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Kynurenine-modified serum proteins are a new marker of cardiovascular disorders developing in type 2 diabetes

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ABSTRACT

Objective(S)*: Cardiovascular disorder (CVD) is the major complication of type 2 diabetes. Tryptophan/kynurenine pathway has been related to CDV progression and diabetogenesis. In this paper, we will assess the presence of Kynurenine-modified serum proteins (KMSP) as a new predictor of CVD development in type 2 diabetes.*
Materials and Methods: *Peripheral blood was obtained from 189 participants. The diabetic patients were classified into two groups: group DC (included 63 type2 diabetic patients with CVD) and group D (included 63 type2 diabetic patients without CVD). The group N (normal people) included 63 volunteers' people. The presence of KMSP was assessed by homemade-ELISA method using highly specific monoclonal antibodies.*
Results: *KMSP were significantly higher in diabetic patients and especially in DC group. Furthermore, KMSP level was only related with the onset of diabetes regardless of the presence of CVD.*
Conclusion: *KMSP predicted CVD in type 2 diabetes and might participate in CVD development.*

Keywords: *Cardiovascular disorders, Diabetes mellitus, Kynurenine-modified serum proteins, prognostic marker.*

1. INTRODUCTION

Millions people worldwide have type 2 diabetes (T2D) [1] .The major complication of T2D is the cardiovascular disease (CVD) which are the cause of death in more than 70% of type 2 diabetic patients [2]. Despite advances in our understanding of some early markers of atherogenesis, the mechanisms underlying the increased risk of CVD in type 2 diabetes remain incompletely delineated [3]. It has been postulated that proinflammatory cytokine –such as interferon γ (IFN-γ) may be crucially involved in the pathogenesis of atherosclerosis and CVD in general population [4-6].

IFN-γ, which is released by activated T-lymphocytes, can stimulate the activity of indoleamine 2.3-dioxygenase (IDO) [7]. This enzyme, by degradation of tryptophan to kynurenine (KYN), induces the kynurenine pathway and the production of different metabolites (Kynurenines). Kynurenines are unstable under physiological conditions; they undergo spontaneous deamination to form reactive α, β-unsaturated ketones that may react with nucleophilic amino acids in proteins and glutathione and modify proteins [8].

Several studies suggest a role of kynurenine pathway dysregulation in the pathogenesis of both diabetes and CVD [9-13]. However, no clinical studies have been done to investigate whether KYN- mediated modification could prognosticate a CVD developing during diabetes. To address this issue, we have evaluated, using highly specific monoclonal antibodies, the presence of Kynurenine-modified serum proteins (KMSP) in type 2 diabetic patients with and without CVD.

2. MATERIALS AND METHODS

2.1 Reagents

anti-Kynurenine-modified protein mouse monoclonal antibody (anti-KYN mAb) was purchased from Advanced Targeting Systems (USA). Nunc Maxisorp flat-bottom 96 well plates were purchased from eBioscience (USA). HRP conjugated goat anti-mouse secondary antibody was purchased from Promega (USA). Bovine serum albumin (BSA) was purchased from Spinreact (Mexico). 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. All participants provided written informed consent. Peripheral blood was obtained from 189 participants. The diabetic patients were classified into two groups: group DC (included 63 type2 diabetic patients with CVD) and group D (included 63 type2 diabetic patients without CVD). The group N (normal people) included 63 volunteers' people. In the DC group: 22 (34.9%) had myocardial infarction, 4 (6.4%) had angina pectoris, 7 (11.1%) had heart failure, 5 (7.9%) was ischemic and 25 (39.7%) had other CVD. All selected patients were without liver/kidney dysfunction, infection and corticosteroid therapy. Each blood sample was placed in tube without anticoagulant and the serum was separated and kept at -80°C until the biochemical evaluations.

2.3 Homemade ELISA for Kynurenine-modified serum proteins detection

Microplate wells were coated with serum proteins in 0.05M carbonate buffer (pH 9.7) at a concentration of 20µg/ml, incubated for 2 h at 4C, and then washed three times with PBST (PBS buffer containing Tween 20) . The wells then were blocked with 2.5% BSA in PBST and washed three times with PBST. The primary antibody, mouse anti-KYN mAb (1:1000 diluted in PBS containing 2.5% BSA), was added, the plates were incubated for 1 h at 37C, washed with PBST, and then incubated with HRP conjugated goat anti-mouse IgG secondary antibody (1:15 000 in PBST). After wells washing, HRP substrate with H2O2 was added the reaction stopped, and color development was measured at 450 nm in comparison with blank and reported in absorbance units (ABSU). The blank was made to verify the primary and secondary antibody specificity by using BSA-coated wells.

2.4 Total Protein, Glucose, cholesterol, and triglycerides measurement

Glucose, cholesterol, total Protein and triglycerides were measured using routine kits.

2.5 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. Stepwise Linear Regression was used to investigate the correlation between each of the clinical parameters and the level of Kynurenine-modified serum proteins expressed as ABSU. The sensitivity and specificity of Kynurenine-modified serum proteins measurement were determined by ROC curve analysis. *P* values less than 0.05 were 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	63	63	63
Male (%)	52.4	58.7	52.4
Age (years)	46.24 ± 8.7	52.56 ± 6.24	57.3 ± 7.99
Current smoking (%)	43	43	41
Glucose (mg/dl)	82.54 ±9.65	210.14 ± 68.4	210.1 ± 57.17
Cholesterol (mg/dl)	134.6 ± 14.174	182.9 ± 43.51	186.8 ±39.115
Triglyceride (mg/dl)	98.6±20.13	182.59± 45.83	160.25 ± 22.347
BMI: Body Mass Index (Kg/m2)	29.1± 4.226	28.8±3.25	30.15 ± 4.21
KMSP (ABSU)	0.175 ±0.026	0.22 ± 0.062	0.25 ± 0.066

For the 189 person in our study, median age was 52 years, 103 (54.5%) were men and 42% were current smokers. Compared with the total population glucose, cholesterol and triglyceride levels were higher in diabetic patients (p <0.05). In contrast, there was no significant difference in gender, body mass index and age distribution between the groups (p >0.05) (Table 1). In addition, there was no influence of gender or age on the other clinical parameters (p >0.05).

Median absorbance values indicating the presence of KMSP, were significantly higher in DC group than among those in the N and D groups (p <0.0001 and p <0.01 respectively) and in D group compared with N group (p <0.0001) (Fig.1). Thus, these results indicate that the level of KMSP is higher in diabetic patients and especially in the presence of CVD. We then examined the correlation between ELISA values and other tested parameters in D and DC groups. A positive relation was only found with the onset of diabetes (P <0.05, R=0.78) regardless of the presence of CVD.

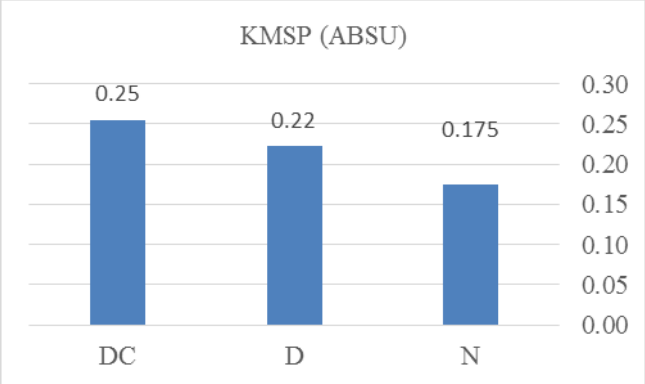

Fig.1: KMSP levels in different groups.

A ROC curve analysis was employed to quantify the overall ability of KMSP to discriminate type 2 diabetic patients from normal individuals. Area under the curve was 0.796 (95% confidence interval [CI], 0.706–0.885) (Fig.2). At the optimum decision point of 0.1935, the sensitivity and specificity of the test for assessing type2 diabetes were 77% and 85% respectively. We then used the same anlaysis to quantify the overall ability of KMSP to discriminate type 2 diabetic patients with CVD from those without CVD. Area under the curve was 0.654 (95% confidence interval [CI], 0.558–0.750) (Fig.3). At the optimum decision point of 0.2325, the sensitivity and specificity of the test for assessing cardiovascular disorder were 63% and 64% respectively.

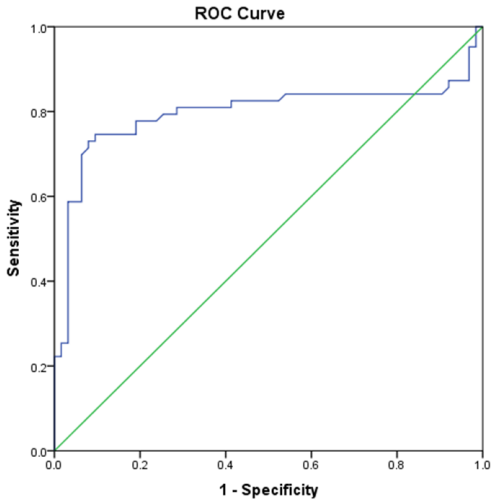

Fig.2: the ROC curve of KMSP test for the predication of type2 diabetes.

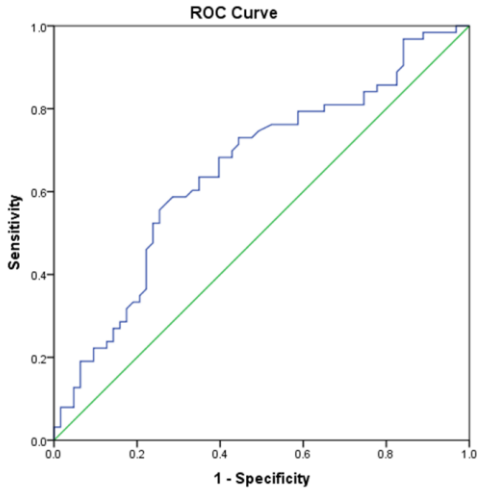

Fig.3: the ROC curve of KMSP test for the predication of CVD in type2 diabetic patients.

4. DISCUSSION

Pre-diabetes state may be present years before the occurrence of overt diabetes [14]. Mechanisms leading to diabetes and its complications development are multiple and incompletely understood. A growing interest was recently given to inflammatory process in the progression of diabetes and CVD [4,5]. CVD is the most dangerous complication in diabetic patients and represents a real defiance in diabetes management.

Several studies suggest diverse and complex effects of tryptophan/kynurenine pathway on glucose homeostasis and vasculature. This pathway is induced during inflammation giving products that can react with proteins and modify them [7,8]. In a recent study, elevated levels of plasma kynurenines was found as a good predictor of risk of acute myocardial infarction [15]. In another study, kynurenine overproduction contributed in cataract formation by modification of lens proteins [16]. Moreover, our previous results demonstrated a prognostic role of ischemia-modified albumin (IMA) in detection of CVD in type2 diabetic patients [17]. Taken

together, we hypothesized that serum protein modification by kynurenine may represent a marker or possible mechanism for CVD developing in type 2 diabetes. Using highly specific monoclonal antibodies, we demonstrated that type 2 diabetic patients had higher kynurenine-modified serum proteins as compared with normal people. In addition, the level of these modified proteins increased significantly in the presence of CVD. We so proposed two cutoff values in order to discriminate type 2 diabetic patients from normal people and to predict diabetic patients who may suffer of CVD. Importantly, the detection of KMSP was only related to the onset of diabetes (P <0.05, R=0.78) regardless of the presence of CVD. These results suggest a potential role of kynurenine pathway in diabetogenesis, disease progression and its CVD complication.

5. CONCLUSION

We concluded that kynurenine-modified serum proteins might be a new predictive marker for cardiovascular disorders in type 2 diabetic patients. Furthermore, they could be a therapeutic target to prevent diabetes and its CVD complication. Future studies should aim to clarify the cellular and molecular mechanisms of these modified proteins.

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