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ROLE OF PARAOXONASE ENZYME STATUS IN CORONARY HEART DISEASE IN THE AL- QUWAYIYAH REGION OF SAUDI ARABIA

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ABSTRACT

Background: Coronary Heart Disease (CHD) is a principal cause of mortality and disability globally. Dyslipidemia and lipid peroxidation are considered an independent risk factor for CHD. Serum Paraoxonase (PON) enzyme is almost exclusively located on the high density lipoprotein (HDL). It will protect against LDL oxidative modification in atherosclerotic lesions and atherogenic indexes in CHD patients and healthy peoples. **Methods:** An open randomized study was case controlled in design. The present study included 60 patients suffering from CHD and 65 age and sex matched healthy controls were included during the period of December 2016 to October 2017. Various parameters like lipid profile, lipid peroxidation marker i.e. malondialdehyde (MDA) and antioxidant enzyme PON levels were measured and compared. **Results:** Increased levels of MDA concentration, total cholesterol, triglycerides, LDL-cholesterol, while decreased levels of HDL- C and PON were significantly low ($p<0.001$) in CHD patients compared to normal healthy controls. **Conclusion:** The study showed strong evidence of CHD associated with low activity of PON and HDL-C and significantly a high level of lipids and lipid peroxides concentrations, atherogenic index, atherogenic coefficient and cardiac risk ratios may be involved in the early pathogenesis of CHD. The present study illustrated that the PON is an important enzyme in oxidant - antioxidant status of atherosclerosis with its antioxidant effect.

KEYWORDS: Coronary heart disease, Lipid profile, Malondialdehyde, Paraoxonase.

INTRODUCTION

Coronary Heart Disease (CHD) is a complex trait caused by a number of genetic and environmental factors. The basis for most cardiovascular diseases is atherosclerosis. Atherosclerosis is the end product of a series of complex cascade of interactions among the cellular and non-cellular components of the arterial wall, blood constituents, mononuclear phagocytes and platelets, focal hemodynamic stresses and environmental and genetic factors.[1]

The prevalence of CHD worldwide is rapidly rising. CHD remains the leading cause of mortality worldwide. Mortality rates associated with CHD have shown an exceptional increase particularly in fast developing economies like the Kingdom of Saudi Arabia (KSA). Over the past twenty years, CHD has become the leading cause of death in KSA and has reached epidemic proportions. The increase of coincided with fast economic growth and urbanization that promotes sedentary life style, smoking, and a diet high in energy-dense fast food and low in fruits and vegetables. These factors have undoubtedly contributed to the epidemic of CHD in KSA.[2]

Genetic, clinical, epidemiological and pharmacological studies implicated that the development of atherosclerosis is closely associated with risk factors such as hypertension, obesity; smoking, dyslipidemia and diabetes have been identified. The high elevated blood levels of low density lipoprotein (LDL), total cholesterol (TC), triglycerides (TG); however a low level of high density lipoprotein (HDL) is a risk factor for mortality from CHD.[3]

Several researchers have indicated that the development of CHD is related to free radical processes. Lipid peroxidation is a free radical related process, which is potentially harmful because its uncontrolled, self enhancing process causes disruption of membranes, lipids, proteins and other cell components. A lot of oxygenated compounds, particularly aldehydes such as Malondialdehyde (MDA) are produced during the attack of free radicals to membranes, lipoprotein and polyunsaturated fatty acids.[4] Thus monitoring of lipid profiles and lipid peroxidation in the blood provides useful information for the prognosis of CHD patients.

Human serum Paraoxonase enzyme (PON) is synthesized in the liver and it is an HDL- C bound enzyme considered to be the major determinant of the antioxidant action of HDL-C. PON has detoxification activity in atherosclerotic processes. Several studies reported that PON and HDL protect against LDL oxidative modifications. PON gene family in humans has three members; PON1, PON2, and PON3. PON1 is the best studied of the family. Human PON1 gene is located on the long arm of chromosome 7. Human serum PON1 shows substrate activity polymorphism.[5] Some studies have shown that serum PON activity is decreased in diseases that are associated with accelerated atherogenesis. Many studies have proposed that PON-192 polymorphism might be a risk factor for atherosclerosis. Some studies have failed to find such a relationship.[6] Hence a present study was planned and conducted to evaluate the role of Lipid profile, Lipid peroxidation which was measured by MDA and serum PON enzyme activity were measured in CHD patients compared with normal healthy subjects.

MATERIALS AND METHODS

Study design and area

An open randomized study was case controlled in design. The descriptive study was taken during the period of December 2016 to October 2017. The study patients were all admitted in the Intensive Coronary care Unit (ICCU) / Intensive Care Unit (ICU) or attending the Outpatient department (OPD) of medicine of the Al-Quwayiyah General Hospital, Kingdom of Saudi Arabia.

Study subjects

The study group consisted of 60 patients with CHD and they are admitted to hospital and they are between 40 – 65 years of both the sexes (32 males and 28 females). The criteria for the diagnosis of a CHD was made on the basis of clinical history, chest pain, history of myocardial infarction, 12 leads electrocardiogram (ECG) and coronary angiography findings. Those patients whose body mass index (BMI) was >30 kg/m^2 were considered as obese. Smoking was defined as regular smoking of cigarettes / Beedies (local type of tobacco). Sixty five healthy volunteers, both age and gender matched who were non CHD subjects were considered as controls. Subjects suffering from renal disease, hepatic disease, strokes, any chronic or acute inflammatory illness, pregnancy and lactating mothers, cerebrovascular accidents, alcoholics, rheumatoid arthritis, autoimmune disease, patients of juvenile, type 1 and type 2 diabetes mellitus were excluded from the study. None of the subjects were on antioxidant supplementation or lipid lowering drugs. All participants gave written informed consent and this protocol was approved by the ethical and human research committee.

Collection of blood sample and biochemical analysis

12 hours overnight fast, 6ml of blood was collected for each subjects. The blood samples were centrifuged at 3000 RPM for 20 minutes, the serum was carefully separated and transferred to micro tubes and stored at + 4^0 C before analysis. The lipid profile was done by fully autoanalyzer Cobas Integra 800 from ROCHE diagnostics, Germany. The concentration of serum Cholesterol was estimated by CHOD- PAP method[7], Triglycerides level was estimated by GPO (trinder) method[8], while HDL-C estimation was done by Phosphotungestic method[9] and LDL-C levels were estimated by enzymatic methods.[10] Serum levels of MDA, a marker of lipid peroxidation were measured by thiobarbituric acid (TBA) method.[11] Serum PON activity was estimated by using 5.5 mmol/l p-nitro phenol acetate (sigma chemicals Co.,) as a substrate, the increase in the absorbance of p- nitrophenol formed at 412 nm was measured by using ELICO spectrometer. The activity of PON was measured in 20mM/L tris buffer at pH 8.0 and which contains 1mM calcium chloride. The generated product of p- nitrophenol was calculated by using molar extinction coefficient of 1700 per moles per cm t pH 8.0 results are expressed as U/l. (1U, 1nmol p- nitrophenol formed per minute).[12]

Statistical Analysis

The statistical analysis was undertaken using SPSS version 17.0 software. All values are expressed as mean ± SD. Student's t - test was used to estimate the significant difference between the groups. Pearson's correlation analysis was used to test the correlation between various biochemical parameters. The level of significance was considered when p value < 0.05.

RESULTS

The clinical characteristics of CHD patients and normal subjects are presented in Table 1. In the present study the number of obesity, hypertensive and smokers were significantly high in the CHD patients compared to controls.

Serum total cholesterol, triglycerides, LDL- C and VLDL levels were significantly increased in CHD patients compared to controls ($p< 0.001$), whereas decreased levels of HDL-C ($p<0.001$) in CHD patients when compared to controls as shown in table 2.

Significantly increased levels of MDA and however decreased activities of PON was observed ($p<0.001$) in CHD patients compared to controls respectively as shown in table 3.

Table 1: The demographic data of the study subjects.

Particulars	Controls (n= 65) Mean ±SD	CHD (n= 60) Mean ±SD
Age (yrs)	52.4 ± 10.6	53.2 ± 11.4 *
Sex (male / female)	35 / 30	32 / 28 *
BMI (kg/m^2)	23.2 ± 2.6	26.5 ± 4.7 *
HTN %	7 %	51 % *
Smokers	-----	40 % *
Family history of CVD	------	22 % *

The values are mean ± Standard deviation (SD), * *P*<0.001, highly significantly compared to controls. BMI= Body mass Index, HTN= Hypertension, CVD= cardiovascular disease.

Table 2: Various Biochemical parameters of the patients and controls.

Parameters	Controls (n= 65) Mean ±SD	CHD (n= 60) Mean ±SD
Total Cholesterol (mg/dl)	156.2 ± 11.3	248.5 ± 8.9 *
Triglycerides (mg/ dl)	88.1 ± 6.2	189.3 ± 7.7 *
HDL-C (mg/ dl)	53.2 ± 4.0	33.4 ± 2.9 *
LDL-C (mg/ dl)	99.2 ± 5.5	201.7 ± 10.8*
VLDL (mg/ dl)	28.2 ± 5.2	41.3 ± 9.2*

The values are mean ± Standard deviation (SD), * *P*<0.001, highly significantly compared to controls. HDL- c=High density lipoprotein, LDL-c = Low density lipoprotein, VLDL= Very low density lipoprotein.

Table 3. MDA and PON activities in the CHD patients and healthy controls

Particulars	Controls (n= 65) Mean ±SD	CHD (n= 60) Mean ±SD
MDA (nmoles/ml)	3.75 ± 1.0	7.11 ± 1.8*
PON (U / L)	142.5 ± 10.5	112.3 ± 8.3*

The values are mean ± Standard deviation (SD), * *P*<0.001, highly significantly compared to controls. MDA= Malondialdehyde, PON= Paraoxonase.

DISCUSSION

According to the INTERHEART and INTERSTROKE studies, hypertension, diabetes, dyslipidemia, obesity, smoking, physical activity, poor diet, and alcohol consumption are the most common risk factors for CHD or heart attack and strokes worldwide.[13] Numbers of research have shown that classical and extrinsic factors such as smoking, high cholesterol levels and high blood pressure have a significant role in the pathogenesis of CHD.[14] Tobacco Smoking is not only dangerous but also a strong risk factor for the development of CHD patients.[15] Our data showed that prevalence of smoking was significantly higher in CHD patients as compared to controls. In the present study we observed, hypertension and obesity was found to be high in CHD patients compare to controls according to the American Heart Association's 2013 recommended that screenings should include assessment of all CHD risk factors including lifestyle habits. Nowadays, overweight and obesity are recognized as a rising pandemic.[16]

Hypercholesterolemia and triglyceridemia are independent risk factors that alone or together can accelerate the development of CHD and progression of atherosclerotic lesions. Many studies have shown that total cholesterol and LDL are major risk factors for atherosclerotic vascular diseases.[17] Also, epidemiological studies have shown a correlation between LDL and CHD. The strong relationship between low levels of HDL and the risk for CHD has been attributed to several distinct mechanisms.[18] Subsequent studies showed that HDL accepts cholesterol from macrophage foam cells, the cellular hallmark of the atherosclerotic lesion. Therefore, HDL might be cardio protective because it prevents cholesterol accumulation in cells of the artery wall. A greater increase of LDL may also cause a greater decrease of HDL as there is reciprocal relationship between the concentration of LDL and HDL.[19] Many studies have shown that atherogenic indices, atherogenic coefficient, and cardiac risk factors are major risk factors for atherosclerotic vascular disease and its complications.[20] The results of our study agree with other researcher's studies showing that increased levels of cholesterol, triglycerides, LDL and decreased HDL in CHD patients than in healthy peoples.

Malondialdehyde (MDA) is a natural product of lipid peroxidation (LPO) and reflects the oxidant status of the

biological systems. LPO can also generate advanced products of oxidation, such as alkanes, aldehydes and isoprostanes. It is a free radical mediated chain reaction ant it is self perpetuating and it plays an important role in ageing, diabetes and atherosclerosis. So many studies revealed that increase of MDA in CHD patients.[21, 22] In our study also, significant increase of MDA was found in CHD patients compare to controls. The estimation of MDA along with lipid profile in the CHD patients is very useful as it may serve as a useful monitor to judge the prognosis of the patient.

Several studies reported that PON and HDL protect against LDL oxidative modification. The results of the Caerphilly study, the first prospective epidemiological study of PON 1 and CHD, also showed that PON 1 activity predicted coronary events independent of all other coronary risk factors, including HDL.[23] Mostafa et al[20] and Rakhi et al[1] observed that PON activity might be developed as a biomarker of HDL function and cardiovascular risk independent of HDL concentrations. In Natalia Ferre et al[24] study, suggest that the differential substrate activity of PON 1 is more critical than the enzyme concentration for its protective effect against atherosclerosis. Smoking reduces serum PON activity whereas alcohol shows no association. So many authors revealed that low serum PON activity has been reported with disease like diabetes mellitus, hypercholesterolemia, myocardial infarction and renal failure also.[20] The association between PON activity and the development of atherosclerosis has been shown in animal and human studies. Also, in epidemiological studies have revealed an association between decreased PON 1 levels and an increased risk for atherosclerosis.[25] Our results agreed with those of earlier studies showing decreased PON activity in CHD patients compared to healthy controls. This report strongly suggests that decreased PON activity may be caused by oxidative stress. It also acts as antioxidant property, because it prevents the increase of ROS quantity by hydrolysing lipid peroxidation products. It also shows protective effect in cell membranes by neutralizing the atherogenic effects of lipid peroxides.

There were some limitations in the present study, our sample size was small and since it was a hospital based study, so we can't represent over the entire population. Additional large-scale of clinical trial and epidemiologic studies are needed to further determine the relationship between PON2 and PON3 polymorphisms and CHD risk.

CONCLUSION

This will be the first report to show the knowledge of Coronary heart disease among the Saudi population in Al-Quwayiyah region of Saudi Arabia. However, they are not very well aware of the diabetes, kidney disease and cardiovascular disease. This study showed strong evidence of CHD associated with low activity of PON and HDL-C and significantly high levels of TC, TG, LDL-C and lipid peroxides concentrations, atherogenic index, atherogenic coefficient and cardiac risk ratios may be involved in the early pathogenesis of CHD. The present study illustrated that the PON is an important enzyme in oxidant - antioxidant status of atherosclerosis with its antioxidant effect. Therefore, assessing of PON and MDA in CHD patients as routine analyses in clinical chemistry laboratories.

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