depressed immune response in patients with type 2 diabetes which might be causes for delayed wound healing and repeated infections. Zinc supplementation may help in healing the wounds by enhancing the immune response. More comprehensive and detailed studies are required in this field.

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