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Original Research Article

Reliability of Serum IL 6 Levels as a Predictor for Early Diagnosis of Diabetic Foot Ulcer

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Conflict of interest: Nil

Abstract:

Introduction: Diabetic foot ulcer is a common complication of uncontrolled diabetes mellitus. DFU leads to lower limb amputation and recurs easily hence affects quality of life. Detection of DFU in early stages may be beneficial in controlling its progression and prevent amputation. IL 6 is a pro inflammatory cytokine produced in early stages of infection.

Aim: The aim of the current study was to assess and compare the levels of IL 6 in diabetic patients with DFU against diabetic patients without DFU.

Materials and Methods: The current study was a prospective cross- sectional study conducted in a private clinical setup for a period of over one year from mid of 2022 to 2023. There were total 100 participants grouped into Group A 50 diabetic patients without DFU and Group B 50 patients with DFU. 5ml of venous blood samples were collected and assessed for HbA1c and IL 6. The difference in values was compared using unpaired student t test and p value of <0.05 was considered to be statistically significant.

Results: In Group A patients belonged to the age group of 37-83 years and in Group B the age range was 41-86 years and the comparison showed a p value of 0.014. The comparison of gender among Group A and Group B showed that though males are more in both groups but was not significant (p=0.380). The comparison of duration of diabetes among Group A and Group B showed a p value of 0.001. The serum HbA1c and serum IL 6 comparison of Group B showed more values than Group A with p value of 0.003 and 0.001 respectively.

Conclusion: DFU is the most common complication of uncontrolled diabetes mellitus. Serum IL 6 is elevated in DFU and hence can be used in the early diagnosis of DFU. It can also be used to differentiate between infected and non-infected DFU. Decrease in level of IL 6 can be used as a measure of healing in patients undergoing treatment for DFU.

Keywords: Diabetic foot ulcer, diabetes mellitus, HbA1c, IL 6.

Introduction

Reliability of serum IL 6 levels as a predictor for early diagnosis of DFU.

Diabetes Mellitus (DM) is a chronic endocrinal condition which leads to impaired blood glucose control ultimately leading to microvascular and macrovascular complications.[1] Microvascular complications include diabetic retinopathy, nephropathy and neuropathy whereas macrovascular complication are coronary artery disease, peripheral arterial disease and cerebrovascular disease.[2] Peripheral arterial disease along with diabetic neuropathy impairs perfusion and sensation to lower limbs. Diabetic foot ulcer DFU is the most common complication of prolonged uncontrolled diabetes mellitus causing significant economic burden and impact on patient's quality of life. Other common causes for DFU apart from uncontrolled diabetes are calluses, foot deformities, improper foot care, ill-fitting footwear, underlying peripheral neuropathy, poor circulation, dry skin etc. [3] Among all the complications of diabetes, patients with DFU require more hospitalization. DFU occurs in patients in areas of the foot which receives repeated trauma and pressure sensation. [4]

DFU's are commonly infected with Staphylococcus organism. DFU is associated with inflammation brought by immune cells like neutrophils, macrophages and T cells. Interleukin 6 IL 6 is a pro-inflammatory cytokine secreted by T cells and macrophages. IL 6 in event of infection and inflammation stimulates immune reaction and can be detected in serum in early stages of infection.[5]Accordingly assessment of IL 6 will enable early diagnosis of DFU and the development of novel treatment methods. Thus the aim of the current study was to assess and compare the levels of IL 6 in diabetic patients with DFU against diabetic patients without DFU.

Materials and Methods

The current study was a prospective cross sectional study conducted in a private clinical setup for over a period of 1 year from mid of 2022 to 2023. This study was structured and conducted according to local and global ethical norms. The total participants of the current study were 100 and were divided into 2 groups as follows.

Group A comprised of 50 diabetic patients without DFU and Group B comprised of 50 diabetic patients with DFU. Patients above 18 years who satisfied the IWGDF criteria for diabetic foot ulcer were included in the current study. Patients suffering from any form of inflammatory condition, Bowel disease and other diabetic or non-diabetic complication, coexisting infection, patients who were on immunosuppressive or antibiotics therapy, patients who underwent surgery in the past 6 month were excluded from the current study.

All willing participants were randomly selected, procedure was explained and written consent was obtained. 5 ml of blood sample was collected from all participants under strict aseptic conditions. The blood samples were centrifuged and serum samples were collected to assess HbA1c and IL 6. HbA1c were measured in a fully automated analyzer whereas IL 6 was measured using ELISA.

The values obtained were entered and stored in an excel sheet. Data was expressed as Mean ± standard deviation (SD). The difference in Group A and Group B was analyzed using student t test and p value of ≤ 0.05 was considered statistically significant.

Results

The current study was a prospective study conducted on 100 diabetic patients who were grouped as Group A-50 diabetics without DFU and Group B-50 patients with DFU. The patients of Group B were selected based on International Working Group on the Diabetic Foot (IWGDF) and the PEDIS (perfusion, extent, depth, infection, and sensation) grading system (Table 1) [6].

Table 1:

PEDIS Grade	Clinical presentation
1	Simple diabetic foot ulcer without infection
2	Presence of 2 or more of: purulence, erythema, tenderness, warmth, or induration. If cellulitis or erythema is present, it is limited to 2cm around the ulcer
3	Infection as above in an otherwise systemically well patient and with the presence of 1 or more of: cellulitis >2cm, deep tissue abscess, gangrene, involvement of bone
4	Infection as above in a patient with systemic involvement (sepsis)

Table 1 showing IWGDF classification system of diabetic foot ulcers and PEDIS grade. The patients in Group A were in the age range of 37-83 years patients and patients in Group B were in the age range of 41-86 years with a p value of 0.014. There were 29 males and 21 females in Group A and 27 males and 23 females in Group B with a p value of 0.380 (Chart 1). The duration of diabetes in Group A is 3-34 years and in Group B is 4-38 years and the p value was 0.001 (Table2).

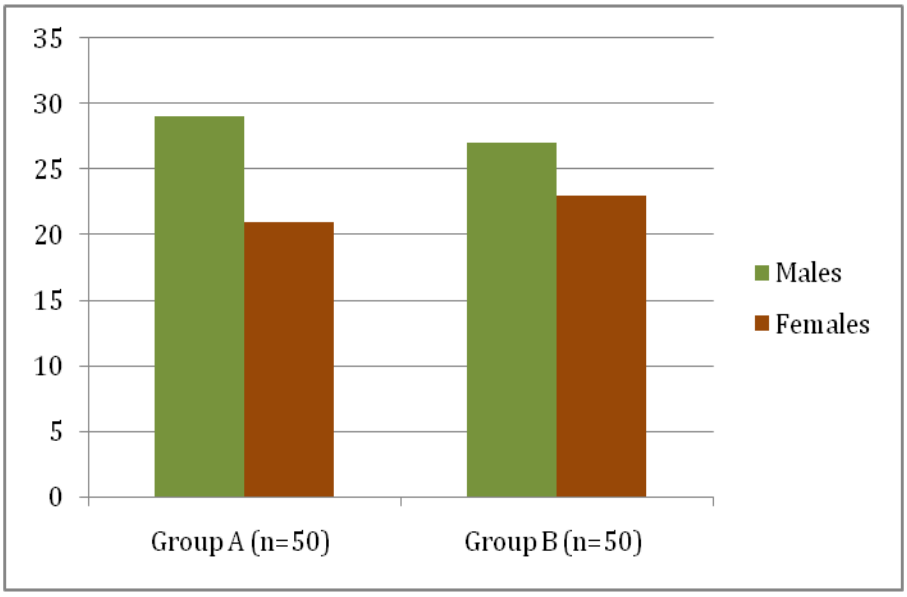

Figure 1: 1 Bar Graph showing distribution of males and females in Group A and Group B

Table 2:

Variables	Group A (n=50) Diabetics without DFU	Group B (n=50) Diabetic with DFU	P value
Age in years (mean± SD)	43.4±5.28	49.1±7.85	**0.014**
Gender Males Females	 29 21	 27 23	 0.380
Duration of diabetes in years (mean±SD)	7.72±5.61	13.15±6.34	**0.001**

Table 2 showing the variables of the study in Group A and Group B and unpaired t test. The serum HbA1c value in Group A showed a mean and standard deviation of 8.23±2.11and in Group B the mean and deviation of 9.55±2.30 and the p value noted was 0.003 (Table 3). HbA1c was noted to be >8% in 23 out of 50 patients in Group A and in 42 patients of Group B (Chart 2).

Table 3:

HbA1c	Group A (n=50) Diabetics without DFU	Group B (n=50) Diabetic with DFU	P value
	8.23±2.11	9.55±2.30	**0.003**

Table 3 showing values of HbA1c in Group A and Group B with p value

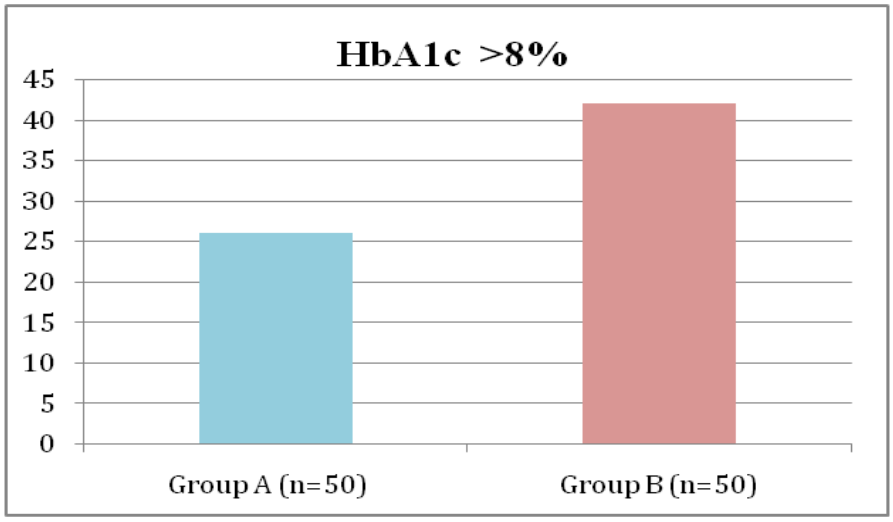

Figure 2: Bar Graph showing distribution of patients with HbA1c >8% in Group A and Group B

The serum IL 6 value in Group A showed a mean and standard deviation of 31.25±23.57 and in Group B the mean and deviation of 276.21±329.54 and the p value noted was 0.001 (Table 4).

Table 4:

IL 6 (pg/ml)	Group A (n=50) Diabetics without DFU	Group B (n=50) Diabetic with DFU	P value
Min- max	31.25±23.57, 4.9 to 102.7 (31.7)	276.21±329.54, 158.9-2002.8 (483.2)	**0.001**
Unpaired t test 0.001			

Table 4 showing serum IL 6 values in Group A and Group B with p value

Discussion

DFU is an injury to all layers of skin that usually occur on the soles of the feet which includes ulcerations, infections and gangrene, as a result of peripheral neuropathy or peripheral arterial disease, poor glycemic control, foot deformity in patients with diabetes mellitus. DFU occurs in 3 stages; in the first stage there is development of callus which is a result of neuropathy. Physical deformity of the foot results from motor neuropathy and sensory loss leading to repeated trauma. Such continued, repeated trauma results in subcutaneous hemorrhage and eventually erodes to become an ulcer. Alternatively severe atherosclerosis of small blood vessels in the leg and feet may also lead to vascular compromise leading to DFU. Blood compromise delays healing and may lead to eventual necrosis and gangrene. DFU is colonized by many microorganism and they may penetrate down to the deeper tissues, and spreads to bone. Thus, progression of infection leads to hospitalization, surgical resection and amputation of lower extremity. The quality of life after lower extremity amputation is very poor and the 5year mortality is similar to that of some of the most mortal cancer types.[6] IL 6 is

a pro inflammatory cytokine secreted by T cells and macrophages. It is a multifunctional cytokine detected in serum at early stages of infection. It is thought to be involved in the inflammatory response associated with insulin resistant state.[7] The level of IL 6 increases earlier than CRP hence can help in early diagnosis of DFU.[8] To the best of our knowledge there are few studies reported in literature related to use of serum IL 6 levels in DFU diagnosis.

The current study was a prospective study conducted on 100 diabetic patients who were grouped as Group A-50 diabetic patients without DFU and Group B- 50 diabetic patients with DFU. The age of patients in Group B was noted to be slightly higher than Group A and the finding of the study was statistically significant. It is generally stated that advancing age is considered as a risk factor for development of DFU and incidence is more in patients above 65 years in 50% of patients, similar to the findings of our study. [9,10]

However contrast to this finding Al Kafrawy 2014 and Mackson Nongmaithem 2016 suggested that age is not a risk factor in diabetic patients for developing DFU.[11,12] The population of males were noted to be more than females in both Group A and Group B however, were not statistically significant. This finding of the study is similar to meta-analysis by Rossboth 2021 and is mainly attributed to disease awareness, accurate foot care in females. They are more attentive to symptoms and seek doctor's advice.[13] Men on the other hand are less prone to chronic diseases hence awareness is less they do not follow foot care and use inappropriate foot wear. [14] Men presenting with DFU have higher prevalence of critical limb ischemia, however women with DFU present with asymptomatic disease diagnosed in more advanced stage. [15] Thanh Dinh 2008 reported that women are at lower risk of developing DFU than men as they have less severe neuropathy, increased joint mobility and lower foot pressure. [16] However Song P 2015 in their meta-analysis suggested that gender is not a risk factor for DFU.[17]

The duration of diabetes in patients with DFU was almost double than that in patients without DFU and was noted to be statistically significant. Longer duration of diabetes is considered as a risk factor for DFU as seen in the current study as well as by Brownrigg JR 2012, Shahi 2012, Mackson Nongmaithem 2016, and Rossboth 2021. [9,12,13, 18]

HbA1c is currently considered as the measure of glycemic control for 3 months and thus is the diagnostic test for Type 2 diabetes mellitus and elevated level of HbA1c is considered as a risk factor for complications of diabetes mellitus. Elevated HbA1c is associated with increased risk of DFU in diabetic patients. This is similar to the findings of Hajieh Shahbazian 2013. [19] The increase in level of HbA1c is associated with increased level of tissue loss and amputation. Wang J 2022 considered HbA1c as an independent risk factor for DFU. [20] Meta-analysis by Tang WH 2023 showed that HbA1c level was one of the factor predicting development of DFU. [21]

Meta-analysis by Lane in 2020 and Lin C 2020 show that HbA1c levels ≥8% is associated with increased likelihood of lower extremity amputation in patients with DFU.[22,23] Thus success of HbA1c is the most important factor for predicting success of amputation. Thus it is of utmost importance to lower the levels of HbA1c to reduce the risk of amputation as 1% reduction of HbA1c reduced all diabetic complications by 21% as reported by Stratton 2000 and Pozilli 2014. [24,25] Conditions like chronic kidney disease, iron, B12, folic acid deficiency anemia, functional asplenia the RBC is decreased hence HbA1c may not be reliable. [1]

Thus discovery of other biomarkers is essential in predicting DFU. Though limited research is available multifunctional cytokine IL 6 is emerging as a marker for DFU. It is produced in a variety of tissues including activated leukocytes, adipocytes and endothelial cells. Elevated levels of IL 6 produce features of insulin resistance syndrome and overt type of T2DM. [26]

In diabetic foot ulcer there is overexpression of pro inflammatory cytokine like IL 6 and hypersecretion of IL 6 which destroys or impairs beta cell insulin secretion. IL 6 produced at sites of diabetic complication may exacerbate atherosclerosis. Atherosclerotic plaque itself produces cytokines which exacerbates local atherosclerosis. In the current study we found that serum IL 6 was significantly elevated in diabetic patients with DFU when compared to diabetic patients without DFU. This finding is similar to studies by Chambers 2001, Weigelt 2009, Ammal AW 2012 and Aruna et al 2021. [26-28]

Serum level of IL 6 was found to be effective in classification of ulcer as well as to effectively differentiate between infected DFU and non-infected DFU. Also elevated serum IL 6 was seen in patients with infected DFU consequently the levels of IL 6 decreased in patients who recovered after antibiotic treatment. [5] Hence IL 6 can be used as a promising inflammatory marker to differentiate infected DFU from non- infected DFU and thus serves a marker for progression of DFU. [29] Serum levels of IL 6 reduced consistently when Platelet rich fibrin combined with hyaluronic acid was applied topically in the treatment of DFU. There is successful formation of healthy granulation tissue for successful wound healing by increasing angiogenesis and fibrogenosis. [30] Inflammation is an

inevitable process of DFU and can be used as a potential target in DFU by using pro inflammatory cytokine like IL 6.

Limitations of the study

In the current study PEDIS grading system with comparison of other variables was not performed. The study performed as randomized control trial on larger sample size with more variables may yield better statistical results.

Conclusion

DFU is the most common complication of uncontrolled diabetes mellitus. Serum IL 6 is elevated in DFU and hence can be used in the early diagnosis of DFU. It can also be used to differentiate between infected and non-infected DFU. Decrease in level of IL 6 can be used as a measure of healing in patients undergoing treatment for DFU.

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