Study of Low Paraoxonase and Arylesterase Enzyme Activity in the Development of Metabolic Syndrome, Diabetes Mellitus and Coronary Artery Disease in Najaf Province, Iraq.

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Abstract
Paraoxonase-1 (PON1) enzyme in human and its associated esterase are high-density lipoprotein and is speculated in performing a great effect in different human diseases such as diabetes mellitus, atherosclerosis and lately stroke. A crucial antioxidant defense system against lipid oxidation has been recorded in relation with these enzymes. Decreased PON-1 activity in several conditions and diseases has been correlated with systemic oxidative stress related to decrease activity of these enzymes. The goal of this research was to ascertain measurement of the level of activity of PON and arylesterase (ARE) enzyme, also, the level of total antioxidant capacity (TAC) in metabolic syndrome (MES) was determined and reveal the likely relationship between them. The study was performed on 110 MES affected patients and 240 controls indicated as healthy. Outpatients selected randomly and samples had been recruited from the clinics of Al-Najaf center of Diabetes and Endocrinology using a case -control study as a design. A spectrophotometry technique was used to measure and determine PON and ARE activities. The ferric reducing ability of plasma assay was used to determine the TAC. We indicated that a significant lowering in serum PON activity in patients with MES (70.5±65.52 IU/l) than healthy subjects (70.5±65.52 and 92.52±78.35 IU/l) (P<0.05). It has been shown that the activity of serum ARE enzyme in MES and control were 43.52±22.62 and 63.85±30.2 kU/l respectively. Additionally, a significant lower of activity ARE enzyme was recorded in affected individuals with MES than control (P<0.0001). Whilst variations between MES and controls regarding TAC were not significant. in their diversity. Probability of lowering in the PON and ARE activities in MES may be theorized as an autonomous risk factor for many cardiovascular diseases, which reside to be clarified and left for further investigation

Keywords: Metabolic syndrome, Paraoxinase enzyme, Arylesterase enzyme

1. Introduction

Metabolic syndrome (METS) comprise a bunch of a complex metabolic abnormality evidenced by three or more out of the following traits, hypertension, atherogenic dyslipidemia, insulin resistance, and central obesity. Multiple genetic and acquired existence are involved in the pathogenesis of METS which include low -grade chronic inflammation and insulin resistance. Consequently, propagation the risk of developing diseases like cardiovascular diseases (CVDs), diabetes (DM) and stroke considerably associated with an MES [1].

A protective antioxidant system against lipid oxidation exerted by human paraoxonase-1 (PON-1) enzyme, which is also recognized to have arylesterase (ARE) activity, which can modulate the vulnerability of high-density lipoprotein (HDL) to atherogenic alteration such as glycation and homocysteinlation, and even exert an anti-inflammatory role. This enzyme is a glycoprotein of 43–45 kDa). In humans, gene location is in the long arm of chromosome 7 (q21–q22). PON-1 enzyme owns anti-inflammatory with calcium-dependent esterase, associated with HDL. Consequently it is used as antioxidant and predominant marker of lipid peroxidation maintaining the intracellular homeostasis as well as redox balance[2].

In many pathological conditions including DM, coronary artery disease CAD, hypercholesterolemia, polycystic ovary (PCO), and renal failure serum PON1 activity was found to be decreased [3]–[7]. In oxidized lipoproteins PON1 hydrolyzes lipid peroxides and considered as an antioxidant enzyme. Presumably, In Iraq information regarding activities of PON and ARE enzymes in MES is restricted. Finding out the levels of activities of PON and ARE in MES would be our aim for this research.

2. Patient Materials and Methods

The subjects were divided into two groups :1. 110 cases of clinically diagnosed metabolic syndrome referred for the outpatient's clinic of Al-Najaf center for Diabetes and Endocrinology in Al-Sadr Medical City 2. 240 age and sex-matched controls.

According to the national cholesterol education program (NCEP) ATP III, the MES was specified as the existence of three or more of five elements (Table 1 and 2)). Lipid profile was estimated by enzymatic methods and serum PON was estimated by spectrophotometric method.

In the presence of 1 M NaCl (salt-stimulated activity)

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using paraoxone (diethyl-P-nitrophenyl phosphate) as a substrate and in the scarcity of salt (essential activity) PON activity assays were carried out [8].

To locate the ARE activity phenylacetate was utilized as a substrate. The production rate of phenol was permanently monitored at 270 nm at 37 C0. As well as, to determine ARE activity we use Molar extinction coefficient of phenol (1310/M per cm) and evident as kU/l serum enzyme [8].

By measuring ferric reducing ability of plasma (FRAP) assay to reduce Fe+3to Fe+2, we determined total antioxidant capacity (TAC) in serum [9].

Using commercial software (SPSS for Windows, V17 IBM corporation, Armonk, New York, US) and Microsoft 2010 based Excel (Microsoft, US) statistical analysis was performed. Additionally, using an independent sample Student t-test and the Pearson correlation coefficient test. A P value of < 0.05 was considered statistically significant.

Assessment of anthropometric measurements

The speculation of anthropometric parts such as weight, height, BMI, waist circumference (WC), and hip circumference (HC) was determined. Briefly, to measure BMI a mathematical equation has been used by providing body weight in kilograms divided by height in square meter [kg/m2].

Additionally, everyone enrolled in this research the assessment of body weight in kilograms (kg) utilizing a measuring digital dimension was accomplished with a lightweight outfit. Also, with a wall-mounted height rod the body height in centimeters was measured. By using a plastic measuring tape WC and HC in centimeters were estimated. At the end by dividing WC by HC waist to hip ratio (WHR) was estimated.

Patient's authority and ethical view

Enrollment of all individual in this research has been subjected to the identity and information which had been kept secured and not published. Written informed consent was achieved in accordance with standard of ethics and regulations of ministry of health in Iraq. All procedures were performed involving human participants.

3. Results

This research plan considered 110 MES (60 females and 50 males; age 46.05±11.03) and 240 healthy individuals (115 males and 125 females; age 32.53±12.11). The levels of activity PON1 enzyme in affected MES and control healthy individual (Table2,1).

Regarding the levels of activity of PON1 in MES and control healthy participants as it shown in (Table 3) were 70.5±65.52 and 92.52±78.35 IU/l respectively. There was a significant lowering in the level of enzyme activity of PON in MES when compared with than normal subjects (P=0.01). Moreover, PON activity with salt-stimulated technique significantly lowered in MES (132.25±112.5 IU/l) than normal subjects (191.26±160.7 IU/l) (P=0.01).

Assessment of activity of ARE enzyme in serum of both MES and control indicated that 43.52±22.62 and 63.85±30.2 kU/l respectively (Table 3).

There was no statistically significant difference in the PON1 enzyme activity regarding MES in male group and the results were (62.47±57.32 U/l) while in control (89.01±81.13 U/l) (P=0.105). Additionally, our results indicated also that there is significant difference in activity of ARE enzyme in MES (62.65±32.05 kU/l) and normal subjects (42.92±15.54 kU/l) and (P<0.001).

Significant lowering in PON activity was recorded in the affected MES group (71.05±61.35 U/l) than in healthy female control group (90.81±71.52 U/l) (P=0.039).

Regarding ARE activity a significant statistical lowering of (P<0.001) in female cases (45.42±23.71kU/l) than in control normal individuals (61.52±24.2 kU/l) was indicated. While no significant statistical correlated value was obtained between PON or ARE activities and age (P>0.05).

Interestingly, for TAC there were no significant differences recorded among affected MES individuals and control healthy ones. A significant statistical difference was documented and recorded among affected MES individual and normal control in relation to the activity of ARE enzyme (P<0.0001). There was a significant positive relationship between activity of PON and ARE enzymes (r=0.368, P<0.0001; r=0.594, P<0.0001) in affected MES and normal control individuals. However, a significant positive relationship was conducted between activity of PON enzyme and HDL-C in normal control individuals (r=0.168, P=0.01), while the relationship between affected MES group and HDL-C was not correlated in significant way (r=0.122, P=0.21). Moreover, regarding activity of PON enzyme and the level of cholesterol, LDL-C, TG, TAC, and body mass index (BMI) (P>0.05) there were no relationship had been recorded between affected MES and normal control individuals.

4. Discussion

To restrain cellular components from damage to a sequela of reaction that occur chemically which involve free radicals' formation that emerging by the action of antioxidants maintaining physiological healthy situation. PON1 is one of the specific antioxidative enzymes which owns both PON and ARE activities.

In this research, a significant lowering in the activities of PON and ARE enzymes were recorded in affected MES individuals when matched with normal control individuals. A habitual free radicals' formation is a consequence for an assortment of substantial biochemical reactions and under pathophysiological situations can be initiated at elevated rates [10], [11]. [12](12) Senti et al.[13] had been established that in normal subjects the activity of serum PON1 enzyme was elevated significantly when compared with affected MES individuals. Our result coincides with

these results. Tabur et al.[14] had found no significant difference among non-diabetic affected MES, non - affected MES obese patients, and healthy control individuals in relation to the levels of PON and ARE activities , while in both obese groups and the affected MES individuals group when comparing with controls group, total antioxidant status was very low.

A miscellaneous PON1 gene polymorphisms assortment have been established. The relationship between systemic PON1 activity and a functional polymorphism (Q192R) leading to increase PON1 activity with predominant CAD and future major dangerous cardiac events (myocardial infarction, stroke, or death [15][16]. Variants of the gene PON1 enzyme [L55M and Q192R] create a great modification of both level of concentration and activity of the enzyme and initiate shift and has been well authenticated and they are the two common coding region [17][18]

Furthermore, PON1 expression and serum concentration has been affected by the promoter variant of PON1 enzyme, especially [K107T / C].[19]

Over and above, the PON1 activity affected by many gained causes e.g., diseases, lifestyle, and diet.

It has been proposed that activity of serum PON1 increases by consuming drinks containing flavonoids or red wine [20] and consuming moderate alcohol [21]

No clearance of the precise ways of working affecting activities of low PON1 and ARE in affected MES individuals has been established until now.

It is well noted that in the general population, increased morbidity and mortality of cardiovascular diseases [22]and wide spread of type 2 DM can be correlated with MES which is associated with PON1 enzyme, an HDL-associated enzyme, is brilliant and a potent factor of restrain LDL oxidation [23].

Depending on the previous studies [13] diminished the level of activity of PON enzyme and ARE in affected MES supposed in these patients to be an independent risk factor for cardiovascular disease. Whereby, genetic factors can be effective on decreased or increased level of this enzyme both in MES and control groups and this was one of limitations of our study and more research for other populations are important to confirm this clearly.

5. Conclusion

In conclusion, PON may play a role in pathogenesis of MES which is the predominant cause of DM and CAD and we demonstrated that, a significant lowering in the activities of PON and ARE had been recorded in affected MES when had been matched with normal control individuals.

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Table 1. Anthropometric and biochemical factors in metabolic syndrome (MES) and control

Parameter	MES	Control	P value
FBG (mg/dl)	120.3±54.5	82.8±15.6	<0.0001
TG (md/dl)	240.6±180.9	112.5±63.9	<0.0001
Total cholesterol (mg/dl)	215.2±45.7	165.8±45.7	<0.0001
HDL (mg/dl)	39.5±9.1	45.3±6.9	<0.0001
LDL (mg/dl)	128.7±42.6	101.5±35.5	<0.0001
Height (cm)	159.91±8.9	162.98±11.21	0.02
Weight (Kg)	130.5±22.3	60.5± 14.3	<0.0001
BMI	40.5±5.5	23.2±4.2	<0.0001
Waist Circumference(cm)	110.3±12.5	75.4±12.5	<0.0001
SBP (mm Hg)	130± 25	110±13	<0.0001
DBP (mm Hg)	80.5± 15	70±11	<0.0001

Table 2. The national cholesterol education program (NCEP) ATP III for diagnosis of the metabolic syndrome.

Standard Criteria	[NCEP ATP III]
Waist [cm] Female Male	 ≥ 88 ≥ 102
HDL [mg/dl] Female Male	 < 50 < 40
TG	≥ 150
FBS	≥ 100
BP	≥ 130/85

Table 3. Activities related to paraoxonase, stimulated PON enzyme by salt, arylesterase enzymes, and measuring of total antioxidant capacity (TAC) in affected metabolic syndrome [MES] individuals and control.

Parameter	MES	Control	P Value
PON activity (IU/l)	70.5 ± 61.52	92.52 ± 78.35	0.01
Salt Stimulated PON (IU/l)	132.25±112.5	191.26± 160.7	0.02
ARE (kU/l)	43.52±22.62	63.85± 30.2	<0.0001
TAC (µmol/l)	985±365.23	970±375	0.9

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