The Review Of
DIABETIC
STUDIES OPEN ACCESS

"Comparative Analysis of Serum IGF-1 Levels in Diabetic Patients with and Without Foot Ulcers across Wagner Grades"

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ABSTRACT

Background: Diabetic foot ulcer (DFU) is a debilitating complication of diabetes mellitus. Impaired wound healing in DFU is associated with dysregulation of growth factors, including Insulin-like Growth Factor 1 (IGF-1), which plays a central role in tissue repair and cellular proliferation. **Objective:** The aim of this study is to evaluate and compare serum IGF-1 levels in diabetic patients with varying grades of DFU compared to diabetic patients without foot ulcers. **Patients and Methods:** A hospital-based case-control study was conducted involving 125 diabetic patients with DFUs (categorized using Wagner grading) and 125 diabetic patients without foot ulcers. Serum levels of IGF-1 was assessed by drawing blood samples and using enzyme-linked immunosorbent assay (ELISA), and color intensity was measured on an automated iMark ELISA reader (Bio-Rad). Statistical analysis included one-way ANOVA and correlation analysis to determine associations between IGF-1 levels and DFU severity. **Results:** Preliminary analysis suggests significantly lower IGF-1 levels in DFU patients compared to diabetic controls without ulcers. Furthermore, serum IGF-1 levels demonstrated an inverse relationship with ulcer severity, with the lowest values seen in Wagner grade IV and V ulcers. **Conclusion:** IGF-1 may serve as a potential biomarker for DFU severity and impaired wound healing. Its therapeutic role warrants further investigation.

Key words: Diabetic Foot Ulcer (DFU), Insulin-like Growth Factor 1 (IGF-1), Wagner Grading, Biomarker, Wound Healing

INTRODUCTION

Diabetic Foot Ulcer (DFU) is one of the most challenging complications of diabetes mellitus, and affects 15% of all diabetic patients contributing to substantial morbidity, prolonged hospitalization, and amputations (1). Five-year risk of mortality for a patient with diabetic foot ulcer is 2.5 times higher than the risk for a patient without DFU (2). Inflammation is a key component in the pathogenesis of DFUs, and various anti inflammatory markers such as Insulin-like growth factor 1 (IGF-1) have been implicated in the development and progression of these ulcers (3). A key factor in wound healing is Insulin-like Growth Factor 1 (IGF-1), a peptide hormone crucial for cellular proliferation regeneration wound healing and tissues repair .IGF-1 is often found to be decreased in chronic non-healing wounds, especially in diabetic individuals (4). Alteration in the

microenvironment due to diabetes mellitus (DM) results in a change in the level of oxygen, chemokines, synthesis of growth factors, extracellular matrix, oxidative stress that in turn alter normal cellular recruitment and activation, and induce impaired or delayed wound healing (5,6). Anti-inflammatory processes are crucial in the different phases of wound healing, it is conceivable that disturbances of the immune system interfere with tissue homeostasis and wound healing after the manifestation of ulcers and lead to the chronic, non healing wounds that are characteristic of diabetic foot syndrome (7). Most studies on DFUs have emphasized inflammatory markers, with fewer examining growth factors like IGF-1. The induction of wound healing by insulin-like growth factor-I (IGF-I) has been demonstrated in several animal studies; however, there are disproportionately fewer studies assessing its value in humans (8) .Given IGF-1's role in angiogenesis, cell migration, and proliferation, it may provide a biological explanation for delayed wound healing (4) .There is a gap in literature assessing IGF-1 across different grades of foot ulcers. Understanding this gradient may support the development of IGF-1–based therapeutic interventions or risk stratification tools. Therefore, more investigation is required to completely clarify the connection between IGF-1 and inflammation in DFUs as well as to investigate the possibility of using IGF-1 as a therapeutic target for these ulcers. Few studies have examined the stratification of IGF-1 levels among DFU grades, despite growing evidence that IGF-1 plays a role in wound healing.In this study, we aimed to investigate the role of IGF-1 in individuals with different grades of diabetic foot ulcers (DFUs) and compare them to diabetic patients without any foot ulcer. The primary objective is to evaluate and compare serum IGF-1 levels in diabetic patients with different Wagner grades of foot ulcers and secondary objective is to compare serum IGF-1 levels between diabetic patients with and without foot ulcers.

MATERIALS AND METHODOLOGY

This study was carried out at a tertiary care hospital and is set up as a case-control investigation. 125 diabetes individuals with foot ulcers and 125 diabetic patients without foot ulcers made up the 250 participants who were included. The cases include 25 patients from each grade (I to V) of Diabetic Foot Ulcer (DFU), classified according to the Wagner grading system. Controls will consist of diabetic patients without any current or prior history of foot ulcers. The inclusion criteria for participation include diabetic patients aged between 35 and 70 years with a confirmed diagnosis of type 2 diabetes mellitus with Diabetic foot ulcer. Exclusion criteria were if the participants had any active infections, autoimmune or rheumatic diseases, malignancies, or if they are undergoing treatment with immunosuppressive drugs. Other exclusion criteria include the presence of non-diabetic foot ulcers caused by trauma, arterial insufficiency, or venous insufficiency, as well as pregnancy or lactation. Participants were recruited using a consecutive sampling method. For biochemical analysis, blood samples will be collected from all participants and serum IGF-1 levels was measured using a sandwich ELISA technique.

STATISTICAL ANALYSIS

All statistical analysis was done after the data was entered into Microsoft Excel. The Shapiro-Wilk test was used to determine whether the data had a normal distribution. The significant difference between two groups of continuous variables was assessed through Student t or Welch t or Mann-Whitney U test depending on assumptions. The summary of categorical variables will be reported in terms of frequencies and percentage.The association between the categories will be assessed using Chi-square test or Fisher’s exact test.It is observed that all the variables do not follow Normality. Thus the statistical difference between the groups was assessed through Kruskal Walli’s test. If null hypothesis is rejected, then post-hoc test (with Bonferroni correction) was carried out for pair-wise comparisons. A p value < 0.05 was considered statistically significant.

RESULTS

A total of 125 diabetic foot ulcer patients and 125 diabetic patients without foot ulcer (free from foot wounds and acute or chronic disease), admitted to surgery wards from Medical College were selected. Age and sex were matched between the two groups. The subjects' ages ranged from 34 to 60 years. Among DFU patients, 25 individuals were enrolled for each Wagner stage (grades 1 to 5) to ensure balanced representation across the spectrum of ulcer severity. The baseline demographic and clinical

characteristics are detailed in Table 1.The mean age was similar between DFU (57.30 ± 11.01 years) and DWO (57.17 ± 10.47 years) groups ($p = 0.965$), with both groups having the same male-to-female ratio (80% male, 20% female).The duration of diabetes was significantly longer in DFU patients (117.81 ± 89.34 months) compared to controls (125.28 ± 69.43 months), ($p < 0.001$).

Figure 1 illustrates a horizontal bar graph showing the percentage prevalence of various clinical symptoms and comorbidities among patients with diabetic foot ulcers (DFU). The analysis shows a significant burden of systemic conditions, with cardiovascular involvement (CVS: 87.1%, CAD: 83.5%), nephropathy (83.5%), retinopathy (77.6%), and autonomic neuropathy (80%) being highly prevalent ($p< 0.05$). Sensory symptoms such as reduced sensation (77.6%), numbness (82.4%), tingling/burning sensations (85.9%), muscle weakness (84.7%), and pain (76.5%) were also markedly common, reflecting advanced neuropathy. Fatty liver (84.7%) and osteomyelitis (60%) were frequent, with osteomyelitis nearing statistical significance ($p = 0.065$). This graphical representation highlights the high burden of neuropathic symptoms and associated conditions in DFU patients, underscoring the need for early screening and comprehensive management. As illustrated in Table 2, a high prevalence of neuropathic symptoms was observed among patients with diabetic foot ulcers when compared to diabetic patients without foot ulcers. The most common symptom was loss of reflex, present in 100% of DFU patients, followed by tingling/burning sensations in 85.9%, muscle weakness in 84.7%, and numbness in 82.4%. Reduced ability to feel sensations, autonomic neuropathy, and pain were also frequently reported in 77.6%, 80.0%, and 76.5% of the patients respectively. Chi-square analysis revealed a highly significant association ($p < 0.001$) between all reported symptoms and the presence of DFU, indicating that neuropathic symptoms are markedly more prevalent in patients with foot ulcers compared to those without. Table 3 and figures 2 present serum Insulin-like Growth Factor-1 (IGF-1) levels across the Wagner ulcer classification system . IGF-1 levels were lowest in control participants without ulcers (0.04 ± 0.03 ng/mL). Among DFU patients, the highest mean IGF-1 level was seen in Grade 1 ulcers (0.44 ± 0.06 ng/mL), progressively declining across increasing ulcer grades Grade 2: 0.37 ± 0.11 ng/mL, Grade 3: 0.33 ± 0.10 ng/mL, Grade 4: 0.15 ± 0.12 ng/mL, Grade 5: 0.06 ± 0.07 ng/mL. A post-hoc test with Bonferroni correction demonstrated that IGF-1 levels were significantly elevated in DFU patients of Grades 1 to 4 compared to the control group ($p < 0.0001$). However, Grade 5 ulcers showed no significant difference from controls ($p > 0.05$), suggesting a depletion of IGF-1 in the most severe stages of ulceration. Figure 3 presents a graphical comparison of serum IGF-1 levels across different Wagner grades of DFU against the control group (diabetic patients without foot ulcers). IGF-1 levels are significantly elevated in DFU patients with Grade 1 to Grade 4 ulcers compared to the control group, with a highly significant p-value (<0.0001). This indicates a possible upregulation of IGF-1 in the early to moderately advanced stages of ulceration, possibly as a response to tissue injury and an attempt at wound healing. However, in Grade 5 DFU, where gangrene is extensive, the IGF-1 levels drop and are not significantly different from the control group ($p > 0.05$, non-significant), suggesting a depletion of IGF-1 or exhaustion of reparative mechanisms in severe ulcerative conditions.

DISCUSSION

The process of wound healing is intricate, involving multiple stages and variables. IGF-I is a hormone that plays an important role during growth and development, and is expressed in several tissues in humans where its exerts several anabolic effects (9, 10). Serum IGF-1 levels in diabetic patients with and without foot ulcers were assessed in this investigation. Our findings demonstrate a distinct pattern of IGF-1 levels significantly higher in Diabetic patients without foot ulcer when compared to cases of diabetes patients with foot ulcer, and the levels declined progressively with increasing ulcer severity, reaching non-significant levels in grade 5 ulcers. Insulin-like growth factor 1 (IGF-1) has been identified as a key regulator of cell growth and differentiation, with an important role in wound healing Many studies suggested that IGF-1 play important roles in the pathogenesis of DFUs. (11)

Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is produced primarily by the liver. Insulin-like growth factor (IGF) is a complex of two peptides: IGF-1 and IGF-2 (12). In diabetic patients expression of IGF-1 is decreased which may explain cell granulation defects (13). IGF-I possesses a crucial role in wound healing via multiple mechanisms; it acts as a chemotactic agent for endothelial cells, stimulates the proliferation and migration of keratinocytes and fibroblasts, and increases the wound strength (14). The initial elevation of IGF-1 in grades 1–4 may reflect an early

reparative response to tissue injury, consistent with IGF-1 established role in stimulating fibroblast proliferation, keratinocyte migration, and collagen synthesis in wound healing. However, the subsequent decline in advanced ulcers (grades 4–5) suggests an exhaustion of the growth factor system, possibly due to chronic inflammation, persistent hyperglycemia, microvascular ischemia, and neuropathy-induced tissue degeneration. The markedly low IGF-1 levels in grade 5 DFUs may also result from systemic catabolism, impaired hepatic production, or downregulation of IGF-1 receptors in the ulcer bed. Our preliminary findings suggest an inverse correlation between serum IGF-1 levels and DFU severity. This is consistent with earlier literature that supports the hypothesis that growth factor depletion impairs wound healing. In addition, a study reported a significant association between serum IGF-1 levels and the severity of DFUs in patients with type 2 diabetes mellitus (15). Previous studies have reported reduced IGF-1 in chronic non-healing wounds, including DFUs, compared to healthy controls or diabetic patients without ulcers. Another study reported that IGF-1 levels were significantly lower in patients with chronic DFUs compared to those with acute DFUs (16). The results highlight IGF-1 as a potential biomarker for disease severity and as a possible target for regenerative therapies. Low IGF-1 levels may predict chronic non-healing ulcers and risk of progression to severe grades. Specifically, both in vitro (17-19) and in vivo (20) studies showed that IGF-I is an important stimulant for the migration of keratinocytes, thus playing a central role in wound epithelialization. As a major growth factor, IGF-1 is responsible for stimulating growth of all cell types and causing significant metabolic effects (21). IGF-1 has been associated with promoting angiogenesis, collagen synthesis, and cell proliferation, making it a key factor in tissue repair. Interestingly, in diabetic patients expression of IGF-1 is decreased which may explain cell granulation defects (22).

In our study there is a high prevalence of neuropathic symptoms and comorbidities in our DFU cohort, such as cardiovascular disease, nephropathy, and retinopathy, underscores the systemic nature of ulcer pathology. These factors may indirectly influence IGF-1 bioavailability by altering endocrine, metabolic, and inflammatory pathways.

From a clinical standpoint, our data suggest that IGF-1 could serve as a biomarker for both the presence and severity of DFUs. Early-stage ulcers with higher IGF-1 might be more amenable to interventions that enhance the growth factor response, whereas advanced ulcers may require strategies to replenish depleted IGF-1 levels. Recombinant IGF-1 or IGF-1–modulating therapies, while not yet standard in DFU management, warrant further exploration in controlled trials.

CONCLUSION

This study demonstrates that serum IGF-1 levels vary significantly across DFU severity, showing an initial elevation in early to moderate grades (1–4) followed by depletion in the most severe stage (grade 5). These findings suggest that IGF-1 plays a dynamic role in the pathophysiology of DFUs—initially acting as a reparative factor but diminishing with chronicity and tissue necrosis.

IGF-1 has potential as a biomarker for DFU severity and prognosis, and possibly as a therapeutic target to enhance wound healing in diabetic patients. Incorporating IGF-1 measurement into DFU assessment protocols may aid in risk stratification and personalized management. Further longitudinal and interventional research is warranted to confirm these observations and to explore IGF-1–based therapeutic strategies in the prevention and treatment of DFUs. IGF-1 may be used not only as a prognostic indicator but also as a candidate for growth factor–based interventions in diabetic wound care.

Table 1: Demographic and baseline data of the Subjects

Variables	DFU n=125	DWO N=125	p value
Age	57.30±11.01	57.17±10.47	0.965[b]
Sex n (%) Male/Female	100/25(80.0%/20.0%)	100(80.0%/20.0%)	1.00[a]
Duration of DM(months)	117.81±89.34	125.28±69.43	<0.001***[b]

Data represented as mean ± SD; n = number of subjects. Gender and other variables are expressed as

frequency with percentage in parenthesis. [a] Chi-square test was performed and [b]Mann-Whitney U test was performed .Abbreviations used: DFU: Diabetic foot ulcer, DWO: Diabetic patient without foot ulcer. Level of significance: * p<0.05 significant, p>0.05 non significant (NS), ***p<0.001

Table 2: Diabetic Neuropathy Symptoms in the Diabetic Foot Ulcer Patients

Variables	Frequency	Percentage	Chi square Value DFU-WO Vs DFU	P value
Reduced ability to feel sensations	66	77.6%	25.98	<0.001***
Numbness	70	82.4%	35.58	<0.001***
Tingling/Burning	73	85.9%	43.77	<0.001***
Pain	65	76.5%	23.82	<0.001***
Muscle weakness	72	84.7%	40.95	<0.001***
Loss of reflex	85	100.0%	85.00	<0.001***
Autonomic Neuropathy	68	80.0%	30.60	<0.001***

DFU-WO: Diabetic patient without foot ulcer ,DFU- Diabetic foot ulcer
* p<0.05,***p<0.001ns : not significant

Table 3: The Wagner-ulcer classification system with 5 different DFU grades

DFU Grades	Symptoms	IGF ng/mL Mean ± SD
Controls	No ulcer in a high-risk foot	0.04 ± 0.03
1	Shallow ulcer in the foot, no infection, neuropathic ulcer	0.44 ± 0.06
2	Soft tissue infections, osteomyelitis or deep abscess	0.37 ± 0.11
3	Deep ulcer, abscess or osteomyelitis	0.33 ± 0.10
4	Localised gangrene, ischemic gangrene	0.15 ± 0.12
5	All gangrene	0.06 ± 0.07

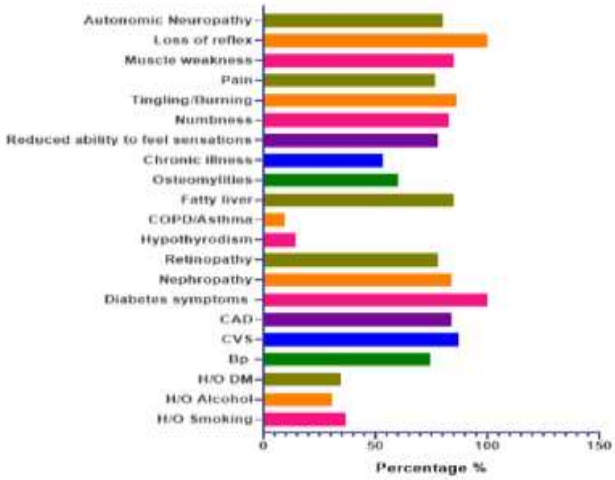

Figure 1: Prevalence of Clinical Symptoms and Comorbidities in DFU Patients

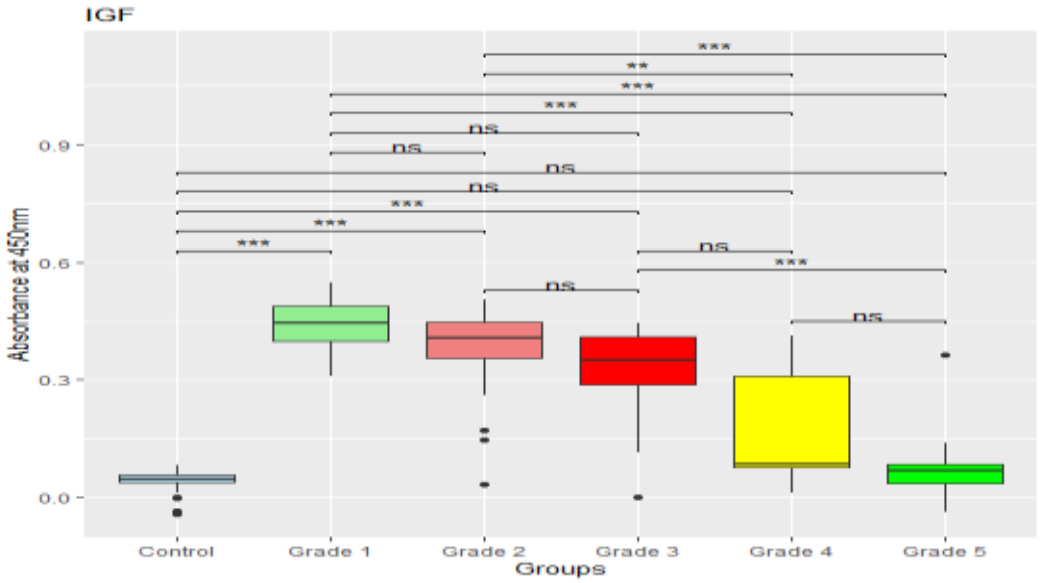

Fig 2: Stages comparisons of levels of Serum IGF-1 in cases and controls
P value is obtained from post-hoc test with Bonferroni correction. Level of significance: * $p<0.05$ significant, $p>0.05$ non significant (NS), ***$p<0.001$

ACKNOWLEDGEMENT
Dr.Shaheen B. shaikh, Dr H. M Ismail and Dr. Yashodhar Prabhakar Bhandary designed the experiment. Dr.Shaheen and Dr. Mossabba performed the experiment and did the statistical analysis. Dr.Shaheen B. shaikh , Dr. M.S Moosabba ,Aleena and Dr. Yashodhar P Bhandary, prepared the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE: The protocol of this study was approved by the Institutional Ethics Committee. The treatment protocol was standard care without any experimental treatment approach or medications and was carried out according to the Helsinki Declaration. All methods were carried out in accordance with relevant guidelines and regulations. Written informed consent was obtained before participation.

CONFLICT OF INTEREST STATEMENT: Not applicable

FUNDING SOURCES
This work was supported by the Seed grant Yenepoya (Deemed to be university),YU/Seedgrant/164-2024 .

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