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Original Article

Evaluation of Ischemia-Modified Albumin and Fibrinogen in Relation with High-Sensitive C-reactive Protein in Diabetic Foot Ulcers

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Abstract

Context: Diabetic foot ulcer (DFU) is one of the most dreaded complication of diabetes which often affects the morbidity and mortality of a diabetic. Biomarkers are required for follow-up of these patients to prevent further complications to the affected limb. **Aim:** The aim of this study is to evaluate ischemia-modified albumin (IMA), fibrinogen in relation with high sensitive-C-reactive protein (hs-CRP) in patients with DFUs. **Methods:** a hospital-based cross-sectional study was carried out among 30 patients with DFU admitted in the surgery department of tertiary care teaching hospital. The duration of the study was 2 months. Serum IMA, hs-CRP, and plasma fibrinogen levels were measured. **Results:** The present study has demonstrated that IMA levels ($P < 0.05$) and fibrinogen levels ($P < 0.05$) are statistically significantly elevated in patients with DFU and had significant correlation with albumin and hs-CRP ($P < 0.05$). **Conclusions:** The use of IMA, hs-CRP, and fibrinogen may be incorporated during the follow-up of type 2 diabetes mellitus patients and may probably prevent the development of DFU and also possibly prevent lower limb amputation. Further studies with a larger number of patients with DFU are necessary to reach a definitive judgment.

Keywords: Diabetic foot, fibrinogen, high-sensitive C-reactive protein, ischemia-modified albumin

Introduction

Diabetic foot ulcer (DFU) is one of the major complications in patients with diabetes, predominantly seen in elderly male patients above 60 years of age. The incidence rate of DFU over the lifespan of a patient with diabetes is 15%–25%.[1] Regardless of extensive efforts, DFUs remain to be predisposed for a large number of amputations of the lower limb and are related to diminished quality of life in addition to higher risk of morbidity and mortality.[2,3] Glycemic status plays a very important role in DFUs. The long-term persistent hyperglycemia creates an environment wherein the cells are exposed to the reducing sugars and amino groups of biomolecules leading to the production of advanced glycation-end products. This reaction is known as the Maillard reaction, which has been hypothesized to be an important mechanism in the pathophysiology of diabetes complications.[4,5]

The abnormal metabolic state in type 2 diabetes mellitus (T2DM) involves chronic hyperglycemia, dyslipidemia, and insulin resistance. This abnormal metabolic condition affects the function of cells of endothelium, smooth muscle, and inflammatory cells. In normal individuals, there is a balance between reactive oxygen species and antioxidant levels. However, in T2DM, this balance is disturbed leading to a condition of increased oxidative stress, due to rise in the oxidant contents and fall in antioxidant level in the serum. There is hypercoagulability of blood in addition to the oxidative stress.[6] Patients with T2DM have diminished fibrinolysis and heightened production of procoagulants. Thus, the oxidative stress, enhanced levels of procoagulants, and

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reduced fibrinolysis favor the development of atherosclerosis that commonly progress to DFU.[7] The etiology of DFUs is multifactorial, the risk factors being peripheral neuropathy, vascular disease, deformities of the foot, limited joint mobility, minor trauma, impaired visual acuity, etc.[8]

The ischemic pathophysiological events, together with hypoxia and free radicals, lead to a characteristic conformational change of N-terminal end of albumin, which results in the development of ischemia-modified albumin (IMA) with decreased affinity binding to transition metals.[8] Currently, IMA is being explored as a general marker for ischemic tissues, and its role in DFU is one of the recent areas of research.[9] C-reactive protein (CRP) and fibrinogen are two of the best known acute-phase proteins that are synthesized primarily by the hepatic tissue in response to a variety of inflammatory cytokines and in the setting of acute inflammation, their concentration may increase several hundred fold.[10]

Low-grade immune activation implicated in the development of T2DM is also responsible for several microvascular and macrovascular complications associated with DM.[11] These patients have diminished fibrinolysis and a higher production of procoagulants, leading to an inflammatory state, playing a chief role in the development of atherosclerosis and its complications.[11,12] Fibrinogen, a simple and effective inflammatory marker, has been estimated to be elevated in the presence of DFU.[13,14] The present study is taken up to explore the role of IMA and fibrinogen levels in relation to high sensitive CRP (hsCRP) in patients with DFU when compared to patients with diabetes without foot ulcers.

Methods

This study is a hospital-based, cross-sectional study conducted on the participants admitted with DFU in the general surgery department of a tertiary care hospital. The study was carried out over a period of 2 months, i.e., from June 01, 2017, to July 31, 2017. The sample size for this study was determined to be 30 cases with 95% confidence interval at 90% power based on the works of Muhtaroğlu S. *et al*.[8] The study was approved by the Institutional Ethics Committee. All T2DM patients presenting with foot ulcers who were admitted in the general surgery department of a tertiary care hospital were considered for this study. However, patients with chronic kidney disease with estimated glomerular filtration rate <30 ml/min/1.73 m^2, liver failure, cardiovascular disorders, venous ulcer, pregnancy, and malignancy were excluded from the study. The history of the patients was obtained, following a clear explanation and understanding of the purpose of the study through an informed consent, which was signed by the patients. A thorough physical examination of the patients was done which included recording the height, weight, blood pressure, pulse rate, and a general physical examination followed by the inspection of the ulcer site in the DFU patients. Data relevant to biochemical parameters such as glycated hemoglobin, plasma sugars, renal function test, and liver function tests were obtained from the patient's medical records at the time of admission.

After obtaining informed consent from the patients, 5 ml blood was collected in a sterile plain vacutainer and 3 ml blood in citrated vacutainer for plasma. The serum and plasma were separated by centrifugation at 2500 × g for 15 min, and stored at minus 80°C until further analysis. Serum hsCRP was measured by immunoturbidimetry method using commercially available kits by Abbott on ARCHITECT ci4100 (USA) analyzer. Plasma fibrinogen was estimated on a semi-automated coagulation analyzer using commercially available kits based on Clauss method.[15] Serum IMA was measured by colorimetric assay based on the biochemical properties of albumin to bind exogenous cobalt, as previously described by Bar-Or *et al*.[16] IMA was reported in absorbance units; 1 ABSU = 100 × ΔOD (Optical Density of Test minus Blank). To adjust for the interference of the serum albumin, albumin adjusted IMA was calculated based on the following formula and was expressed in ABSU.[17]

$$\text{AAIMA} = \frac{\text{Serum albumin level of the patient}}{\text{Median albumin level of the population}} \times \text{Serum IMA level of the patient}$$

Statistical analysis

Data analysis was done using (trial version IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). The data were tested for normality by using the Kolmogorov-Smirnov test; further data were compared using student's independent sample *t*-test for parametric data and Mann–Whitney U-test for nonparametric data. Pearson's correlation was done to assess the relation of biomarkers with hsCRP. *P* values were considered statistically significant at < 0.05.

Results

Following the inclusion criteria, a total of sixty patients were enrolled in the study after obtaining informed consent from them. The control group comprised of patients with diabetes without foot ulcer and the cases were the patients with DFU, based on the inclusion criteria. Among them,

Table 1: Clinical characteristics of the study subjects

Parameter	Controls (*n*=30)	Cases with DFU (*n*=30)	*P*
Age (years), mean±SD	55.97±11.16	57.73±8.57	0.495
Gender (male/female), *n* (%)	13 (43.3)/17 (56.7)	27 (90)/3 (10)	<0.001*
Duration of T2DM (years), median (IQR)	5.50 (6.50)	8.00 (7.25)	0.377
Hypertension, *n* (%)	12 (40)	13 (43.3)	0.798
Smoking, *n* (%)	4 (13.3)	11 (36.7)	0.038*
Alcohol, *n* (%)	3 (10)	10 (33.3)	0.028*
On insulin, *n* (%)	2 (6.7)	22 (73.3)	<0.001*
On oral hypoglycemic agent, *n* (%)	30 (100)	23 (100)	<0.005*

*P<0.05 is considered statistically significant. DFU: Diabetic foot ulcer, SD: Standard deviation, IQR: Inter-quartile range, T2DM: Type 2 diabetes mellitus

20 were females and 40 were men, with more percentage of men (90%) suffering with DFU. In this study, the cases and controls were matched for age and duration of having diabetes [Table 1]. The biochemical parameters of the two study groups are given in Table 2. Based on the HbA1c status, 66.7% of cases with DFU were found to have very poor glycemic control.

The IMA varied from the lowest 43.00 ABSU to a maximum of 95.00 ABSU in the cases with DFU with a mean ± standard deviation of 67.1 ± 14 ABSU. On comparing the levels of glycemic groups with IMA levels, it showed an increased trend, implying raise in glycated hemoglobin level is associated with increase in the serum IMA level [Figure 1]., Linear regression analysis between IMA and HbA1c revealed a significant moderate positive correlation coefficient, $r^2 = 0.30$ ($P < 0.05$).

When glycated Hb was correlated with hsCRP, IMA, and AAIMA, all the three parameters correlated positively with the HbA1c ($P < 0.05$) [Table 3]. Further, on correlating the values of serum albumin with hsCRP, IMA, and AAIMA, it revealed that all these parameters had an inverse correlation with serum albumin [Table 3]. Based on the inverse correlation between hsCRP and serum albumin, it is suggested that high hsCRP levels may accompany the decrease in serum albumin [Figure 2]. Similar is the scenario with serum fibrinogen and serum albumin. Furthermore, plasma fibrinogen levels correlated positively with serum IMA indicating rise in fibrinogen levels is associated with rise in IMA levels in the serum [Figure 3].

Discussion

The prevalence of diabetes mellitus (DM) shows an increased propensity worldwide, the most important complication being the lower-extremity vascular disease associated with diabetes.[18,19] Patients often ignore the initial signs and symptoms of the peripheral vascular disease. The lower extremity injury either due to trauma, infection, or both in the presence of diabetic neuropathy often presents as foot ulcers of varied degree. One of the major causes of hospitalization and amputation among patients with diabetes are the complications of the foot ulcers leading to significant health-care costs. This is evidenced by the fact that around 20%–40% of health-care resources are spent on diabetes-related diabetic foot.[20]

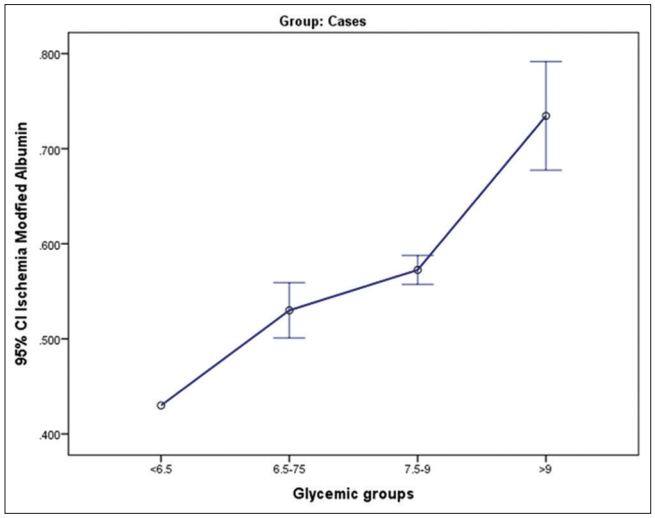

Figure 1: Serum ischemia-modified albumin levels correlated with HbA1c in the study subjects

When the cases were grouped according to American Diabetes Association[21] into good glycemic control (HbA1c ≤7.0%) and poor glycemic control (HbA1c >7.0%), about 25 patients with DFU (83.3%) were found to be having poor glycemic control. Long-term testing and maintenance of glycemic targets especially HbA1c in the normal range is of utmost importance for delaying and preventing the onset of vascular complications of DM.[22]

The IMA levels were not correlated with the duration of diabetes implying that it could be more of an acute

Table 2: Biochemical parameters of the study subjects (Mean±SD)

Biochemical parameter	Controls	Cases with DFU	*P*
HbA1c (%)	7.60±1.73	10.44±2.39	<0.001*
FPG (mg/dl)	150.92±46.75	159.50±73.55	0.598
PPPG (mg/dl)	223.96±62.58	236±86.84	0.536
Blood urea (mg/dl)	29.87±16.54	27.63±12.92	0.578
Serum creatinine (mg/dl)	1.14±0.63	1.09±0.43	0.704
Total protein (g/dl)	6.95±0.34	6.88±0.77	0.659
Serum albumin (g/dl)	4.01±0.22	3.38±0.47	<0.001*
IMA (ABSU)	43.3±16	67.1±14	<0.001*
Albumin adjusted IMA (ABSU)	0.432±0.12	0.660±0.14	<0.001*
hs-CRP (mg/dL)	0.66±0.15	3.84±1.18	0.014*
Fibrinogen	156.03±24.67	491.53±46.35	<0.001*

*P<0.05 is considered statistically significant. HbA1c: Glycated hemoglobin, FPG: Fasting plasma glucose, PPPG: Postprandial plasma glucose, IMA: Ischemia modified albumin, ABSU: Absorbance units, hs-CRP: High-sensitive C reactive protein, DFU: Diabetic foot ulcer

Table 3: Correlation of glycated hemoglobin and serum albumin with high-sensitive C reactive protein, ischemia modified albumin, albumin adjusted ischemia modified albumin and fibrinogen among patients with diabetic foot ulcer

Parameter	Correlation coefficient (*r*)		*P*
	With HbA1c	With serum albumin	
hs-CRP (mg/dL)	0.32	−0.41	<0.05
IMA (ABSU)	0.30	−0.48	<0.05
AAIMA (ABSU)	0.39	−0.27	<0.05
Fibrinogen (mg/dL)	0.72	−0.54	<0.05

*P<0.05 is considered statistically significant. IMA: Ischemia modified albumin, ABSU: Absorbance units, hs-CRP: High-sensitive C-reactive protein, HbA1c: Glycated hemoglobin, AAIMA: Albumin adjusted IMA

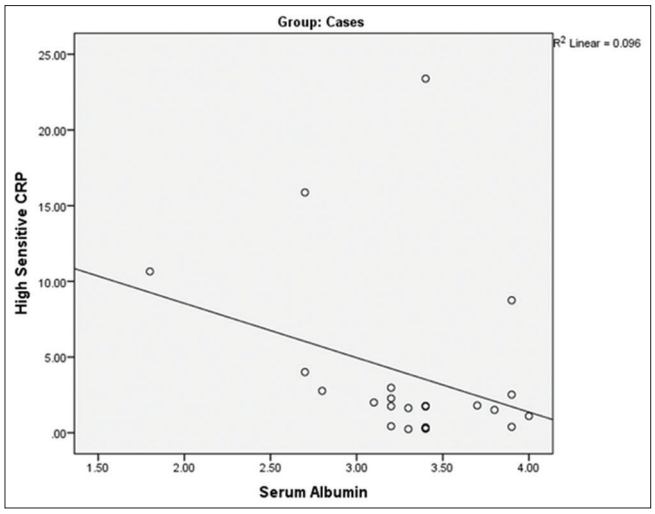

Figure 2: Correlation of serum albumin with high sensitive-C-reactive protein in cases with diabetic foot ulcer

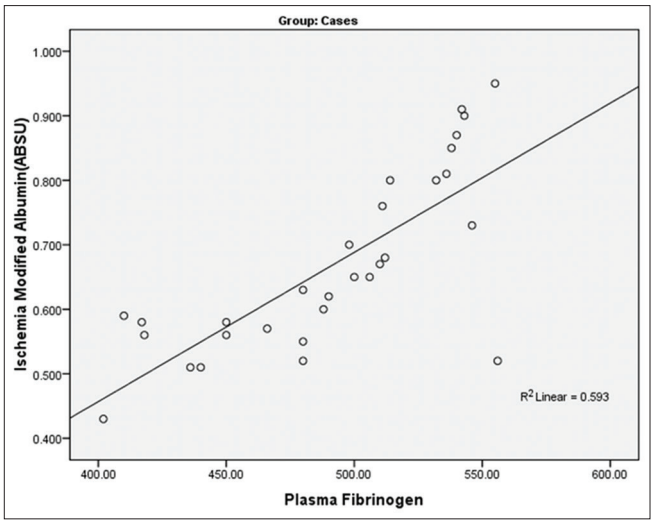

Figure 3: Correlation of plasma fibrinogen with serum ischemia-modified albumin levels in cases with diabetic foot ulcer

phenomenon rather than a chronic one. The serum IMA levels correlated with the HbA1c levels showing an increased trend of serum IMA levels with increase in the HbA1c levels. Similar observations have been reported by other studies.[7,23,24] The serum IMA levels correlated negatively with serum albumin levels ($P < 0.001$).

Hs-CRP an acute-phase protein has been used in the diagnosis and monitoring of acute inflammation and infection and DFU is invariably such a condition, where inflammation is prone to occur and thus hsCRP levels are expected to be elevated. From this study, it is evident that hsCRP levels are significantly on a higher side in DFU when compared to patients with DM without DFU. Elevated levels in DFU could be due to various reasons such as atherosclerosis due to endothelial damage, underlying inflammation, peripheral arterial disease that has led to DFU.[25] It is also noted that hsCRP levels are elevated in the controls, i.e., diabetics without foot ulcer which could be due to underlying inflammatory process.

Fibrinogen is an acute-phase protein, in addition to being a major blood coagulation protein and a prime determinant of blood viscosity and platelet aggregation.[26] This protein is expected to be elevated in the inflammatory settings and is an important risk factor in the vascular events that occur in DFU.[27] Kunutsor *et al*.[28] have shown that fibrinogen is an independent risk factor for sudden cardiac death. Fibrinogen also plays a significant role in endothelial injury, formation of fibrin clot which has low permeability, thrombosis, hyperactivity of platelets, and blood flow abnormalities. All these contribute to subclinical atherosclerosis.[29] Based on the current study, it is noteworthy that fibrinogen levels are considerably much elevated in patients with diabetes with DFU when compared to the values in patients with diabetes without DFU, which is similar to the findings in other studies.[13,14]. In controls, the elevated levels could be due to the initial stages of progression to vascular diseases. Therefore, this could be another good marker in DFU as DFU is generally associated with inflammation.

The present study was done at a single tertiary care center, with a very limited sample size in a limited time frame. Furthermore, the IMA should have been estimated on follow-up in the same set of patients but could not be executed due to the lack of time and appropriate funds. As, this study was done with a small sample size, it is warranted that further studies with larger sample size comprising of patients presenting with various stages of DFU would be required to optimize the fibrinogen and IMA cut-off levels in estimating the disease severity at initial evaluation as well as during follow-up.

Conclusions

The present study has demonstrated that IMA levels and fibrinogen levels are significantly elevated in patients with DFU and had significant correlation with serum albumin and hs-CRP. Higher IMA levels were associated with elevated HbA1c levels, in patients with DFU. In addition to IMA, fibrinogen levels also play an important role in estimating the disease severity. The elevated fibrinogen levels and the serum IMA levels can give a significant clue to the clinician for estimating the disease severity and decide on initiation of the treatment earlier. These two markers can serve as potential prognostic markers in T2DM patients with DFU, and early intervention can prevent lower limb amputation, thereby decreasing the morbidity and mortality of patients with diabetes.

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Conflicts of interest

There are no conflicts of interest.

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