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Research Article

Correlation between IL-10 and IL-23 Levels and Aerobic and Anaerobic Bacteria Isolated from Diabetic Foot Ulcer Patients in Mosul City

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ABSTRACT

Introduction: The occurrence of Foot ulcers among diabetes Mellitus is a common complication and its prevention is of utmost importance.

Aims: To find a relationship between the levels of interleukins 10 and 23 and the bacterial species isolated from diabetic foot ulcer patients to identify the antibiotics used to treat the ulcers the bacterial species that are resistant to antibiotics. Methodology The study was conducted at Al-Salam Teaching Hospital in the city of Mosul during the period from December 1, 2022 to the end of June 2023. The study included 65 samples from patients between the ages of 35 and 80 years.

Results: Of 65 participants, there were 40 males and 25 females , revealing a significantly higher incidence of diabetic foot ulcers in males compared to females in cases of type 2 diabetes. The investigation included an evaluation of IL-10 and IL-23 levels and the results showed no significant difference in the IL-23 levels between patients and controls, while significant negative differences in IL-10 levels were observed. The results of antibiotic susceptibility tests conducted on diabetic foot ulcer isolates showed the presence of bacteria at the site of diabetic ulcers that were resistant to multiple antibiotics.

Conclusion: We conclude from this study that there is a relationship between the levels of IL -10 and IL 23 and the increase in the development of inflammation in diabetic foot ulcer patients. It was seen that there are bacterial species that are resistant to antibiotics. The development of a new drug is needed to treat bacterial infections in antibiotic-resistant diabetic foot ulcer patients.

Keywords: T2DM, Diabetic Foot, IL-10, IL-23, Antibiotics

Introduction

The term "Diabetic Foot Ulcer" (DFU) refers to one of the complications that can occur as a result of diabetes. It can be characterised as a skin slough and full loss of foot skin, frequently brought on by neuropathic or vascular abnormalities in individuals with diabetes type 1 or 2. According to research, it happens to 26% of diabetic patients and has a significant lifelong impact on 34% of them. Foot ulcers are a major health concern for the 5 to 6 million Americans with type 2 diabetes who live in the United States. In addition, lesions connected in some manner to diabetes resulted in the amputation of around 73,000 lower limbs in 2010. According to previous research, DFUs account for more than 85% of foot amputations.[1] One of the worst problems that people with diabetes suffer from is bedsores and sore feet. Gangrene is often a consequence of this, and can lead to amputation of the lower extremities, both small and large. At the microvascular level, sympathetic failure and thermoregulatory issues impair normal tissue perfusion and the body's ability to respond to damage. The primary factor that generates ulcers is foot damage associated with peripheral sensory neuropathy.[2] Aerobic gram-positive cocci such as *Staphylococcus aureus* and coagulase-negative *Streptococcus* species usually cause more serious infections that can be life-threatening and affect deeper layers in the limb. Gram-negative pathogens include *Escherichia coli*, *Klebsiella species*, *Proteus species*, *Pseudomonas species*, and *nterobacteriaceae* species. The three most commonly isolated anaerobes are Pepto*streptococcus* species, Bacteroides species, and *Clostridium perfringens*. Stage 3–4 ulcers are polymicrobial and may involve deeper exposed tissues.[3] An essential function in controlling inflammation is played by the immunoregulatory cytokine interleukin-10 (IL-10). It is thought that IL-10 may be used to treat a variety of inflammatory disorders since it has inhibitory effects on proinflammatory cytokine production and activity, both *in vitro* and in.[4] Low levels of IL-10 have been demonstrated to contribute to the development of non-healing wounds in individuals with diabetes. The expression of anti-inflammatory biomarkers, such as IL-10, which is crucial for accelerating wound healing, is increased when inflammatory biomarkers are inhibited.[5] IL-23 is an important inflammatory cytokine that has a broad effect on the immune response. The formation of endless inflammatory loops that may arise from positive responses to both IL-17 and IL-22 levels is thought to be a contributing factor to a number of autoimmune diseases. This makes IL-23 a critical target for treating autoimmune diseases, which has already been shown to reduce inflammation and disease.[6]

This study aims to isolate and identify bacteria from diabetic foot ulcers, as well as to find the relationship between the levels of interleukin 10 and 23 in diabetic foot patients compared to healthy controls.

Materials and Method

Sample Collection

The study was carried out at the Al-Salam Teaching Hospital in Mosul City during the period from December 1, 2022, to the end of June 2023. The study included 65 samples from patients with ages ranging between 35 and 80 years. After checking their conditions through medical and clinical tests by a specialist physician in this aspect. as well as choosing a random group include (25) samples of healthy of ages between 35 and 80 years old as a control group.

Sample Culture

Samples were collected from patients of both genders with diabetic foot infections under sterile conditions using sterile cotton swabs. Swabs were taken from the deep areas of the infection and were kept in a transport medium to prevent any damage/ contamination. Then they were taken to the laboratory to perform culture cultivation, which was done on Macconkey medium, chocolate agar, and blood agar medium. Incubation was done in aerobic conditions at a temperature of 37 °C. A smear was made on the slide to examine the gram stain, and then the sample was placed in a medium of thioglycollate broth to promote the growth of anaerobic bacteria. HiCrome Clostridial Agar Base was used to isolate *Clostridium perfringens* bacteria and was placed in an anaerobic jar to provide the anaerobic conditions for the growth of the bacteria.

Diagnosis

Bacterial isolates were subjected to morphological, microscopical, and biochemical investigations.

An antimicrobial susceptibility test was performed using the Kirby-Bauar disk diffusion method according to CLSI guidelines (2022). Antibiotic discs manufactured by Oxoid (England) were used. Types and concentrations of these antibiotic discs were as follows:

Imipenem (10 µg/disc), augmentin (20/10 µg/disc), amikacin (10 mg/disc), levofloxacin (5 µg/disc),ceftriaxone (10 µg/disc) , and gentamycin (10 mg/disc).

Patients with wound infections had their serum samples tested using ELISA technology and a test kit provided by the manufacturer. We used group instructions to calculate IL-10 and IL-23 levels.

Statistical Analysis

In order to carry out statistical analysis, version 25 of the SPSS program was utilised. Categorical data was presented using frequencies. Continuous variables were analysed using an independent t-test and were reported through means and standard deviations.

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Results and Discussion

In the examined cohort of 65 diabetic foot patients, 40 were male and 25 were female, revealing a notable male predilection for DFUs in the context of type 2 diabetes mellitus (T2DM), as noted by[7-8] males develop diabetic foot syndrome at a younger age and have more lower limb amputations. Age, body mass index, and systolic blood pressure were found to be independent risk factors for disease development in males, while age, uric acid levels, and insulin therapy were the independent risk factors for disease development in women. Male sex, peripheral vascular disease, and kidney failure are all risk factors for long-term death. It was found through this study that the highest percentage was in the ages ranging from 61 to 70 years, with a percentage of 38% as shown in Figure 1.

Al-Rubeaan[9] found that the prevalence of foot issues rose with age and duration of diabetes, with male patients being the most affected. Diabetic foot is more frequent among type 2 diabetics. Moreover, some studies found that patients over the age of 50 years were more likely to develop chronic complications than patients younger than that age, while another study found that the younger the age at which diabetes is diagnosed, the higher the risk of complications risk of death and cardiovascular disease.[10] In this study, bacteria were isolated from the feet of patients, and diagnosed by several methods, including API and Vitek 2, with the use of different culture media for diagnosis. Through the results, it was found that the percentage of gram-positive bacteria was 51%, while gram-negative bacteria were 49%, as shown in Figure 2.

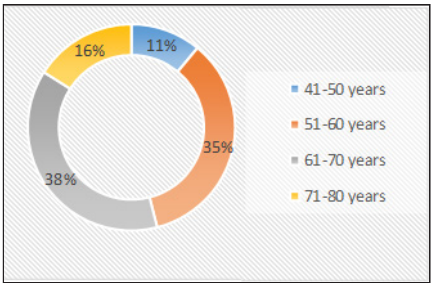

Figure 1.Age-Wise Distribution of Participants

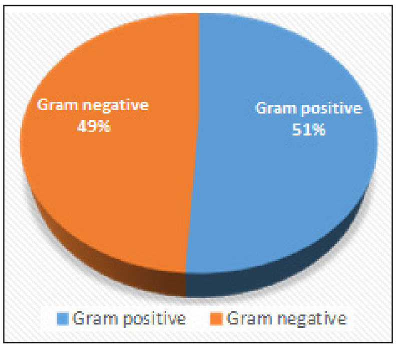

Figure 2.Percentages of Gram-Negative and Gram-Positive Bacteria in the Sample

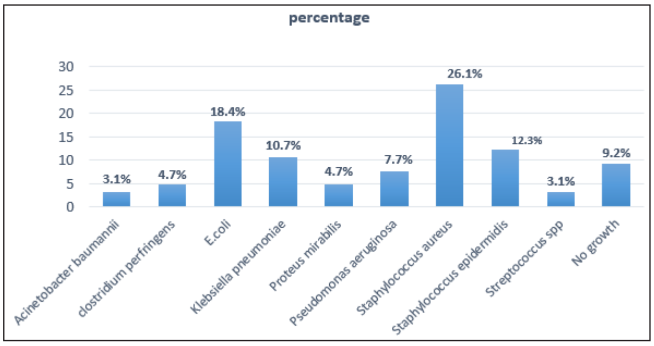

Figure 3.Percentages of Bacterial Species in Diabetic Foot Ulcer Patients

The percentages of isolated bacteria were as shown in Figure 3, where the highest percentage was of *Staphylococcus aureus*, followed by *E. coli*, and the rest of the species, including the anaerobic bacteria *Clostridium perfringens*.

According to previous research[11-13], *Staphylococcus aureus* haplotypes were the most often found in DFUs, followed by. *Escherichia coli* Previous research conducted in countries like Morocco and Brazil (Perim et al., 2015[14]& Belefquih et al., 2015[15]) found that gram-negative bacteria were more common than gram-positive ones. The prevalence of *Escherichia coli* and Pseudomonas aeruginosa was consistent with prior research. The observed variations in microbial cultures across different studies may be attributed to the heterogeneous nature of risk factors associated with the development of foot ulcers in individuals with diabetes.[16-17]

In this study, the levels of IL-10 and IL-23 were also measured, and a comparison was made between patients and controls. It was found that there was no significant difference between patients and controls for IL-23, while the results were significantly negative for IL-10, as shown in Table 1.

Table 1.Levels of Interleukin-10 and Interleukin-23 in Diabetic Foot Patients and Controls

S. No.	Parameters	Controls (N = 25) (Mean ± SD pg/mL)	Patients (N = 65) (Mean ± SD pg/mL)	p Value
1	IL-23	4.305 ± 1.588	4.883 ± 1.822	0.527
2	IL-10	8.386 ± 7.204	6.852 ± 3.678	0.000*

*p value ≤ 0.05 significant

The decrease in IL-10 levels may contribute to the development and progression of diabetes. T2DM patients with foot ulcers exhibited lower IL-10 levels than T2DM patients without foot ulcers, IL-10 is a better biomarker for the development of DFU. This is consistent with the findings of our current investigation[18]. Tang et al (2012)[19] described

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IL-23 as a key participant in the central regulation of cellular mechanisms involved in inflammation and that IL-23 forms a novel axis via Th17 cells that has evolved in response to human diseases associated with immune activation and immunopathology, such as bacterial, viral, and chronic infections are all possible. While studies conducted by Al-Bayatim(2020)[20] and Maboudi et al. (2019)[21] showed lower levels of IL-23 in the patient group compared to the control group, there were no statistically significant differences. When comparing the levels of IL-23 and IL10 between patients and controls and according to the bacterial type diagnosed in the patients, the results showed that in the case of IL-10, there were some significant differences at the probability level (p ≤ 0.05) between the bacterial species, and there were types that did not show any significant difference when compared to the control, but for IL- 23, no significant differences appeared between the types of bacteria as shown in Tables 2 and 3.

Table 2.Relationship between IL-10 Serum Levels and Bacterial Isolates Obtained from DFU Patients

S. No.	Bacterial Type	IL-10 (Mean ± SD pg/mL)	P Value
1	Acinetobacter baumannii	5.320 ± 1.397	0.246
2	Clostridium perfringens	6.080 ± 0.807	0.123
3	E. coli	5.880 ± 1.010	0.040*
4	Klebsiella pneumoniae	5.943 ± 2.585	0.161
5	Proteus mirabilis	6.943 ± 2.059	0.141
6	Pseudomonas aeruginosa	6.138 ± 2.040	0.050*
7	Staphylococcus aureus	7.735 ± 3.771	0.183
8	Staphylococcus epidermidis	7.303 ± 6.045	0.432
9	Streptococcus spp.	4.713 ± 6.045	0.113
10	Controls	8.386 ± 7.204	(p ≤ 0.05)

*p value ≤ 0.05, significant

Table 3.Relationship between IL-23 Serum Levels and Bacterial Isolates Obtained from DFUs Patients

S. No.	Bacterial Type	IL-23 (Mean ±SD pg/mL)	p Value
1	Acinetobacter baumannii	5.532 ± 1.743	0.216
2	Clostridium perfringens	4.480 ± 1.672	0.849
3	E. coli	5.161 ± 1.763	0.727
4	Klebsiella pneumoniae	3.795 ± 1.741	0.972
5	Proteus mirabilis	5.223 ± 1.733	0.956
6	Pseudomonas aeruginosa	4.693 ± 0.484	0.419
7	Staphylococcus aureus	4.810 ± 1.411	0.765
8	Staphylococcus epidermidis	3.968 ± 2.748	0.869
9	Streptococcus spp.	4.582 ± 2.039	0.398
10	Controls	4.305 ± 1.588	(p ≤ 0.05)

*p value ≤ 0.05, significant

According to Yanam et al. (2012)[22], IL-23 has a role in the regulation of peripheral bacterial pathogens, opportunistic infections, viral infections of the central nervous system (CNS),autoimmune diseases, and carcinogenesis. Data from antibiotic susceptibility assays performed on DFU isolates indicated the presence of multi-antibiotic-resistant bacteria . Analysis of the organisms' multidrug resistance showed that more than 85% of the 59 bacterial isolates were resistant to more than two drugs in the same class. This is called "multidrug resistance" (MDR). On the contrary, less than 15% of the isolates were classified as non-MDR. The bacterial strains Acinetobacter, Pseudomonas, and *Clostridium perfringens* showed resistance to the given antibiotics. However, there are differences in the profiles of MDR among the different species. Gram-positive and

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gram-negative bacteria were more resistant to gentamicin, amoxiclav, and ceftriaxone than to imipenem, levofloxacin and amikacin.

The results of the study indicate that a new drug is needed to treat bacterial infections in antibiotic-resistant DFU patients as shown in Table 4.

Banu et al. (2015)[23] showed that the organisms causing chronic DFIs are usually resistant to many drugs because these bacteria possess a biofilm mechanism that makes them highly resistant to antibiotics. Biofilms have a crucial role in DFIs and contribute to delayed healing. While Hassan; et al. (2019)[24] discovered that the majority of the isolates were sensitive to amikacin, our analysis revealed that the sensitivity of bacteria to amikacin differed between bacterial species, and Acinetobacter baumannii was 100% resistant to this antibiotic. In a study conducted by Rastog et al. (2017)[25], it was demonstrated that Pseudomonas aeruginosa was the predominant pathogen identified in DFIs. Furthermore, the study revealed that only 64% of P. aeruginosa isolates and 44% of Acinetobacter isolates exhibited susceptibility to quinolones and cephalosporins, respectively. The results conducted by Saleh and Abbas in 2022[26] indicated that 48% of the samples were gram-negative. They confirmed that Enterobacter spp. and *Escherichia coli* are the most prevalentgram-negative bacteria that causes the formation of DFUs. They showed that the bacteria were less resistant to imipenem (15%) but more susceptible to meropenem (about 43%). Microorganisms isolated from DFUs were highly sensitive to meropenem and vancomycin, according to recent research from.[27] The present investigation demonstrated that DFU infections in the study sites were caused by aerobic and anaerobic pathogenic bacteria of both gram-positive and gram-negative nature. These bacteria, because they possess antimicrobial resistance, present difficulties in patient management and may lead to additional complications, including osteomyelitis and possibly necessitating amputation.

Table 4.Antibiotic Sensitivity for Gram-Positive and Gram-Negative Bacteria

Type of Bacteria (N)	-	Imipenem (10 µg) n (%)	Levofloxacin (5 µg) n (%)	Amoxiclav (30 µg) n (%)	Ceftriaxone (10 µg) n (%)	Gentamicin (10 µg) n (%)	Amikacin (10 mg) n (%)
Acinetobacter	S	2 (100.0)	1 (50.0)	-	-	-	-
baumannii (2)	R	-	1 (50)	2 (100)	2 (100)	2 (100)	2 (100.)
Clostridium	S	2 (66.6)	1 (33.3)	-	-	-	1 (33.3)
perfringens (3)	R	1 (33.3)	2 (66.6)	3 (100.0)	3 (100)	3 (100)	2 (66.6)
Escherichia	S	11 (91.6)	10 (83.3)	6 (50.0)	7 (58.3)	7 (58.3)	8 (66.6)
coli (12)	R	1 (8.3)	2 (16.6)	6 (50.0)	5 (41.6)	5 (41.6)	4 (33.3)
Klebsiella	S	5 (71.4)	5 (71.4)	4 (57.1)	2 (28.5)	3 (42.8)	4 (57.1)
pneumoniae (7)	R	2 (28.5)	2 (28.5)	3 (42.8)	5 (71.4)	4 (57.1)	3 (42.8)
Staphylococcus	S	14 (82.3)	12 (70.5)	7 (41.1)	9 (52.9)	5 (29.4)	11 (64.7)
aureus (17)	R	3 (17.6)	5 (29.4)	10 (58.8)	8 (47.1)	12 (70.5)	6 (35.2)
Staphylococcus	S	6 (75.0)	5 (62.5)	3 (37.5)	2 (25.0)	3 (37.5)	4 (50.0)
epidermidis (8)	R	2 (25.0)	3 (37.5)	5 (62.5)	6 (75.0)	5 (62.5)	4 (50.0)
Pseudomonas	S	4 (80.0)	3 (60.0)	-	1 (20.0)	-	2 (40.0)
aeruginosa (5)	R	1 (20.0)	2 (40.0)	5 (100.0)	4 (80.0)	5 (100.0)	3 (60.0)
Streptococcus	S	2 (100.0)	2 (100.0)	-	-	1 (50.0)	1 (50.0)
spp. (2)	R	-	-	2 (100.0)	2 (100.0)	1 (50.0)	1 (50.0)
Proteus	S	3 (100.0)	2 (66.6)	-	1 (33.3)	2 (66.6)	2 (66.6)
mirabilis (3)	R	-	1 (33.3)	3 (100.0)	2 (66.6)	1 (33.3)	1 (33.3)

S: Sensitive, R: Resistant

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Conclusion

We conclude from this study that there is a relationship between the levels of IL-10 and IL-23 and the increase in the development of inflammation in diabetic foot ulcer patients. It was also observed that there are bacterial species that are resistant to antibiotics and that a new drug is needed to treat bacterial infections in antibiotic-resistant DFU patients.

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Conflict of Interest: None

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