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Study of Risk Factors of Diabetic Foot and Role of Hypoxia- Inducible Factor I Alpha in Egyptian Type 2 Diabetic Patients

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ABSTRACT

Background: Diabetes mellitus (DM) causes elevated blood sugar levels and over time can cause problems of the heart, blood vessels, kidneys, retina, and nervous system. One of the most severe complications of diabetes, diabetic foot ulcers (DFU) are a major factor in the majority of non-traumatic lower-limb amputations. A heterodimer of HIF-1α and HIF-1 β, hypoxia-inducible factor I (HIF-1) is a transcription factor that is stabilized by hypoxia and acts as a fundamental regulator of oxygen homeostasis and adaptive cellular responses to hypoxia. **Objective:** The current study aimed to evaluate the risk factors of DFU and investigate the serum level of hypoxia inducible factor 1 alpha as biomarker for incidence of DFU. **Patients and methods:** The study was conducted on 80 patients. Participants were divided into two groups: *Group I* (diabetic foot) subdivided according to Wagner Meggitt 1987 Classification into grade (0), grade (1), grade (2) and grade (3). Each group included 10 patients. *Group II* patients (control group) included 40 healthy subjects. Patients were subjected to detailed history and complete examination, and complete laboratory evaluation which included serum Hypoxia- inducible factor I alpha. **Results:** The associated risk factors of DFU identified in our study were peripheral neuropathy, peripheral vascular disease, diabetic nephropathy, age, duration of DM, lack of education about care of diabetic foot and male gender. There was higher level of serum hypoxia inducible factor 1 alpha in diabetic patients than control subjects with significant increase in DFU patients.

Conclusion: HIF-1α may have a role in pathogenesis of DFU.

Keywords: Diabetic foot, Hypoxia- inducible factor I alpha, Egypt, type 2 diabetes mellitus.

INTRODUCTION

The World Health Organization (WHO) describes diabetes mellitus (DM) as a chronic metabolic disorder characterized by high blood sugar that eventually leads to issues with the heart, arteries, kidneys, retina, and nervous system [(1)].

According to the International Diabetes Federation (IDF), 700 million people between the ages of 20 and 79 were estimated to have diabetes in 2045, up from an estimated 463 million in 2019. Worldwide, diabetes claimed 4.2 million lives in 2019. Around 90% of occurrences of diabetes worldwide are of type 2, making it the most common kind of the condition [(2)].

The main pathophysiological factor driving type 2 diabetes, in addition to cell failure, is insulin resistance, which is frequently correlated with abnormal insulin production [(3)]. Diabetic foot ulcer (DFU) is one of the most severe complications of diabetes based on the two primary etiological factors of diabetic peripheral neuropathy and peripheral arterial disease (PAD), which together account for the majority of causes of non-traumatic lower-extremity amputations (LEA) and raise mortality rates. The long-term outlook is rather bleak following LEA's revelation, which is highly related to DFU, with a 3-year mortality rate ranging from 35% to 50%. The total 5-year death rate was considerably greater over the longer term, ranging from 53% to 100% for those who had any amputations to 52% to 80% for those who had severe amputations [(4)].

Adaptive cellular responses to hypoxia are governed by the transcription factor hypoxia inducible factor-1 (HIF-1, a heterodimer of HIF-1α and HIF-1 β), which is stabilized by hypoxia and controls angiogenesis, erythropoiesis, metabolic changes, proliferation, migration, proliferation, and cell survival [(5)]. The increased cytosolic ratio of free NADH to NAD+ in hyperglycemia might create metabolic abnormalities despite appropriate tissue oxygenation. This high ratio was the root cause of pseudohypoxia [(6)].

The current study aimed to evaluate the risk factors of DFU and investigate the serum level of hypoxia inducible factor 1 alpha as biomarker for incidence of DFU among Egyptian type 2 diabetic patients.

PATIENTS AND METHODS

The study was conducted on 80 patients selected from DM Outpatient Clinic at Minia University Hospital in the period from May 2020 to December 2020.

All subjects were divided into two groups: *Group I* (diabetic foot) patients which included 40 diabetic foot patient with type 2 diabetes subdivided according to Wagner Meggitt 1987 Classification [(7)] into grade (0) 10 diabetic patients with intact skin, grade (1) 10 patients with superficial skin ulcer, grade (2) 10 patients with deep skin ulcer and grade (3) 10 patients with deep skin ulcer associated with abscess or bone involved. *Group II* patients (control group) included 40 healthy subjects (free of any acute or chronic medical disease).

Patients with type 2 DM with and without diabetic foot were included in the study. Patients were

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excluded if they had any of the followings: non-diabetic peripheral vascular disorders, traumatic foot ulcers, joint diseases, cancer, autoimmune diseases, ischemic cerebrovascular stroke, and neurodegenerative diseases.

All patients were subjected to full medical history, meticulous clinical examination including vascular, neurological and dermatological assessment, calculation of body mass index (BMI) and laboratory parameters including fasting blood glucose, 2hour post prandial blood glucose, HbA1c, serum urea and creatinine, Lipid profile, eGFR using EPI CKD, uric acid and hypoxia inducible factor 1alpha by ELISA. Radiological assessment included plain X ray on both feet and peripheral color Doppler ultrasonography. Toshiba Xario 200 Ultrasound with Linear Array 6.2-14 MHz transducer. Latitudinally, posterolaterally, anteriorly, and transversely were among the transducer locations employed. For shallow arteries, such as the superficial femoral artery, a transducer frequency of 10 MHz was employed, whereas a frequency of 5 MHz was used for deeper arteries, such as the tibio-peroneal arteries. Using a linear array transducer positioned directly above the vessel at an angle of incidence between 45 and 60 degrees, scanning of the lower leg arteries was carried out while the patient was lying flat.

Ethical consent:

The Academic and Ethical Committee of Minia University granted its clearance for the project. All study participants provided written informed permission after being informed of our research's goals. The Declaration of Helsinki for human beings, which is the international medical association's code of ethics, was followed during the conduct of this study.

Statistical analysis

The IBM SPSS 20.0 statistical package software was used to analyze the data (IBM; Armonk, New York, USA). Using either the Shapiro-Wilk or Kolmogorov-Smirnov tests, the data's normality was determined. For quantitative measurements, data were reported as mean, standard deviation (SD), median, range, and both numbers and percentages for qualitative data. For comparison between two independent groups, and Student's t-test and Mann-Whitney U test for parametric and non-parametric data, respectively, were employed. Comparing categorical variables was done using Chi-square test (X^2) or Fisher's exact test. Pearson correlation analysis was used to examine correlations between the quantitative parameters. P value equals or less than 0.05 was regarded as significant.

RESULTS

The study included 80 patients divided into 2 groups and the diabetic group subdivided into 4 groups. The demographic and clinical data are shown in **Table 1**, while the laboratory data are shown in **Table 2**.

Table (1): Sociodemographic and clinical data of the studied groups.

Variable	Control N=40	Grad 0 DF N=10	Grad 1 DF N=10	Grad 2 DF N=10	Grad 3 DF N=10
Age (years)					
Mean ± SD	45.7 ± 5.9	48.5 ± 5.3	53.7 ± 13.7	61.8 ± 11.6	66.1 ± 7.7
Median	45	49	52	64	67.5
Sex					
Male	16 (40%)	2 (20%)	1 (10%)	10 (100%)	8 (80%)
Female	24 (60%)	8 (80%)	9 (90%)	0 (0%)	2 (20%)
Duration of DM (y)					
Mean ± SD	----	7.5 ± 2.9	12.6 ± 5.4	11.9 ± 5.5	13.8 ± 5.6
Median	----	7.5	12	12	11.5
Not educated about of care of foot	----	4 (40%)	10 (100%)	10 (100%)	10 (100%)
Smoker					
Current smoker	8 (20%)	1 (10%)	1 (10%)	5 (50%)	2 (20%)
Non-smoker	32 (80%)	9 (90%)	9 (90%)	5 (50%)	8 (80%)
SBP					
Mean ± SD	115.8 ± 5	119 ± 12	130 ± 22.6	137 ± 25.8	135 ± 23.2
Median	120	120	120	140	130
DBP					
Mean ± SD	75.8 ± 5	78 ± 9.2	83 ± 14.2	86 ± 14.1	88 ± 17.5
Median	80	80	80	90	85
BMI					
Mean ± SD	22.3 ± 2.1	25.3 ± 2.6	27.5 ± 3.4	26.5 ± 2.9	28.2 ± 6.2
Median	23.9	25.9	26.2	27	28.4
Peripheral neuropathy	0 (0%)	2 (20%)	5 (50%)	10 (100%)	10 (100%)

Table (2): Laboratory investigations of the studied groups:

Variable	**Control N=40**	**Grad 0 DF N=10**	**Grad 1 DF N=10**	**Grad 2 DF N=10**	**Grad 3 DF N=10**
FBG (mg/dl) Mean ± SD	74.8 ± 6.6	135.3 ± 30.2	140.4 ± 30.8	141.2 ± 29.4	153.8 ± 34.1
2h PP BG (mg/dl) Mean ± SD	107 ± 9.1	235.1 ± 7.1	271.7 ± 66.6	278.8 ± 59.6	321.3 ± 78.4
HbA1c (%) Mean ± SD	4.8 ± 0.5	7.8±0.4	7.9±1.2	7.1±1.4	8.5 ± 1.1
LDL mg/dl Mean ± SD	92.3 ± 5.2	96.8 ± 3.2	101.3 ± 20.5	121.7 ± 22.1	115.8 ± 22.7
T C mg/dl Mean ± SD	158.5 ± 23.5	164.1 ± 23.7	173.5 ± 29.8	201 ± 41.7	198 ± 31.7
T G mg/dl (Mean ± SD)	130 ± 18.2	142 ± 33.2	160.5 ± 38.6	161.5 ± 39.5	195.5 ± 46.1
Urea mg/dl (Mean ± SD)	34.9 ± 4.3	39.3 ± 9.7	44.6 ± 11.1	50.7 ± 12.4	60.9 ± 15.1
Uric acid mg/dl Mean ± SD	5.1 ± 0.8	5.9 ± 0.8	7 ± 1.5	7.2 ± 1.7	8.9 ± 2.1
Albumin(g/dl) Mean ± SD	4.2 ± 0.2	4.3 ± 0.5	3.8 ± 0.5	3.8 ± 0.5	3.2 ± 0.7
HIF-1α ng/ml Mean ± SD	0.9 ± 0.2	1.1 ± 0.2	3.1 ± 0.6	4 ± 0.8	5.6 ± 1.2
Creatinine mg/dl Mean ± SD	0.8 ± 0.1	1 ± 0.2	1.3 ± 0.3	1.4 ± 0.3	1.9 ± 1.1
EGFR ML/min Mean ± SD	111.3 ± 15.2	94.4 ± 22.3	80.2 ± 18.9	67.2 ± 15.5	59.2 ± 13.1

The comparison between diabetic patients with DFU and diabetic patient without DFU showed significant difference in age, sex, duration of diabetes mellitus, peripheral neuropathy and absence of education about care of foot (**Table 3**). Also, there was significant difference in postprandial blood sugar, eGFR, uric acid, albumin, LDL and HIF-1α (**Table 4**).

Table (3): Comparison of sociodemographic and clinical data between grade 0 (diabetic with intact skin) and grade 1-3 (diabetic with foot ulcer).

Variable	**Grade 0 N=10**	**Diabetic foot (G1,2,3) N=30**	**P value**
Age (year) Mean ± SD Median	48.5 ± 5.3 49	60.5 ± 12.1 62.5	0.006*
Sex Male Female	2 (20%) 8 (80%)	19 (63.3%) 11 (36.7%)	0.028*
Duration of DM (y) Mean ± SD Median	7.5 ± 2.9 7.5	12.8 ± 5.4 12	0.004*
Not educated about care of foot	4 (40%)	30 (100%)	<0.001*
SBP Mean ± SD Median	119 ± 12 120	134 ± 23.3 130	0.159
DBP Mean ± SD Median	78 ± 9.2 80	85.7 ± 15 85	0.23
BMI Mean ± SD Median	25.3 ± 3.6 25.9	27 ± 4.8 27.2	0.988
Peripheral neuropathy	2 (20%)	25 (83.3%)	0.001*

Table (4): Comparison of laboratory parameters between grade 0 (diabetic with intact skin) and grade 1, 2 and 3 (diabetic with foot ulcer).

Variable	Grade 0 DF N=10	Diabetic foot (G1,2,3) N=30	P value
Fasting blood glucose (mg/dl) Mean ± SD	135.3 ± 30.2	142.2 ± 34.6	0.79
2h PP BG (mg/dl) Mean ± SD	235.1 ± 57.1	290.6 ± 7.3	0.041*
HbA1c (%) Mean ± SD	7.8 ± 0.4	8 ± 1.3	0.888
Serum creatinine (mg/dl) Mean ± SD	1 ± 0.2	1.5 ± 0.3	0.020*
Estimated GFR (ml/min) Mean ± SD	94.4 ± 22.7	68.9 ± 6.4	0.042*
Urea (mg/dl) Mean ± SD	39.3 ± 9.6	52.1 ± 12.8	0.077
Uric acid (mg/dl) Mean ± SD	5.9 ± 0.8	7.7 ± 1.7	0.014*
Serum albumin (g/dl) Mean ± SD	4.3 ± 0.5	3.6 ± 0.7	0.010*
Serum cholesterol (mg/dl) Mean ± SD	164.1 ± 23.7	201 ± 41.7	0.137
Serum triglycerides (mg/dl) Mean ± SD	142 ± 35	166.3 ± 40.7	0.79
LDL (mg/dl) Mean ± SD	96.8 ± 21.2	121.7 ± 22.1	0.041*
HIF-1α (ng/ml) Mean ± SD	1.1 ± 0.3	4.2 ± 0.8	<0.001*

We found that HIF-1α was correlated with age, eGFR, Fasting blood glucose, 2 h postprandial blood glucose, HbA1c and peak systolic velocity of ATA (**Table 5**).

Table (5): Correlation between Hypoxia inducible factor 1 alpha and other variables in all study participants (N=80).

Variable	Hypoxia inducible factor 1 alpha	
	Pearson's Correlation (**R**)	**P-value**
Age (years)	0.358	0.001*
Duration of DM (y)	0.201	0.213
SBP	0.384	<0.001*
DBP	0.279	0.012*
BMI	0.291	0.009*
Estimated GFR (ml/min)	-0.282	0.011*
Serum albumin(g/dl)	-0.270	0.015*
2 h postprandial blood glucose (mg/dl)	0.493	<0.001*
HbA1c (%)	0.467	<0.001*
Fasting blood glucose (mg/dl)	0.487	<0.001*
Serum cholesterol(mg/dl)	0.017	0.884
Serum triglycerides (mg/dl)	0.094	0.409
LDL (mg/dl)	0.114	0.315
Uric acid(mg/dl)	0.304	0.006*
PSV of ATA (cm/sec)	-0.357	0.001*
PSV of PTA (cm/sec)	-0.210	0.062

DISCUSSION

A fast growing global public health problem that affects both industrialized and developing nations is DM [8]. Diabetes patients' foot ulcers are becoming more common, which has put a tremendous strain on the healthcare system. There are 347 million diabetics worldwide; it is anticipated that one in 20 of these persons will get foot ulceration within a year, and that more than 10% of these people may need to undergo an amputation [9].

In our study, we assess all patients and evaluate the risk factors for DFU. we found that there is a strong association between age, duration of diabetes and DFU p value (0.006 and 0.004) respectively and this with agreement with **Al Rubeaan *et al.*** [10] study, diabetes duration of less than ≥10 years was a significant risk factor for all affected foot, foot ulcer, and gangrene cases, with odds ratios of 7.22, 6.7, and 9.7, respectively, with gangrene having a higher odds ratio.

The predominance of male gender was found to be another risk factor with significantly higher in DFU patients 63.3% with significant p value (p=0.028). **Jiang *et al.*** [11] noted that there is significant statistical variation between the gender ratios (p=0.001) and hypothesized that a male diabetes patient was more likely (2.062 times more likely than a female patient) to develop a foot ulcer.

We also found that all DFU patients never had any prior foot care education. We found that 80% of DFU patients have lost of vibration perception and 10gm monofilament, 63.3 % loss of pain sensation and 56.7% abnormal ankle reflex with p value (<0.001, =0.001 and 0.002, respectively). This result is consistent with the findings of the **Younis *et al.*** [12] research indicating PN is a diabetic complication that increases the risk of foot ulcers. PN was found in 26% of diabetic participants, and there is a substantial relationship between DFU and PN, with a significant odds ratio of 23.9 (95% confidence interval: 5.41-105.6) and p value of 0.001.

There was significant increase in serum creatinine and serum uric acid with p values (0.028 and 0.014, respectively) with significant decrease in estimated GFR with p value 0.048, in concordance with **Young *et al.*** [13], who discovered that diabetic patients with amputations had a higher incidence of diabetic renal disease than diabetic patients without amputations (29.6 vs. 9.8%, p value <0.001).

We found that hypoxia inducible factor 1 alpha was significantly increased in DFU with p value <0.001 and there was significant increase in diabetic patients than control p value <0.001. This study showed that HIF-1α positively correlated with fasting blood sugar, 2hours postprandial and HbA1c (r=0.486 p<0.001, r=0.493 p<0.001 and r=0.467 p<0.001, respectively).

These agree with **Li *et al.*** [14], who discovered a substantial association between serum HIF1α levels and HbA1c and FBG in type 2 DM (r=0.242, p=<0.001 and r=0.244, p=0.029, respectively). Serum HIF-1α levels are increased in hyperglycemic patients.

Also, in agreement with results by **Isoe *et al.*** [15], which showed that the glucose-responsive sensor carbohydrate response element binding protein (ChREBP) is a transcriptional factor binding to the ChRE; ChREBP's binding to the HIF-1α promoter in glomerular mesangial cells exposed to high glucose mediates the upregulation of the HIF-1 α mRNA by high glucose.

Our findings disagreed with those of **Pichu *et al.*** [16], who found that DFU patients' levels of HIF-1α gene expression were lower than those of type 2 DM and control group. Wider investigations on both serum level and genetic expression on a larger scale of patients may be required, despite the fact that he did not compare the blood level of HIF-1 α. According to a research by **Jiang *et al.*** [17], constitutively active HIF1 α overexpression in adipose tissue causes obesity, insulin resistance, and glucose intolerance.

Similarly, we found that HIF-1α positively correlated with BMI and body weight (r=0.291 p=0.009 and r=0.334 p=0.002, respectively). This present study showed negative correlation between HIF-1α and estimated GFR (r= -0.282, p=0.011). This agrees with **Shao study** that reported serum levels of HIF-1 was significantly elevated in patients with type 2 DM compared with the control group and increased as urinary protein (as a marker of renal impairment) increased. The correlation analysis showed that serum HIF-1 was negatively connected with eGFR and favorably correlated with serum creatinine (r=0.174, p<0.001) [18]. This may suggest that serum HIF-1 was independent factors associated with DKD.

CONCLUSION

Proper control of DM and dyslipidemia with good education of diabetic patients about care of foot may have beneficial effect in prevention of DFU. Serum levels of HIF-1α may have a role in pathogenesis of diabetic wound healing. Future study on a larger scale of patients with DFU is needed to fully understand the role of HIF-1α in this disease and possibility of therapeutic strategy based on this role.

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