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Immunological Base Behind the Increased Susceptibility of Diabetic Patients for Infections

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ABSTRACT

A 120 middle-aged adults and elderly were enrolled in this case – control study, including 48 type 2 diabetes without foot infection patients, 36 diabetic foot patients and 36 apparently healthy individuals matched as control group. The current study was carried out to evaluate the immunological status displayed by antimicrobial peptide and their role in diabetes mellitus patients and diabetic foot infection patients. The results of demographic data examination among patients with diabetes mellitus in this work revealed that out of all 84 diabetic patients, 78% had their first signs of diabetes during their forties. Otherwise, the most common age interval for acquirement of diabetic foot infection among diabetic patients were in 50s-60s leading to 60% of diabetic foot patients underwent foot amputation at that age interval. Regarding gender as a demographic factor among diabetes patients in this study, the results showed that the gender of females and males were equal in frequency among diabetes patients without foot ulcers. On the other hand, the results demonstrate that the gender of male was found to have susceptibility to get diabetic foot infection more than the female gender (63.89% versus 36.11%). In this study, biochemical test and hematological parameters that affected on foot amputation were random blood sugar (RBS), hemoglobin A1c, neutrophils (high-significant difference between the groups in the study ($P=0.0001$)) and neutrophil-lymphocyte ratio (high statistical significant difference between the study groups ($P>0.003$)). Regarding the evaluation of immune parameters reflected by antimicrobial peptides in present study, serum HNP1-3 and DEF- β 2 levels were non-significant difference between the study groups ($P>0.05$). Otherwise, LL-37 level was significant difference between the study groups ($p<0.05$). These results suggest that although most AMPs are expressed in DFU, this production is not appropriate to promote wound healing and contain secondary infections but present decrease in human LL-37 can be cause in don't wound healing in diabetic foot patients.

Keywords: *Diabetes mellitus; Diabetic foot ulcer; HNP1-3; LL-37; DEF- β 2*

Introduction

Diabetes is widely recognized as an emerging epidemic that has a cumulative impact on almost every country, age group, and economy across the world¹⁷. Type 2 diabetes mellitus (DM) is a chronic metabolic disorder in which prevalence has been increasing steadily all over the world. As a result of this trend, it is fast becoming an epidemic in some countries of the world with the number of people affected expected to double in the next

decade due to increase in ageing population, thereby adding to the already existing burden for healthcare providers, especially in poorly developed countries¹⁶. Diabetes complications are common among patients with type 1 or type 2 diabetes but, at the same time, are responsible for significant morbidity and mortality. The chronic complications of diabetes are broadly divided into microvascular and macrovascular. Microvascular complications include neuropathy, nephropathy, and retinopathy, while macrovascular complications consist of cardiovascular disease, stroke, and peripheral artery disease (PAD). Diabetic foot syndrome has been defined as the presence of foot ulcer associated with neuropathy, PAD, and infection, and it is a major cause of lower limb amputation¹⁷. For a long time, alterations of immune defences have been implicated in the susceptibility

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to infection of diabetic patients. Louis Pasteur was reported to acknowledge in 1895 on his deathbed that the microbe is nothing, the terrain is everything, quoting Claude Bernard's motto. A lot of immunological defects, mostly related to innate immunity, have been reported in diabetic patients¹⁴. The most consistent involves altered function of PMNs, specially impaired phagocytosis and bactericidal activity. Twenty years ago, it was shown that ingestion and killing of *Staphylococcus aureus* by PMNs were significantly reduced in diabetic patients with poor metabolic control²¹. The neutrophils play an essential role in the inflammatory reaction and in the response to an infection. They are one of the first lines of defense against pathogens and during inflammation. Their immunological capacity is linked to their property of migrating to the inflammatory site and phagocytosing the pathogen or killing it via their microbicidal activity^(19,24).

Materials and Method

Patients group: This study was conducted in the department of Microbiology of Medicine College/ Babylon University. A hundred and twenty middle-aged adults and elderly were enrolled in this case – control study, including forty-eight diabetes mellitus only, thirty-six diabetic foot and thirty-six healthy individuals matched as control group. All patients in the study were referred and diagnosed in the Diabetes Center at Marjan hospital Teaching Hospital during the period from November 2018 to February 2019. A full questionnaire including demographic information's set, residence, history of diabetes mellitus, taking medication subjects. All were matched regarding the age & set to the patients group.

Specimens collection: Blood samples were taken from all subjects in the study for biochemical, hematological and immunological parameters. Blood samples were collected by drawing 3ml of blood from each subject included in this study using sterile 5ml syringes with sterile needle G-22. Blood samples of study groups were collected in sterile 10ml capacity sterile gel tubes and labeled with name, age, gender as well as date of their collection. After blood clot formation at room temperature within 30minutes, clot blood sample were centrifuged at 2500 r.p.m. for 5 minutes. Separated sera samples were collected, distributed in 0.5ml quantities in sterile containers, labeled and stored at -20°C until used. Two ml of venous blood was collected by disposable syringe then withdrawn in EDTA container and mixed with the anticoagulant to avoid clot formation.

Complete blood count: Complete blood count (HB, RBCs, WBC (neutrophil and lymphocyte), MCV, MCH, MCHC, HCT, platelet indices) was tested by automated method (Sysmex).

Biochemical tests: Random blood sugar (RBS) was detected by Glucose kit {Company: Biomaghreb, France, Lot number (20121)}. HbA1c was perceived by Finecare HbA1c kit {Company: Wondf, China, Lot number (F20712402AD)}. Test should be performed at room temperature.

Serological Markers: HNP1-3, Human DEFβ2/DEFB2 and Human LL-37 detected by enzyme linked immunosorbent assay {Human HNP1-3(Neutrophil Peptide 1-3), Human DEFβ2/DEFB2 (Defensin Beta 2) and Human LL-37 (Antibacterial Protein LL-37) ELISA Kit, Elabscience, U.S.A., Lot number(E-EL-H2299, E-EL-H0996 and E-EL-H2438 respectively)}. Parameters were measured according to instruction of the manufacturing company.

Statistical Analysis: This done by using statistical package for social sciences (SPSS) version 22, in which the researcher used analysis of difference (ANOVA) and independent sample T-test for measuring the data and chi square (X²) for a categorical data. Set p value < 0.05 as significant (95%).

Results and Discussion

This result was supported by the recommendation of the American Diabetes Association (ADA) that doing annual diabetes screening tests after the age of 45. But the age at which someone develops the condition depends on too many differing factors to accurately predict. Many people have diabetes for years before being diagnosed, causing a large variation between the age of onset and age of diagnosis. Coming in line with this study data about the age distribution among diabetic patients, according to the CDC, the majority of new diabetes diagnoses were among adults aged between 45 and 64. While there might not be a set age for onset for type 2 diabetes, age greatly increases the chances of developing diabetes complications most importantly diabetic foot infection⁹. Otherwise, the most common age interval for acquirement of diabetic foot infection among diabetic patients were in 50s-60s leading to 60% of diabetic foot patients underwent foot amputation at that age interval (Table: 1)

Table 1: Percentages of study groups according to their age groups

Factor	DM only				Diabetic foot			
	40-49	50-59	60-69	70-79	40-49	50-59	60-69	70-79
No.	6	24	13	5	7	12	12	5
Total No.	48				36			
% of No.	12.5%	50%	27.08%	10.42%	19.44%	33.33%	33.33%	13.89%

Table 2: Frequency distribution of gender among the study groups

Factor	DM only		Diabetic foot		Total No.	
	Female	Male	Female	Male	Female	Male
No.	24	24	13	23	37	47
Total No.	48		36		84	
% of No.	50 %	50 %	36.11%	63.89 %	44%	56%
% of total	28.57%	28.57%	15.48%	27.38 %	100%	
	57.14%		42.86%			

Table 3: The distribution of the study groups according to RBS, HbA1c, Neutrophil, NLR, HNP1-3, DEF-β2 and LL- 37

Factor	DM only	Diabetic foot	Healthy control	P-value
RBS	9.8906 ± 3.31763	12.5625 ± 4.96911	4.7125 ± 0.91856	0.0001
HbA1c	6.8138 ± 1.09464	7.7425 ± 1.13673	5.4608 ± 0.06143	0.0001
Neutrophil	4.2344 ± 0.73193	7.3521 ± 3.09883	4.2767 ± 1.22586	0.0001
NLR	1.5795 ± 0.48765	3.6191 ± 3.33925	2.1003 ± 0.81056	0.003
HNP1-3	7.5645 ± 2.79812	6.8089 ± 4.00288	6.1982 ± 2.46817	0.824
DEF-β2	151.1111 ± 115.88834	230.6481 ± 141.67729	196.4815 ± 161.53972	0.166
LL- 37	30.8833 ± 19.10989	28.0639 ± 15.09763	44.4611 ± 13.42676	0.045

In this study, blood sugar was studied as a biochemical diagnostic parameter for diabetic patients. Random blood sugar level among the three groups through the current study established that there was a high-significant difference between their serum levels. While HbA1c in the diabetic foot group were significantly higher than diabetes and control groups, and there was a high-significant difference between the groups in the study (P=0.0001) (Table: 3). In recent studies about the distribution of random blood sugar mean value among diabetic patients, the risk factors of foot ulceration varied in studies, loss of glycemic control for any reason may lead with time to diabetes become progressed and less responsive to treatment and resultant increase in DFU risk. Improving ability to predict and prevent diabetic foot ulceration is imperative because of the high personal and financial costs of this complication. Furthermore the better glycemic control the fewer complications

from hyperglycemic attacks^(12, 13). The results regarding HbA1c reached through this study were in consistence with pervious study which reported that higher HbA1c levels were positively correlated with future diabetes complications such as risks of stroke, coronary heart disease, and all-cause death. The risks of ischemic stroke increased by 1% increment of HbA1c regardless of diabetes diagnosis. In addition, HbA1c levels 7.5% (58.5 mmol/mol) incrementally and significantly increased future risks of ischemic stroke, coronary heart disease, and all-cause death compared to the reference group of HbA1c levels <5.5% (36.5 mmol/mol) (Chen et al., 2015). Likewise, these results about HbA1c were agreed with earlier study which found that Hba1c in diabetic foot ulcer was 9.76 ± 2.5, diabetic control group 7.93 ± 2, statistically high significant differences were also observed according to HbA1c (P = 0.000). Short-term glycemic control as measured by HbA1C was variable

in regression models among patients with diabetic foot ulcers in the study; however, the association between poor glycemic control and development of diabetes complications demonstrated that health-related quality of life was not identified in the diabetic patient group. These findings have implications for clinical and policy decisions, as well as for the design for future studies with larger sample sizes. In particular, the findings underscore the importance of quality of life in the management of diabetic patients with or at risk of foot disease ²⁶.

Likewise in the present study neutrophil count was studied as a hematological parameter for diabetic patients, the results showed that neutrophils count in the diabetic foot group were significantly higher than diabetes patients and control group and there was high-significant difference between the study groups ($P=0.0001$) (Table: 3). In the present study we hypothesized that the phagocytic activity disturbances in patients with poorly controlled diabetes may play an important role in the pathogenesis of infectious complications in these patients and may augment the progression of immune dysfunction to increased morbidity and mortality rates. These results regarding neutrophils count among diabetic foot patients identified in the present study was in agreement with earlier study who found a higher mean laboratory result for neutrophils count than control. Among laboratory finding neutrophils had a significant impact on extremity amputation, the presence of coronary artery disease, duration of hospital stay, presence of osteomyelitis, decreased levels of neutrophil are crucial for predicting amputation in patients referring to emergency units with DFU ¹⁰. While the neutrophil-lymphocyte ratio in the diabetic foot were higher than both the diabetes patients and control groups, also the healthy control group had high ratio than diabetes mellitus group and there was high statistical significant difference between the study groups ($P>0.003$) (Table: 3). The NLR is a result of dividing the number of neutrophils to lymphocytes acquired by the differential blood cell count test. It is an inexpensive and more accessible way of evaluating immune system activity compared with many other wound-specific markers. These results in the existing study were confirmed with a prior study which mentioned that there was a statistically significant differences observed groups according to NLR ($P = 0.001$). Patient with diabetic foot ulcers and non-ulcer has been analyzed regarding NLR level as inflammatory marker. Determining NLR in the presence of high levels of diabetic foot ulcers

is not a local inflammation alone but also showed that there is a systemic inflammatory response. In patients with diabetic foot other macrovascular complications of diabetes can be seen much more and using NLR has been suggested as cheap and accessible inflammatory markers for developing and following of macrovascular complication ¹⁹.

Conclusions

In this work foot ulcer of type 2 diabetes mellitus is fairly common. Lack of awareness of foot care and delayed treatment request will increase the extent of tissue destruction. Neutrophils are the main source of antimicrobial peptides which are expressed in DFU, although their expression levels are not appropriate to promote wound healing and contain secondary infections. Administration of antimicrobial peptides may be recommended to be used as augmentation therapy to fasten diabetic foot infection.

Source of Funding: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Medical Microbiology, College of Medicine, University of Babylon, Hilla, Iraq and all experiments were carried out in accordance with approved guidelines.

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