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Evaluation of Fructosamine and Ischemia–Modified Albumin as prognostic markers for cardiovascular disorders in type 2 diabetic patients

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ABSTRACT *Cardiovascular disorders (CVD) are a major complication of diabetes mellitus. They have always been underestimated in patients with diabetes. Therefore, a delayed recognition of various forms of CVD worsens the survival prognosis for many diabetic patients. In this study, we assessed Ischemia-modified albumin (IMA) and Fructosamine as prognostic markers for CVD in Type 2 Diabetic Patients (T2DP). In contrast to Fructosamine, There was a significant difference ($p < 0.0001$) between levels of IMA in diabetic patient with CVD as compared with those without CVD. No correlation has been found between Fructosamine and IMA in T2DP with CVD ($r = -0.169$, $p = 0.245$). For IMA, We proposed a calculated Cutoff to predict T2DP who may sufferer of CVD.*

Keywords: *Biomarker, Cardiovascular Disorders (CVD), Diabetes mellitus, Fructosamine, Ischemia-modified albumin (IMA).*

1. INTRODUCTION

The most prevalent form of diabetes mellitus is type 2 diabetes (T2D). This disorder typically makes its appearance later in life. The underlying metabolic causes of type 2 diabetes are the combination of impairment in insulin-mediated glucose disposal (insulin resistance) and defective secretion of insulin by pancreatic β cells [1]. Diabetes is an independent risk factor for CVD in both men and women [2-4]. CVD are the cause of death in more than 70% of T2D patients [5]. To make matters worse, when patients with diabetes develop clinical CVD, they sustain a worse prognosis for survival than do CVD patients without diabetes [6,7]. Therefore, we need markers that diagnose early cardiovascular events in T2D.

A number of candidate biomarkers have been proposed for the detection of cardiac ischemia; however, only Ischemia Modified Albumin (IMA) has been released for clinical use [8]. The ischemia-modified albumin (IMA) is a novel ischemia marker developed by quantifying the decrease in the metal binding capacity of albumin [9-12]. This decreased binding reflects changes to the NH2 terminus of albumin, the binding site for the transition metals Co(II), Cu(II), and Ni(II) [11-13]. Conditions that can alter albumin's N-terminal region, and therefore albumin cobalt binding, can occur within minutes of an ischemic event via induced endothelial and extracellular hypoxia, acidosis, free radical injury, and sodium and calcium pump disruptions. This effect on albumin could be detectable up to 6 h after the ischemic event [14,15].

Fructosamine is a glycated protein formed via a non-enzymatic mechanism that involves the binding of plasma glucose to serum proteins to form ketoamines [16]. Fructosamine has a short half-life and reflects the physiology of glucose metabolism in the extracellular space; therefore, Fructosamine provides information on blood glucose over the previous 2 - 4 weeks. It is still unclear whether Fructosamine is associated with the risk of cardiovascular disease in diabetic patients with CVD [17].

However, to our knowledge, no previous study has assessed the relationship between Fructosamine and ischemia-modified albumin in diabetic patients with cardiovascular disorders. Therefore, our objective was to identify whether ischemia-modified albumin and Fructosamine are a nontraditional prognostic markers for CVD in T2DP.

2. MATERIALS AND METHODS

2.1 Reagents

Cobalt chloride was purchased from Sigma-Aldrich (France). DTT was purchased from Vivantis (Malaysia). All diagnostic reagents were purchased from Roche (France).

2.2 Patients

This study was performed at Albasel Hospital, Aleppo, Syria, and was approved by the local ethics committee. Peripheral blood was obtained from 149 participants. The diabetic patients were classified into two groups: group DC (included 49 type2 diabetic patients with CVD) and group D (included 50 type2 diabetic patients without CVD). The group N (normal people) included 50 volunteers' people. In the DC group: 17 (35%) had myocardial infarction, 12 (25%) had angina pectoris, 7 (14%) had heart failure, 6 (12%) were ischemic and 7 (14%) had other CVD. All selected patients were without liver or kidney dysfunction, infection and corticosteroid therapy. Each blood sample was placed in two separate tubes, tube without anticoagulant and a tube with heparin. The serum or plasma was separated and kept at -80°C until the biochemical evaluations.

2.3 IMA Measurements

The IMA levels was measured by albumin cobalt binding test (ACB test) [12,18]. Briefly, 50 μl water solution of 0.1% cobalt chloride (CoCl26H2O) was added to 200 μl of serum, gently mixed and after 10 min the 50 μl of Dithiothreitiol (DTT) solution (1.5 mg/ml H2O) was added as a colorizing agent and the reaction was quenched two minutes later by adding 1.0 ml of 0.9 % NaCl. Color development with DTT was measured spectrophotometrically at 470 nm in comparison with a serum cobalt blank without DTT and reported in absorbance units (ABSU).

2.4 Fructosamine determination

Plasma Fructosamine was determined by SPOTCHEM II reagents strips using a SPOTCHEM Analyzer (Medical Group Company, distributor of Arkray, Syria) according to manufacturer's instructions.

2.5 Glucose, cholesterol, and triglycerides measurement

Glucose cholesterol and triglycerides were analyzed using routine kits.

2.6 Statistical Analysis

Each sample was measured in duplicate and the mean value was reported. Statistical analyses were done with SPSS software. ANOVA and Tukey tests were used to evaluate the significance of differences between test groups. Pearson's correlation was used to investigate the correlation between the levels of IMA and Fructosamine levels. The sensitivity and specificity of the IMA test were determined by ROC curve analysis. A p value <0.05 was considered statistically significant.

3. RESULTS

We first investigated the basic characteristics of the total study population (Table 1).

Table 1: basic characteristics of the total study population

Group	**N**	**D**	**DC**
n	50	50	49
Male (%)	55.1	46	67.3
Age (years)	29.2±4.6	48.2±8.6	58.6±8.6
Glucose (mg/dl)	77.6±10.7	206.3±78.8	210.1±91.4
Cholesterol (mg/dl)	123.5±30.7	175.1±35.9	139.27±39.2
Triglyceride (mg/dl)	68.7±25	173.7±70.1	126.4±69.2
BMI: Body Mass Index (Kg/m^2)	23.1±4	32.1±7.8	29.5±5.5
IMA (ABSU)	0.755±0.115	0.801±0.991	1.099±0.205
Fructosamine (µmol/L)	189.8±16.3	278.8±75.1	273.3±63

Despite the difference in gender and age distribution between the groups, there was no influence of gender or age on the other clinical parameters (p >0.05).The levels of IMA were distinctly higher in DC group than among those in the N and D groups (p <0.0001). On the other hand, there was no difference in the levels of IMA between the D and N groups (p=0.452 > 0.05).

Median Fructosamine values were significantly higher in DC group compared with N group (p <0.0001) and in D group compared with N group (p <0.0001). In contrast, no significant difference was found between DC and D groups (p=0.081>0.05). Thus, these results indicate that the IMA level but not the Fructosamine level has the ability to discriminate type 2 diabetic patients with CVD.

Although Fructosamine level did not distinguish between DC and D groups, it was higher in diabetic patients as compared with healthy persons. Therefore, it may be used for monitoring short-term glycemic control in persons with diabetes. The Fructosamine test is more expensive than fasting plasma glucose, for this reason, we used linear regression analysis to predict Fructosamine value using age, BMI and glucose level (mg/dl) as independent variables (Fig.1).

Fructosamine (µmol/L) = 163.221 + 1.568*Age + 0.275*Glucose - 1.689*BMI

Fig.1. Linear regression analysis to predict Fructosamine value. The r of equation was 0.615 and standard error of estimation was 51. 9178.

A ROC curve analysis was employed to quantify the overall ability of IMA to discriminate type 2 diabetic patients with CVD from those without CVD. Area under the curve was 0.909 (95% confidence interval [CI], 0.851–0.966) (Fig.2). At the optimum decision point of 0.89, the sensitivity and specificity of the IMA test for assessing cardiovascular disorder were 84% and 87.5 % respectively.

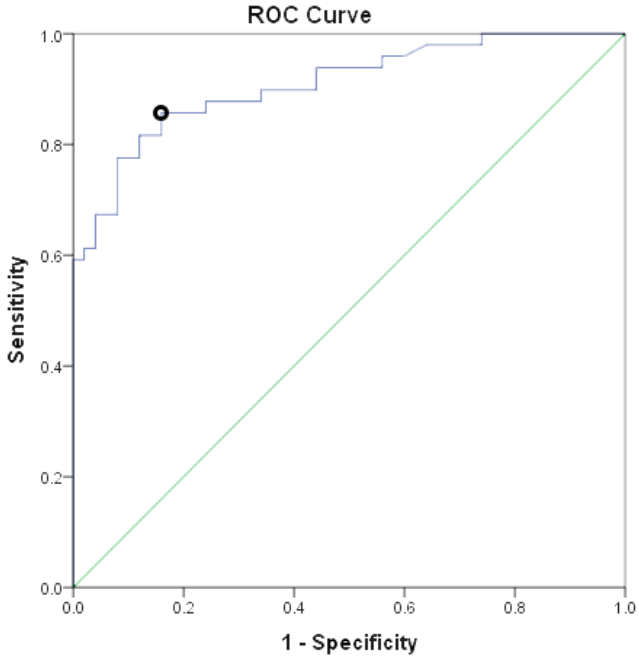

Fig.2. the ROC curve of the IMA test for the predication of CVD in type2 diabetic patients.

We finally examined the correlation between the levels of IMA and Fructosamine levels in the three groups. As shown in Table 2 no correlation was observed.

Table 2: the correlation between IMA levels and Fructosamine levels in the three groups.

Group	r- value	P-Value
DC	-0.169	0.245
D	-0.054	0.711
N	-0.113	0.443

4. DISCUSSION

Cardiovascular disorders (CVD) remain the major cause of morbidity and mortality in developed and developing word [19]. Cardiovascular complication of diabetes mellitus with a two- to four folds increased risk [5]. Although much progress has been made in the definition and treatment of CVD risk factors, there is a growing interest in the identification of novel biomarkers to help identify diabetic patients at risk of future cardiovascular events [17].

A small number of studies debated the use of IMA in primary prevention or its ability to detect asymptomatic cardiovascular disorder in type 2 diabetic patients [18,20,21]. In our study, diabetic patients with CVD had higher modified albumin levels than diabetic patients without CVD and this result suits with previous studies [20,21]. The biological explanation of association between CVD and ischemia-modified albumin is related to a decrease in lower limit perfusion and oxygenation, thus triggering albumin modification. High serum modified albumin levels in diabetic subjects might indicate subclinical vascular disease. Microvascular dysfunction is a potential pathophysiological mechanism of CVD in such cases [19]. Thus, levels of ischemia-modified albumin might be a gold prognostic marker of developing CVD in type 2 diabetic patients.

Fructosamine is a biomarker used to monitor short-term glucose control and when HbA1c measurements can be affected by non-physiological hemoglobin turnovers [16]. Fructosamine is available, but is not routinely used in Syria. More recently, observational studies have also focused on the role of Fructosamine as risk factor for cardiovascular complication. The association of Fructosamine and cardiovascular disorders in diabetic patient has been recently described (Elizabeth Selvin. et al) [22]. In contrast to that study, our results indicated non-significant difference in Fructosamine levels between DC and D groups. One of the reasons for this may well be the modest number of patients in each group of our study. Nonetheless, Fructosamine levels were higher in diabetic patients as compared with healthy persons. We then proposed a calculated value of Fructosamine using common parameters. Fructosamine test can be quite costly, so this calculated value could permit physicians to easily, inexpensively and rapidly monitor their patients.

No correlation has been found between IMA and Fructosamine in the three groups. Nonetheless, the DC group, which had higher IMA levels, also presented elevated levels of Fructosamine. Therefore, we need to extend the study to large scale to confirm or denounce these results. Moreover, IMA or Fructosamine combination with other prognostic biomarkers may be needed.

5. CONCLUSION

Our study approved that ischemia-modified albumin (IMA) could be a predictive marker for cardiovascular disorders in type 2 diabetic patients. Even though, levels of Fructosamine was not as important as IMA levels, we created an equation to calculate the value of Fructosamine by age, BMI, and glucose value of the patient.

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