Assessment of Some Immunological Parameter Related to Wound Healing in Diabetes Foot Ulcer

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Abstract
Diabetic foot ulcer (DFU) are one of the most common and serious complications of diabetes that consider cause high morbidity and mortality in patients, in addition to increase bacterial resistance against wide range of antibiotics, the study aim certain whether Assessment of Some Immunological Parameter Related to Wound Healing in Diabetes Foot Ulcer. The present study included 30 diabetic type 2 without foot ulcers and 60 patients suffering from diabetic foot ulcer aattending to the private laboratory in Baghdad city, Iraq from beginning September 2021 to March 2022, with age (20-67) years old. The present study also observed that the concentration of Hypoxia-inducible factor-1 (HIF-1), Stromal cell derived factor-1 (Sdf-1) in diabetic foot ulcer infection increase significantly($P< 0.05$) than diabetic patients without foot ulcer infection.
Keyword: Diabetic foot ulcer, Hypoxia-inducible, Stromal cell derived

1-Introduction

Foot infections are common and serious problems in persons with diabetes. Diabetic foot infections (DFIs) typically begin in a wound, most often a neuropathic ulceration. While all wounds are colonized with microorganisms. Infections are then classified into mild (superficial and limited in size and depth), moderate (deeper or more extensive), or severe (accompanied by systemic signs or metabolic perturbations) (Mponponsuo *et al.*,2021). This classification system, along with a vascular assessment, help determine which patients should be hospitalized, which may require special imaging procedures or surgical interventions, and which will require amputation(Lipsky *et al.*,2020).
Wound healing is a complex physiological process comprised of discreet but inter-related and overlapping stages, requiring exact timing and regulation to successfully progress, yet occurs spontaneously in response to injury. It is characterised by four phases, coagulation, inflammation, proliferation and remodelling. Each phase is predominated by particular cell types, cytokines and chemokines (Portou, 2019).
Hypoxia is defined as a state of reduced oxygen levels because of a decrease in the oxygen supply and/or an increase in oxygen consumption. Following injury, the wounded area becomes increasingly hypoxic as a result of vascular disruption and increased oxygen consumption accompanying inflammatory responses (Catrina and Zheng, 2016).
SDF -1α is a peptide chemokine, referred as chemokine ligand 12 (CXCL12) which is located on chromosome 10q 11.1 and detected in stem cells and stromal tissues of multiple organs (Lang, 2017).

2. Materials and Methods

The study included 90 specimens collected during the period from beginning September 2021 to March 2022 who attending to the private laboratory in Baghdad city, Iraq. The patients were divided into two groups, the first group included patients with Diabetic foot ulcer while, the second group represent Diabetic without foot ulcer for both sexes with different age groups.

2.1. Collection of samples

Three to five milliliters of venous blood sample was taken from all individual. A tourniquet was applied directly on the skin around the arm, the skin over the vein sterilized with 70% ethyl alcohol from the patients and control group before blood collection was transferred into Gel tube for serum separation, the blood is left about to 30 minutes in room temperature for clotting and then centrifuged at 3000 g for 2 minutes, then serum was collected in sterile appendrofe tube in three repeaters and kept frozen at -20 C ° for determination of Human HIF-1,and Sdf-1 ELISA kit .

2.2. Estimation of serum HIF-1 and Sdf-1:

This accomplished according to ELISA kit (Ye *et al.*,2020).

4. Results & Discussion

The current study includes 90 samples, which were separated into two groups for further analysis in the private laboratory in Balad city, Iraq, DFU = 60 (66.66%) and D without FU= 30 (33.34%), as illustrated in figure (1).

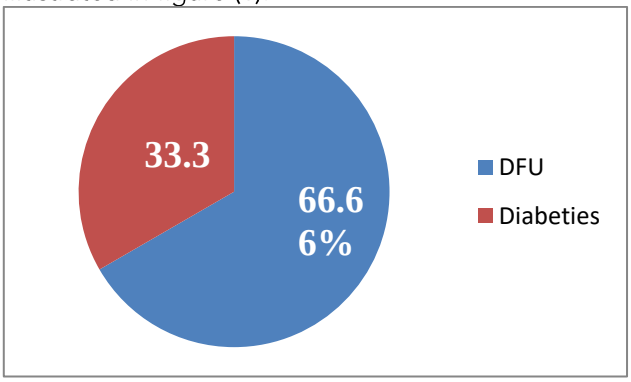

Figure (1): Prevalence of DFU among diabetic patients

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4.1. Gender distribution of patients

The results indicated that clinical specimens were 90 distributed according to the gender, the number of male patients outnumbered the number of female patients by a margin of 36(60%) to 24(40%) for DFU and male 13(43.33%) and female 17(56.67%) for D without FU as illustrated in figure (2).

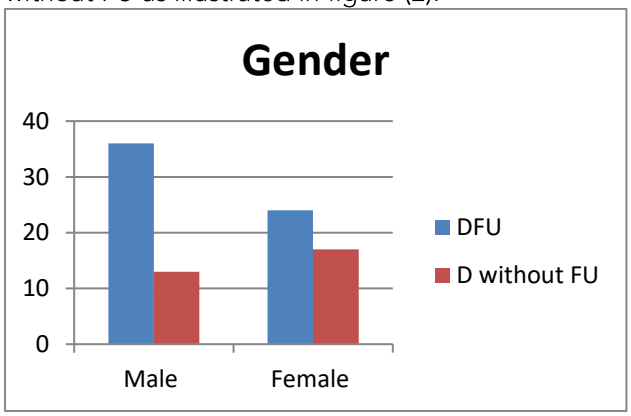


Figure (2):- Gender distribution of the DFU patients

Increased male to female frequency similar with the result of (Shekhar *et al.*,2014) they illustrated that male recorded 72.2%. (Bansal *et al.*,2008) they observed that male more frequency than female (78.64% vs 21.36%) and dissimilar with (Parisi *et al* .,2016) they found The majority of diabetic foot patients were female (58.6 %).

DM has become one of the most common chronic diseases, thereby posing a major challenge to global health and one third of diabetic patients develop serious complications. Diabetic foot disease is a major health problem, which affects 15% of the 200 million patients with diabetes worldwide (Manzi, 2018). Hence there is an imperative need for a better understanding of the molecular mechanisms underlying the accelerated rates of complications under diabetic conditions in order to develop more effective therapeutic regimes and early diagnosis. Diagnosis and treatment of an ischemic diabetic foot is a major challenge in daily routine.

4.2. Distribution of the patients according to Age

Patients were placed into five groups based on their age ranges, with each group being separated into two groups. According to figure (3) the distribution of DFU due to age groups that show 51-60 had the highest frequency, followed by those aged 41-50, 61-70, 31-40, and those aged 21-30, who had the lowest frequency, which were 29, 12, 11, 5 and 3, respectively, and the distribution of D without FU that show 51-60 had the highest frequency, followed by those aged 41-50, 31-40, 21-30 and those aged 61-70, who had the lowest frequency, which were 10, 9, 6, 3 and 2, respectively.

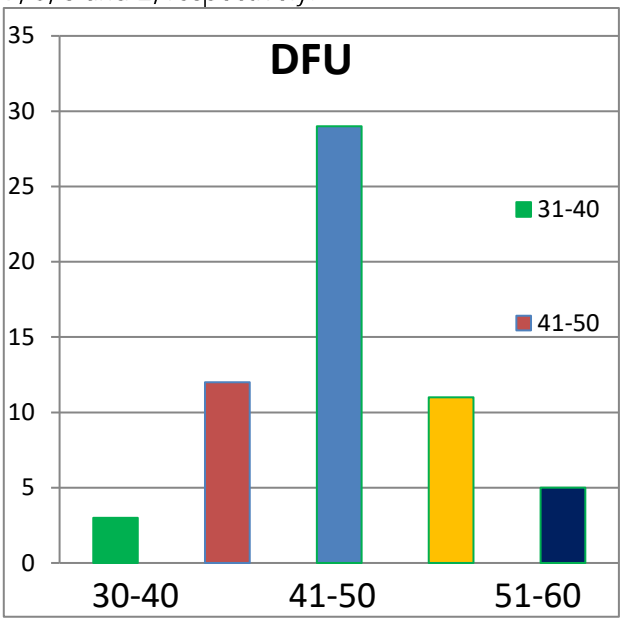


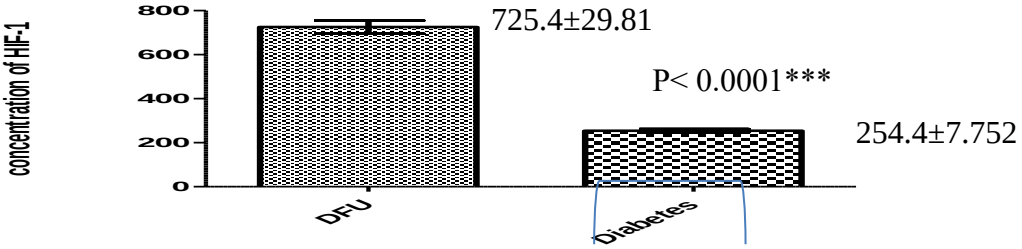
Figure (3):- Distribution of the DFU patients according to Age

The results obtained during this study were similar to the findings observed in(Deribe *et al* .,2014) they revealed that the prevalence of DFU in the age group of 48-57 was 50 % . Also, consistence with other study which revealed that there was a higher frequency of DFU among older age groups (Al-Rubeaan *et al.*, 2015). (Danmusa *et al.*,2016) illustrated that the prevalence of DFU in the age group 51-60 was 32 (31%) .

Age was the risk factor of amputation, peripheral vascular disease (PVD),Neuropathy among diabetic patients (Kravos and Bubnic,2009).With their increasing ages and disease course, elderly patients with diabetes frequently also have peripheral neuropathy and vascular lesions, leading to diabetic foot ulcers that poorly heal over a long treatment time (Shahbazian *et al.*,2013).

4.3. Evaluation of Hypoxia-inducible factor-1 (HIF-1) in DFU

The present study also observed that the concentration of MIP-1α (pg/ml) in DFU infection was 725.4±29.81pg/ml is increase significantly than, diabetes patients without FU infection was 254.4±7.752pg/ml as shown in figure (4).



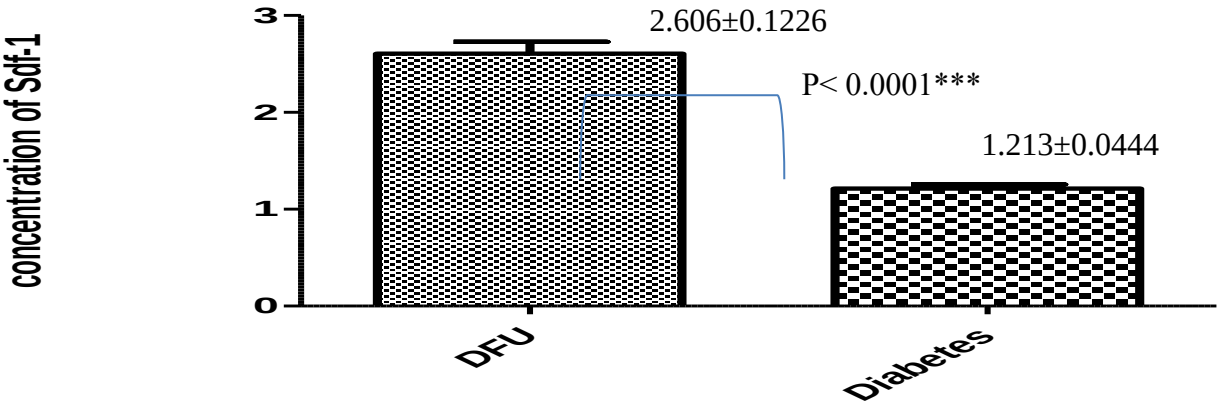
Figure(4): Comparison of HIF-1concentration (pg/ml) between DFU with and DF without ulcer

The result in our study that demonstrated the serum HIF-1 was increase in the diabetic foot ulcer than diabetes patient. This result agreement with Shao *et al.*, (2016) who found the level of serum HIF-1α was significantly increased in patients suffering from DFU compared to the T2DM group.

The result in our study that show in figure (4) was disagreement with Lin *et al.*,(2019) who found the level of serum HIF-1α was decreased in patients suffering from DFU compared to the T2DM group. Also, Pichu *et al.*,(2015) also discovered that the HIF-1α expression level in DFU patients was dramatically lower than that in T2DM patients. Mechanically, the upregulated expression of HIF-1α in DM patients may be caused by the hypoxia and ischemia of local tissues, which activated the endogenous defense and repair mechanism, and thereby leading to the increase of HIF-1α (a transcriptional complex responding to hypoxia) to affect glucose metabolism and up-regulate the expression of HIF-1α inducible genes, like VEGF (Lee *et al.*,2013 ; Rahtu-Korpela *et al.*,2014).

4.4. Evaluation of Stromal cell derived factor-1 (Sdf-1) in DFU

The present study also observed that the concentration of Sdf-1 (pg/ml) in DFU infection was 2.606±0.1226 pg/ml is increase significantly than, diabetic patients without FU infection was 1.213±0.04446 pg/ as shown in figure (5) .



Figure(5): Sdf-1 serum level in DFU patients and D without FU.

The result in our study that demonstrated the serum Sdf-1 was increase in the diabetic foot ulcer than diabetes patient. This result agreement with Fang *et al.*,(2021) who found the level of serum Sdf-1 was significantly increased in patients suffering from DFU patients compared to the T2DM group.

Generally, SDF-1 promotes the recruitment of bone marrow stem cells and inflammatory cells to the injured site in the case of tissue or organ injury, making it a key cytokine for regulating the repair of tissue or organ injury (Zachar *et al.*,2016). Several studies have confirmed that SDF-1/CXCR4 is involved in the repair of tissues and organs (Sun *et al.*,2019 ; Chen *et al.*,2020). For example, after myocardial infarction, increased levels of SDF-1 can be detected in the damaged myocardial areas (Huang *et al.*,2019). After myocardial injury, SDF-1 expression is up-regulated and released rapidly from the injured tissue, forming a concentration gradient of SDF-1 that promotes stem cell mobilization and migration to the injured site for repair (Chen *et al.*,2018). In some severely affected patients, the level of plasma SDF-1 is also increased. According to current research, there is a close relationship between the SDF-1/CXCR4 pathway and neovascularization (Fang *et al.*,2021).

This result agreement with Dhamodharan, *et al* (2015)that found The serum levels of SDF-1 were significantly elevated in DFU–DN groups while most subjects with DFU–PVD had undetectable levels.

4.5. Correlation between HIF-1 with Sdf-1 according to DFU.

The correlation coefficient as showed in (Figure 6) which illustrated low positive and non-significant correlation between HIF-1 with Sdf-1 according to DFU.

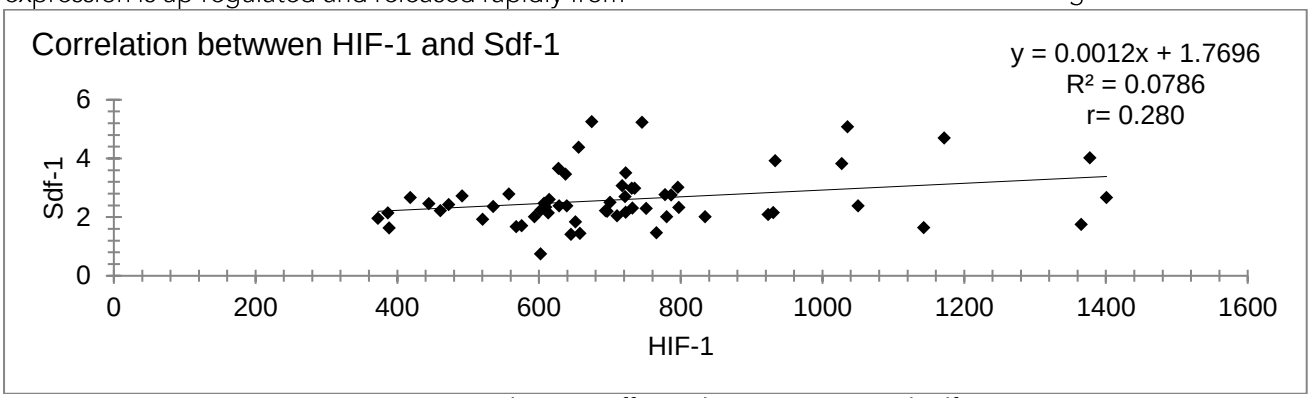


Figure (6): Correlation coefficient between HIF-1 and Sdf-1.

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