Evaluation of the Relationship Between Bacterial Infection and Immunological Factors with Diabetic Foot Ulcer

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ABSTRACT
Diabetes represent a chronic disorder that involve the decline in the levels of insulin produced by the pancreas due to many genetic and environmental factors. The disease involves the elevation of blood sugar in the circulation which may lead to vascular, neurological, ocular and renal complications. In addition, diabetic patients tend to develop ulceration due to wound infection swiftly in the lower limbs due to the reduce in the neurological sensation as well as to elevated sugar circulation which may greatly provoke wound infection and diabetic foot ulcerations. The current study aimed at isolation of the common causes of bacterial infection associated with diabetic foot ulcer as well as the assessment of IL-17 and IL-18 and in those patients and in control healthy group. The present study was conducted in Salah Aldeen province during the period from 1st of November 2023 to 15th of May 2024 and included 150 patients with diabetic foot infection who attended Salah-Alden Hospitals: (Tikrit Teaching Hospital, Balad General Hospital and private surgery clinics). Swabs and whole blood from patients with diabetic foot ulcer were taken and submitted to routine culture and identification of the causative bacterial infection followed by biochemical identification and antibiotics susceptibility test for the isolated microbe. The serum specimens from those positive bacterial isolates were submitted for IL17 and IL-18 ELISA assessment. IL-17, significant elevation $P<0.05$ of this cytokine was recorded in diabetic patients with foot ulcer with mean 21.94 pg/ml compared to control group. In the same way significant increase of IL-18 ($P<0.05$) was noted in diabetic patients with foot ulcer with mean 347.15 pg/ml in comparison to control group. In conclusion, diabetic foot ulceration is the result of in controlled elevation of blood glucose and bacterial infection with various pathogens can complicate the scenario, and the significant elevation of IL17 and IL18 in those patients can insight the role played by these cytokines in signaling the immune to recruit cellular and humoral response.

1. Introduction

A collection of conditions collectively referred to as diabetes mellitus, or just diabetes, are typified by high blood glucose levels that arise from anomalies in the body's production and/or use of insulin. Diabetes and related illnesses were once thought to be a subset of metabolic syndrome disorders. 1. Over the past few decades, diabetes mellitus has become more common; by 2035, 592 million people worldwide are predicted to have the condition. 2. A collection of metabolic disorders known as diabetes are characterized by high blood sugar levels that are caused by abnormalities in the synthesis or action of insulin. Diabetes's long-term hyperglycemia is associated with macrovascular and microvascular issues that affect organ function, particularly in the kidney, eyes, heart, nerves, and blood vessels. Patients with diabetes are at grave risk for these consequences. 4. Type 1 diabetes (T1DM), Type 2 diabetes (T2DM), and gestational diabetes are the three most prevalent kinds of diabetes mellitus, while there are more. Moreover, DM 5 comes in more specialized varieties. 463 million people in Iraq, or 7.6% of the country's population, are estimated by the International Diabetes Federation (IDF) to have diabetes (type 1 and type 2 combined, diagnosed and undiagnosed). This is equivalent to 9.3% of the world's population. As a result of impairment or a lack of reaction to the pandemic, 578 million people, or 10.2% of the population, are expected to develop diabetes by 2030. By 2045, the figure is predicted to climb to 700 million (10.9%) worldwide. 6. Patients frequently misdiagnose diabetic foot ulcers (DFU) due to peripheral neuropathy, neglecting to examine their feet, or lack of pain perception 7. Neuropathic ulcers were more common in those with poorer glycemic control, while ischemic ulcers were more common in those with greater diastolic blood pressure and total cholesterol. The skin, blood vessels, nerves, and musculoskeletal system are all carefully examined while diagnosing diabetic foot. A skin inspection includes evaluating the legs, foot, and each toenail. Clinical measurements of deep tendon response, vibration, pain, and pressure sensitivity can be used to detect peripheral neuropathy. During the presentation, it was difficult to characterize around half of the nine DFIs. According to some specialists, a wound infection is defined as having more than 105 colony forming units (CFU/g) 10. This threshold separates colonization from infection. Other authors recommend using clinical indications and symptoms of inflammation, such as warmth, redness, swelling, and discomfort 11.

Immunology to diabetic foot ulcers.

An increasing number of individuals suffer from diabetes, which is linked to chronic and long-term inflammation. Chronic wounds heal slowly due to diabetes consequences such as ischemia, neuropathy, hypoxia, and hyperglycemia. Angiogenesis is regulated by the pro-inflammatory mediator monocyte chemoattractant protein-1 (MCP-1). Its relevance to the healing of diabetic wounds is examined, along with current studies that use MCP-1 and other chemokines to lower inflammation and accelerate healing. Diabetic patients experience a delayed healing response, which can lead to the development of diabetic foot ulcers (DFUs), which usually affect the lower limbs. The categorization schemes and available treatments for DFUs—debridement, off-loading, and amputation—are discussed in this article. In order to address and expedite chronic wound healing, common dressing strategies are delineated, and current research on the production of biocompatible and bioactive hydrogels is highlighted11. One diverse subset of T-cells are T-Natural Killer (TNK) cells. These cells are able to recognize the specific lipid molecules that CD1d molecules supply as well as the polypeptides that non-major histocompatibility complexes offer. TNK cells are therefore also known as CD1d-dependent natural killer-like T-cells.11 The NK cellular markers CD56 and CD16, as well as the T lymphocyte markers CD3 and TCR12, are expressed on the surface of these cells.

Aim of the study

Find out if diabetes individuals with foot ulcers have higher levels of interleukin (IL-17 and IL-18) in their blood than diabetic patients without foot ulcers.

Materials and Methods

In order to identify the bacterial pathogens linked to foot ulcers, diabetic patients with foot infections had their feet swabbed and blood drawn. Every bacterial isolate underwent an antibiotic susceptibility test in addition to a standard culture and identification process. Serum samples from both the patient and control groups were subjected to IL-17 and IL-18 assessments.

Patients and Sample Collection.

Between November 1, 2023, and May 15, 2024, a cross-sectional study involving 130 diabetic patients with foot ulcers and 20 diabetic patients without a history of foot ulcers was conducted. Blood and swab specimens were collected from every case at the Salah-Alden Hospitals (Tikrit Teaching Hospital, Balad General Hospital, and private surgery clinic). The patients' informed consent was also acquired through the use of an investigator-designed questionnaire form. Every patient received assurances on the confidentiality of their information.

3.6. Serological parameters

3.6.1. Detection of human interleukin-17 (IL-17)

Using the ELISA method as directed by the manufacturer, the amount of human IL-17 in the serum of study participants was ascertained.

Detection of human interleukin-18 (IL-18)

Using the ELISA method as directed by the manufacturer, the amount of human IL-18 in the serum of study participants was ascertained.

Statistical Analysis.

With the use of SPSS (Statistical Package for Science Services), computerized statistical analysis was carried out. A comparison between probability (P value) and Chi-square (X2) was made. P values less than 0.01 were regarded as highly significant (H.S.), P values more than 0.05 were regarded as non-significant (N.S.), and P values < 0.05 were considered statistically significant (S).**Results**

Bacterial isolation and identification

The current investigation identified a number of bacterial species as the cause of diabetic foot ulcer infections. based on biochemical tests, microscopy appearance, and culture properties. According to table (1), the most frequently isolated bacteria were 27 (38.02%) for P. aeruginosa, 16 (22.53%) for E. coli, 14 (19.71%) for Klebsiella pneumonia, 8 (11.26%) for S. aureus, and 6 (8.45%) for Strep. pyogenes.

Table (1): Primary bacterial isolates in patients' group.

Primary bacterial isolates	**Gram stain**	**No.**	**%**
Pseudomonas aeruginosa	Gram negative	**27**	38.02
E. coli	Gram negative	**16**	22.53
Klebsiella pneumonia	Gram negative	**14**	19.71
Staph. aureus	Gram positive	**8**	11.26
Strep. pyogenes	Gram positive	**6**	8.45
Total		71	100

Biochemical tests result of isolated bacteria

The results of biochemical tests used for identification of isolated bacterial species in our study illustrated in Table (2).

Table (2): Biochemical tests used for identification of isolated bacterial species

Wound swab from diabetic foot	**Citrate**	**Indole**	**Urease**	**Motility**	**K/G**	**Catalase**	**Oxidase**
Pseudomonas aeruginosa	+	-	-	-	**Alk/Alk**	+	+
E. coli	-	+	-	+	**Alk/Alk**	+	-
Klebsiella pneumonia	+	-	+	-	**Acid/Acid**	+	-
Staph. aureus	+	-	+	-	**Acid/Acid**	+	-
Proteus spp.	+	-	+	+	**H2S +**	+	-
Strep. pyogenes	-	-	-	-	**Acid/Acid**	-	-

+: positive, - : negative, NT not tested.

Production of virulence factors among isolated bacteria.

All of the isolated bacteria were able to create virulence factors, as Table (3) in the appendix illustrates. With the exception of E. coli, every bacterial isolate made capsules and had negative lecithinase tests. While all P. aeruginosa isolates were lipase and protease positive, Klebsiella pneumoniae isolates displayed varied lipase production. S. aureus isolates were able to produce DNase and hemolysin on blood agar plates.

Table (3): Production of virulence factors [124.]

Bacteria Virulence	Hemolysin production	Capsule	Lipase	Lecithinase	Protease	DNase
S. aureus	+	+	-	-	-	+
E. coli	-	-	-	-	-	-
P. aeruginosa	-	+	+	-	+	-
Klebsiella pneumonia	-	+	V	-	-	-
Strep. pyogenes	+	+	-	-	-	-

Biofilm formation in isolated bacteria by using Congo red medium method.

P. aeruginosa 17 (62.963%) was the most frequent biofilm producer in the current study, followed by E. coli 13 (81.25%) and Klebsiella pneumonia 12 (85.71%), as shown in Figure (4-5) in the appendix, Table (4–10). S. aureus 2 (25%) and Strep. pyogenes 2 (33.3333%) were the least common biofilm producers.

Table (4): biofilm formation of isolated bacteria

Biofilm formation	**Positive**		**Negative**	
	No.	**%**	**No.**	**%**
P. aeruginosa	17	62.96	10	37.037
E. coli	13	81.25	3	18.75
Klebsiella pneumonia	12	85.71	2	14.286
S. aureus	2	25	6	75
Strep. pyogenes	2	33.3333	4	66.667

Associations of diabetic wound infection with immunological markers.

According to the current findings, diabetic patients' levels of interleukin-17 and IL-18 were significantly higher in them than in control diabetic patients without foot ulcers (P=0.0048 and P=0.0003, respectively) (5).

Table (5): Immunological markers in Diabetic patients and control

Immunology markers	**Diabetic foot ulcer patients**		**Non-diabetic foot ulcer patients**		**P value**
	Mean	**±SD**	**Mean**	**±SD**	
IL-17 pg/ml	21.94	11.58	7.62	3.07	**0.0048 ****
IL-18 pg/ml	347.15	201.29	141.62	71.24	**0.0003*****

Significant levels if P<0.05, non-significant if P>0.05

Discussion

Our results demonstrated that almost every microbe in the study had grown and demonstrated biofilm formation. It is more difficult to define infection in chronic wounds than in acute wounds due to the presence of biofilms. Biofilms can induce excessive inflammation by inducing nitric oxide, inflammatory cytokines, free radicals, immune complexes, and complement, all of which can postpone recovery. As of right now, there is no diagnostic for the existence of biofilms. Controlling biofilm is a crucial part of addressing chronic wounds [1]3. According to Corehtash et al. (2014), 92 of his P. aeruginosa isolates (92.4%) were biofilm formers, In contrast, Saffari et al. (2015) found that all 92 of their isolates in Iran produced biofilm. These outcomes agree with the recently discovered information. The ability of P. aeruginosa to produce and secrete a range of virulence factors, including pyocyanin, pyoverdine, and proteases, as well as to form biofilms, in which the bacterial cells are embedded in an extracellular matrix made of alginate, is thought to be the pathogenesis of P. aeruginosa infection. Even with intensive antibiotic therapy, P. aeruginosa is difficult to eradicate after it has established a stable colony on patient 16. The current investigation validated a recent work that shown, when cultivated in a collagen wound biofilm model, Staph. aureus, P. aeruginosa, and Strep. pyogenes could be successfully differentiated based on their production of volatile chemicals 17. Previous research has demonstrated that K. pneumoniae's strong adhesive potential is one of the essential elements in the construction of a biofilm architectonics, which is characterized by an increase in optical density and various drug resistance 18Moreover, Sachivkina et al., 19 found that 57.1% of K. pneumoniae strains had the ability to form biofilm in another study. Biofilm production of S. pyogenes has been demonstrated both in vitro and in vivo. Proteins, DNA, and polysaccharides are known to be present in mature Streptococcus pyogenes biofilms. The latter material is known as glycocalyx20.

Associations of diabetic wound infection with immunological markers.

The data presented here illustrated significant elevation of IL-17 and IL-18 in diabetic foot ulcer patients compared to control group. These data were in line with Akhter *et al*., who reported an elevated levels of IL-17 in diabetic patients with foot ulcer[2]1. Also, it agrees with another study conducted by Kaleli *et al*., who denoted significant elevation of IL-17 in diabetic patients [2]2.

Another study found that those patients with diabetes exhibited elevated levels of IL-17 in comparison to the healthy participants. The concentration of IL-17 in individuals with complex diabetes was shown to be greater compared to those with type 2 diabetes mellitus (T2DM) but without any problems [2]3.

The reason behind the increment in IL-17 in diabetic patients could be due to the role played by this interleukin as a proinflammatory cytokines that produced by several immune cells which help in the recruitment of various inflammatory cells and cytokines that help in the combat against the invading pathogens in the affected site. in diabetic patients with foot ulcer the inflammatory is exacerbated due to the insulin resistance status and the elevated levels of glucose which vastly enhanced capability of the bacteria to colonize and proliferate [2]4.

IL-17F as well as IL-17C. By acting on keratinocytes, these cytokines cause the expression of several chemokines, which attract and gather neutrophils, T cells, and dendritic cells 25. IL-18: A previous study found that serum IL-18 levels rose markedly with the advancement of diabetes and were strongly correlated with the severity of type 2 diabetes, with patients with Wagner grade 4 ulcers 20 showing the greatest quantities of IL-18. Age, the stage of diabetes, and IL-18 were found to be risk variables for DF in the study analysis. The development of DF illness was strongly correlated with elevated serum levels of IL-18 and Lp-PLA2. Lp-PLA2 and IL-18 detection can help physicians determine how severe DF is. The understanding of disease progression and therapy efficacy can also be aided by dynamic detection [2]0. According to late research, it was shown that diabetic individuals with acute diabetic foot ulcers had increased levels of blood IL-18 concentrations. Nevertheless, these data do not establish whether the increase in IL-18 is a causal factor or a consequence of the development of diabetic foot ulcers. Additional research is required to demonstrate the specific function of IL-18 in the progression of these ulcers [2]5. Recent study reported that Interleukin-18 have an important role in recruiting the immune system as a pro inflammatory cytokine. It plays a crucial role in signaling other inflammatory cells to migrate toward the side of confliction where it allows it neutrophils and local microphage to move easily toward the side of infection and consume that cells and bacteria on top of this the side kind promoted by interleukin 18 enhance the inflammatory response by the immune system, which will cause more chronic inflammatory response hence more damage to the infected tissue, which explains the elevated increment of this inter and diabetic food also [2]6.

Conclusions

The study concluded the following: The ability of the isolated microbes to form biofilm was recorded in all the bacterial species with highest rate in *K. pneumonia*. IL17 was significantly upregulated in diabetic patients with foot ulcer. IL-18 was elevated significantly in foot ulcer from those diabetic cases.

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