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ORIGINAL ARTICLE

Effect of Atherosclerosis Developed from Diabetes on Ceruloplasmin Concentration, Ceruloplasmin Ferroxidase Activity and its Specific in Sera of Diabetic Iraqi Patients with Atherosclerosis

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ABSTRACT

Atherosclerosis is one of the common complications in patients with advanced type 2 diabetes (T2DM). This study aims to detect the influence of this complication on ceruloplasmin concentration, ceruloplasmin ferroxidase activity and its specific activity. Samples were collected in the morning from males and females' individuals (50-69years) who attended Ibn Al-Bitar center for cardiac surgery. Fasting serum glucose (F.S.G), malondialdehyde (MDA), Ceruloplasmin concentration, Ceruloplasmin ferroxidase activity and its specific activity were all measured after the participants fasted for about 8-12hours.The F.S.G, Ceruloplasmin Ferroxidase activity and its specific activity had a high significant increase at p<0.001. Meanwhile and Ceruloplasmin concentration had a significant increase at p<0.05.

Keywords: Diabetes, Atherosclerosis, Ceruloplasmin, Ferroxidase.

INTRODUCTION

Diabetes mellitus (DM) is one of the most common metabolic diseases that is described by chronic hyperglycemia, as a consequence of a deficiency in the secretion or action of insulin.[1] The major characteristic of T2DM is the diminution of insulin sensitivity where the patients are resistant to the action of insulin, in which the progressive of insulin secretory disorder on the background of insulin resistance (IR) [2-3] However, when both the dysfunction of β -cell and IR are existent, hyperglycemia is amplified causing T2DM progression [4]. Inflammation have elicited as remarkable pathophysiological factors on such disease, as well obesity affects T2DM, adipose tissue enhances IR via many inflammatory mechanisms, including: increasing the free fatty acid (FFA) release, dysregulation of adipokine, abnormalities in gut microbiota, immune dysregulation[5-6]. In addition, the raise of FFA levels cause an increasing of glucose levels by gluconeogenesis, this increase leads to β cells decompensation; together with its compensation ultimately leads to impaired glucose tolerance, causing T2DM development[7]. It is believed that T2DM is one of the chronic diseases which has essential effects on the quality of life and constitutes a real health problem of the world population. It triggers microvascular and macrovascular complications [8-9] , prolonged exposure to hyperglycemia is recognized a main factor in the pathogenesis of atherosclerosis, which can lead to a variety of vascular diseases, including cerebral vascular disease, cardiovascular disease (CVD), and different vascular diseases[5] Diabetic atherosclerosis pathogenesis includes the direct effects of chronic hyperglycemia, in parallel with IR, FFA production, dyslipidemia, impaired response to injury, and hypercoagulability. [10] As well chronic hyperglycemia with dyslipidemia cause enhancement of OS resulting in enhancement of ox-LDL formation, activation of immune cells[11] The studied enzyme **(**CP**)** is a serum ferroxidase and is a an acute-phase reactant (positive) where its levels increase as a response of inflammation and cell injury. It oxidizes the ferrous iron (2+) to ferric iron (3+) also, it assists the iron and transferrin (tf) binding. It also may has a role in controlling the membrane lipid oxidation[12]. Also, Cp ferroxidase possesses a significant oxidase activity towards numerous aromatic amines and phenols[13]. Malondialdehyde (MDA) which is is a 3-carbon with lower molecular weight aldehyde formed by free-radical-mediated chain reactions and bigly utilized as a lipid peroxidation (LPO) marker [11]. Since MDA is highly cytotoxic and carcinogenic agent it is frequently used as a biomarker of OS during major health problems such as T2DM and, or CAD[14].

METHODS AND METHODS

To achieve the aims of this study 146 Apparently control individuals and patients who attended Ibn Al-Bitar center for cardiac surgery over the period (September 2020- December 2020) were participated

in the current study, they were distributed to 3 major groups:

Apparently control group (**AC**), T2DM patients' group (**P_1**), and T2DM patients with atherosclerosis group (**P_2**): The duration of the disease for P_1 group was (4-13yrs) and for P_2 group the duration for T2DM was (6-25yrs) and they were newly diagnosed with atherosclerosis. Based on the HbA1c tests performed by patients before coming to the hospital; the patients were diabetes (HbA1c>7), Both patient's groups underwent a Cardiac catheterization in order to diagnose atherosclerosis and both groups had a positive CRP. Individuals with peripheral atherosclerosis, chronic renal failure, chronic inflammation, smokers, alcohol consumers and pregnant women were excluded from this study. From all fasting participants, blood samples were collected, centrifuged for (15min at 3000 rpm), distributed in Eppendorf tubes (200µl in each tube), then F.S.G was estimated and remained tubes were stored in the freezer at (-20°C) for the later use.

Determination of Fasting Serum Glucose: Glucose levels were determined spectrophotometrically (at 340 nm) [15-16] using the Abbott architect c4000.

Determination of Malondialdehyde (MDA): Malondialdehyde (MDA) was measured using Thiobarbituric acid (TBA) method (Satoh's modified method), which is considered a very sensitive and specific method for determining serum lipid peroxidase activity. The MDA is one of the final products of lipid peroxidation which forms a colored product with TBA[17].

Determination of CP concentration: The modified rice method (automated spectrophotometric method) was used for the estimation of ceruloplasmin concentration At the wavelength of 605mn then the absorbance was multiplied by Holmberg-Laurel factor = (87.5) [18 19].

The specific enzyme activity is determined using the equation:

$$\mathbf{Sp.act}\left(\frac{\mathbf{U}}{\mathbf{g}}\right) = \mathbf{Enzyme\ activity}\left(\frac{\mathbf{U}}{\mathbf{L}}\right) \div \mathbf{Protein\ concentration}\left(\frac{\mathbf{g}}{\mathbf{L}}\right)$$

Statistical analysis: It was carried out by Statistical Package for the Social Science program (SPSS version 25.0). The differences between the three groups was tested by ANOVA test.

RESULTS

As shown in Table 1, The F.S.G showed a high significant difference at p<0.001 while comparing the three groups together and a significant increase between the mean of group P_1 and group P_2 in comparison with the AC group. Also, there was a significant increase in group P_2 compared to group P_1. The MDA showed a significant increase at p<0.05 and a significant increase

when comparing group AC with P_1 and AC with P_2 also showed a significant increase when comparing group P_1 with P_2. Cp concentration showed a significant increase at $p<0.05$ when comparing the three groups together but when comparing each group with AH group only P_2 had a significant increase also it had a significant increase when compare to group P_1. Cp Ferroxidase activity and Cp Ferroxidase sp. act both showed a high significant difference at $p<0.001$ while comparing the three groups together and a significant difference when comparing each patient group with AH group but no significant difference when comparing patients' group with each other.

Table 1: Mean ± SD of F.S.G, MDA, Cp concentration, Cp Ferroxidase activity, Cp Ferroxidase and its sp. act, in serum of the three studied groups.

Parameters	Groups			p-value
	AH	P_1	P_2	
F.S.G (mg/dL)	107.21±31.76	155.33±50.59	188.25±77.67	0.000
MDA (mg/L)	0.5±0.22	1.12±0.27	1.7±0.42	0.001
Cp conc (mg/dL)	15.1±5.22	16.6±4.88	19.38±3.64	0.03
Cp Ferroxidase activity (U/L)	986.81±278.1	1225.53±321.19	1376.19±233.6	0.000
Cp Ferroxidase sp. act (U/g)	136.9±40.59	165.48±45.78	184.09±31.7	0.000

DISCUSSION

This study concluded that the lack of glycemic control in T2DM (both P_1 group and P_2 group had elevated levels of F.S.G)) results in increased ROS levels with increased lipid peroxidation (meaning higher MDA levels) and decreased antioxidant capacity, resulting in MDA being a major risk factor that could be considered as a parameter with antioxidants to assess OS in T2DM patients[21]. The serum Cp levels in some studies showed an increase in patients with T2DM and found that glucose is associated with the increase in serum Cp[22]. elevated glycation accompanied with an altered lipid profile in T2DM causes enhanced generation of this enzyme which could be used as possible biomarker for identifying accelerated glycation and atherosclerosis[23]. Another study revealed a highly significant increase in CP concentration in sera of patients with T2DM[24]; There is metabolic and OS in uncontrolled diabetes[25], CP is thought to be a scavenger so its levels increase. But high levels of CP can cause vascular injury by generating FRs and oxidizing LDL making it more atherogenic. The ROS disrupt the binding of copper to CP, therefore impairing its normal protective function as liberated copper probably could promote oxidative pathology[26]. The mechanism where CP may affect the progression of CVD is still to be figured

out. Probably ROS (such as hydrogen peroxide or superoxide) may associate in the underlying mechanisms. Elevated ROS levels, antioxidant systems such as SOD, catalase and glutathione are overwhelmed and the CP structural integrity is damaged. Studies explaining the relation between CP and atherosclerosis had different suggestions, a study suggested that CP levels could promote CHD where CP could promote an inflammatory environment, in parallel with the activation of ROS cascade by indirectly or may be directly producing of the ox LDL. According to another mechanism, CP could possibly have an influence on the progression of atherosclerosis by affecting the NO pathway, which have an important role in a protective effect in the ischemic and failing heart in addition to its important role in normal cardiac physiology [27]. A study found that patients with T2DM had a decreased levels of Cp ferroxidase activity and its sp.act, the study claimed that changes in the activity of Cp ferroxidase is compensate by the increase of LDL levels. also, hyperglycemia increases the concentrations of Cp and a drop in its sp. act[28]. A study which was first to research the relationship of Cp activity with VHD found that the patients had significantly elevated Cp ferroxidase activity[29].

CONCLUSION

Cp ferroxidase and Cp concentrations had a significant increase, this increase is linked to the ongoing increase in hyperglycemia, inflammation, higher levels of oxLDL and oxidative stress. More studies need to be done to further explain this connection.

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