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Evaluation of lipid peroxidation and antioxidant enzyme status in ischemic heart disease patients

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ABSTRACT

Background: Oxidative stress is an independent risk factor by induction producing of oxygen free radicals in cardiovascular diseases. Although, oxygen free radicals are known to contribute to the development of Ischaemic Heart Disease (IHD). Disturbed lipid profile and increased level of lipid peroxidation as well as decreased in total antioxidant capacity is one of the most important and potent risk factors in IHD.

Aim: In the present study, our aim was to investigate the lipid peroxidation, lipid profile and Antioxidant enzymes in IHD patients in Al- Quwayiyah region of Kingdom of Saudi Arabia.

Materials and methods: A total of 52 patients suffering from IHD and 63 healthy volunteers (control group) were randomly selected for the study. Various biochemical parameters like lipid profile, the levels of thiobarbituric acid reactive substances (TBARS), which is a predictor of lipid peroxidation and antioxidant enzyme activities like Glutathione peroxidase (GPx), Superoxide dismutase (SOD), Catalase and Paraoxonase (PON) was measured.

Results: Increased levels of cholesterol, triglycerides, LDL- C and TBARS, whereas decreased levels of HDL- C, GPx, SOD, Catalase and PON in IHD patients when compared to controls ($P<0.001$).

Conclusion: The hypothesis of the present study indicates that an imbalance between oxidant and antioxidant molecules in IHD patients. Therefore, assessing of these biomarkers of oxidative stress may be useful in diagnosis of patients with IHD.

Key Words: Ischemic heart Disease, Lipid Profile, Lipid peroxidation, Antioxidant enzymes.

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1. INTRODUCTION

Since we are not designed and destined to live forever, the "Aging" process is a natural phenomenon not only in human but in all organisms. During its prospective journey, the genetic ribbon energetically moves onward with crescendo till youth and then deterioratingly slows down progressively. Aging is contemplated to be due to genetic dictation, entwined with turbulent environmental interactions which may lead to age related disease such as ischemic heart disease (IHD), cancer, diabetes mellitus, neurological disorders and many others and set faster under inclement settings (Maharjan et al., 2008; Gil et al., 2006).

Heart disease is one of the major health problems of developed as well as developing countries of the world. IHD is now the leading cause of death worldwide, responsible for more than 7 million deaths annually worldwide. It is also the major cause of death in the Kingdom of Saudi Arabia (KSA), according to data published by the Saudi Ministry of Health. In KSA, cardiovascular diseases (CVD) accounted for approximately 20% in 2005, 22% in 2006, and 19% in 2007of the total death (Ahmed Osman et al., 2011).

IHD is a progressive disease arising when the supply of oxygen in the myocardium is compromised by impeded blood flow in the coronary vasculature caused by luminal occlusion. IHD leads to the development of atherosclerosis and hypertrophy of musculature. The pathogenesis of atherosclerosis involves damage to the capillary endothelium caused by various factors including oxidized low density lipoprotein (LDL) (Kumar et al., 2012). Recent experimental findings suggest that overproduction of reactive oxygen and nitrogen species have been implicated in various cardiovascular disorders including ischemia / reperfusion (I/R), atherosclerosis, hypertension, cardiotoxicity induced by drugs etc (Debasish et al., 2013).

Oxidative stress is defined as the interruption of balance between oxidants and reductants within the body due to the excess production of peroxides and free radicals. The development of IHD is closely associated with risk factors such as hypertension (HTN), obesity, smoking, and dyslipidemia (Mahbood and Rahman, 2005). Lipid peroxidation is a free radical related process, which is potentially harmful because its uncontrolled, self-enhancing process causes disruption of membranes, lipids and other cell components. Thus lipid peroxidation in the blood provides useful information for the prognosis of IHD patients. This imbalance will cause damage to cellular components and tissues in the body leading to oxidative stress and as well as the decrease in total antioxidant capacity (Pavlova et al., 2007).

Sufficient levels of antioxidants are important to live with healthy condition for human beings and most important for the prevention of chronic diseases such as cancer, diabetes and heart diseases (Van der Gaag et al., 2000). Enzymatic Glutathione peroxidase (GPx), Superoxide Dismutase (SOD), Catalase and Paraoxonase (PON) play an important role in alleviating tissue damage due to formation of free radicals. Hence a present study was planned and conducted to evaluate the role of Lipid peroxidation which was measured by thiobarbituric acid reactive substances (TBARS) and stress related enzymes such as GPx, SOD, Catalase and PON were measured in IHD patients compared with normal healthy subjects.

2. MATERIALS AND METHODS

2.1. Study design and study area

An open randomized study was case controlled in design. We have selected the patients as they are presented. The study cohort patients were all admitted in the Intensive Cardiac Care Unit (ICCU) / Intensive Care Unit (ICU) and some were admitted in the medicine unit or attending the Outpatient department (OPD) of medicine of the Al- Quwayiyah Government General Hospital, Shaqra University, Kingdom of Saudi Arabia. The study was carried from February 2013 to January 2014.

2.2. Selection of Patients

The study group consisted of 52 patients with Ischemic Heart Disease (IHD) of both the sexes (34 males and 18 females) they were 40—70 years all they are admitted in hospital. The criteria for the diagnosis of IHD was made on the basis of clinical history, history of myocardial infarction, 12 leads electrocardiogram (ECG), Echocardiography and Coronary angiography findings. Detailed present and past history of patients was collected with the help of pre-tested proforma. The proforma included primary patient information (such as name, age and sex), socioeconomic status, food habituation, smoking, family history of disease, past medical and surgical history. In addition to this we also measured the body mass index (BMI) whose >25 we considered as an obese. 63 healthy volunteers age and gender matched, non MI, and non diabetes were included as a control. All IHD patients selected for these studies were or irregular treatment. Subjects suffering from diabetes, acute renal failure, hepatic disease, strokes, any chronic or acute inflammatory illness, pregnancy and lactating mothers were excluded from the study. All participants gave written informed consent and this protocol was approved by the ethical and human research committee.

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Table 1
The demographic features of Controls and IHD patients

Particulars	Controls (n=63) Mean ±SD	IHD (n=52) Mean ±SD
Age (yrs)	49.6 ± 6.8	56.8 ± 10.2 *
Sex (male / female)	40 / 23	34 / 18 *
BMI (kg/m^2)	22.1 ± 6.9	27.9 ± 5.2 *
HTN (%)	9 %	49 % *
Smokers (%)	11 %	55 % *
Family history of Cardio vascular Disease	5 %	39 % *

* P<0.001, Highly significantly compared to controls. IHD= Ischemic heart disease,
BMI= Body mass Index, HTN= Hypertension

Table 2
Various Biochemical parameters of the study subjects

Parameters	Controls (n=63) Mean ± SD	IHD (n=52) Mean ± SD
Total Cholesterol (mg/dl)	151.0 ± 18.1	232.2 ± 22.1*
Triglycerides (mg/dl)	91.2 ± 10.9	193.8 ± 19.5*
HDL-C (mg/dl)	53.2 ± 4.8	33.1 ± 5.9*
LDL-C (mg/dl)	75.6 ± 15.5	181.3 ± 20.1*
TBARS (nmoles/ml)	3.1 ± 0.4	8.3 ± 2.1*

*P<0.001, Highly statistically significantly vs controls. IHD= Ischemic heart disease,
HDL-C= High density lipoprotein cholesterol, LDL-C= Low density lipoprotein cholesterol, TBARS= thiobarbituric acid reactive substances.

2.3. Blood sample collection and Laboratory 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 lipid profile was done by fully autoanalyzer Cobas Integra 400 from ROCHE diagnostics, Germany. The concentration of serum Cholesterol was estimated by CHOD- PAP method (Richmond, 1973), Triglycerides level was estimated by GPO (trinder) method (McGowan et al., 1983), while HDL-C estimation was done by Phosphotungestic method (Rifal and Warnick, 1994) and LDL-C levels were estimated by enzymatic methods (Pissani et al., 1995). Lipid peroxidation in the serum was determined as thiobarbituric acid reactive substances (TBARS) method (Buege and Aust, 1978). The haemolysate prepared from the red cells was used for the estimation of antioxidant enzyme activities. GPx was measured by the method of Paglia and Valentine (Paglia and Valentine, 1967). SOD estimation was based on the reaction between superoxide radicals and *2-4-iodophenyl 3-4- nitrophenol- 5-phenyl tetrazolium chloride* to form a red formazon dye (Arthur and Boyne, 1985). Catalase was assayed by the method of Beers and Seizer (Beers and Seizer, 1952). PON activity was estimated by using *5.5 Mm p-nitro phenyl acetate* (sigma chemicals Co.,) as a substrate. The change in the absorbance at 412 nm due to the formation of *p- nitro phenol* was measured by using ELICO spectrometer (Mackness et al., 1991).

3. STATISTICAL EVALUATION

All values are expressed as mean ±SD. Student t- test was used to estimate the significant difference between the groups. Pearson's correlation analysis was used to test the correlation between various parameters and considered significant when p<0.05. SPSS for windows 13.0 was used for statistical analysis.

4. RESULTS

The average age of the normal subjects and IHD patients were enrolled in the study. In the present study number of BMI, smokers, and hypertensive were more in the IHD patients when compared to controls as shown in Table 1. Serum cholesterol, triglycerides, LDL- C were significantly increased in IHD patients compared to controls (p< 0.001), whereas decreased levels of HDL-C (p<0.001) in IHD patients when compared to controls. Significantly increased levels of TBARS was observed in IHD patients than normal subjects (p<0.001) (Table 2). In table 3, antioxidant enzyme activities of GPx, SOD, Catalase and PON were significantly decreased (p<0.001) in IHD patients as compared to controls respectively.

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Table 3
Antioxidant enzyme activities in patients and controls

Parameters	Controls (n=63) Mean ± SD	IHD (n=52) Mean ± SD
GPx (U / gHb)	31.2 ± 5.1	20.1 ± 2.8 *
SOD (U / gHb)	7.5 ± 1.5	5.5 ± 0.9 *
Catalase (U / mg protein)	17.1 ± 1.9	10.2 ± 0.7 *
Serum PON (U / ml)	168.1 ± 11.1	120.3 ± 5.5 *

*P<0.001, Highly statistically significantly compared to controls. IHD= Ischemic heart disease,
GPx= Glutathione peroxidase, SOD= Superoxide dismutase, PON= Paraoxonase.

Table 4
Correlation Analysis of TBARS levels vs Antioxidant enzymes in IHD patients

	IHD patients 'r'
TBARS / GPx	- 0.49*
TBARS / SOD	- 0.61*
TBARS / Catalase	- 0.39*
TBARS / PON	- 0.72*

*significant at p<0.05, r = correlation coefficient, IHD = Ischemic heart disease,
TBARS= thiobarbituric acid reactive substances, GPx=Glutathione peroxidase,
SOD= Superoxide dismutase, PON= Paraoxonase.

In order to ascertain any relationship between TBARS levels (Stress factor) with various antioxidant enzymes, linear correlation analysis was carried out. Results revealed that GPx, SOD, Catalase and PON activities were negatively correlated with TBARS level in IHD patients as shown in Table 4. The increased levels of TBARS and decreased levels of antioxidant enzyme activities may be result of the oxidative stress of the disease.

5. DISCUSSION

The IHD remains the first cause of death worldwide including KSA. IHD is a process for which there is substantial evidence of a role for oxidative stress. The prevalence of IHD and relatively mortality risk associated with several risk factors. Tobacco Smoking is not only dangerous but also a strong risk factor for the development of IHD patients (Al-Nozha et al., 2004). Our data showed that prevalence of smoking was significantly higher in IHD patients as compared to controls. The lower incidence of IHD as seen in females probably due to the protective effect of estrogens. Like other studies (Chen et al., 1995; Zimmerman et al., 1995) in our study also, hypertension and obesity was found to be significantly high in IHD patients compared to controls.

Hypercholesterolemia and triglyceridemia are independent risk factors that alone or together can accelerate the development of IHD and progression of atherosclerotic lesions. HDL is regarded as one of the most important protective factors against arteriosclerosis. HDL's protective function has been attributed to its active participation in the reverse transport of cholesterol. Numerous cohort studies and clinical trials have confirmed the association between a low HDL and an increased risk of IHD (Kaviarasan et al., 2005). The concentration of LDL correlates positively whereas HDL correlates inversely to the development of coronary heart disease. Smokers have significantly higher serum cholesterol, triglyceride, and LDL levels, but HDL is lower in smokers than in non-smokers (Kharb and Singh, 2000). Evidence suggests that oxidatively modified LDL contribute to the pathogenesis of heart disease. Increased oxidative stress and the generation of the free oxygen radicals can result in modification of LDL to oxidized LDL that could lead to cardiac diseases (Tomas et al., 2004). Recent studies demonstrated that disturbed lipid profile is one of the most important and potent risk factors in IHD. According to these researchers, an elevated levels of undesirable pathways including the formation of oxidized LDL (O-LDL) and oxidized cholesterol which encourages cholesterol accumulation in arterial tissues (Varbo et al., 2013). In the present study also we observed increased levels of cholesterol, triglycerides, LDL and decreased levels of HDL in IHD patients.

Lipid peroxidation (LPO) level is an index of oxidative stress. LPO is a free radical mediated chain reaction and it is self perpetuating. Tissue damage is considered proportional to lipid peroxide contents and thus cell membrane damage is tested by measuring lipid peroxide content by various ways and it plays an important role in ageing, diabetes and atherosclerosis (Jung et al., 2004). Here, we measured the TBARS levels as a product of LPO. Several

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authors have reported increased levels of TBARS in IHD patients (Mooradian, 1991; Griesmacher et al., 1995). In our study also, the significant increased levels of TBARS in IHD patients as compared to controls, because due to a greater degree of oxidative stress and poor enzymatic and non- enzymatic antioxidant defense system. The estimation of LPO along with lipid profile in the IHD patients is very useful as it may serve as a useful monitor to judge the prognosis of the patient.

The antioxidant enzymes such as GPx, SOD, Catalase and PON play a very important role in the protection against LPO. Reduced glutathione (GSH) is an important constituent of the cellular defense mechanism of the body against various exogenous as well as endogenously produced xenobiotics. It plays the role of a sulfhydryl (SH) group provider for direct scavenging reactions. GPx protects the cell damage by catalyzing the reduction of lipid hydroperoxides and also protects the heart from damage by oxidative stress due to oxygen free radicals through its antioxidant effect (Shazia et al., 2012). A few authors reported decreased activity of GPx in IHD patients (Al Ghonaim Mohammed et al., 2012; Kumar et al., 2012). Similarly, in our study also we observed decreased activity of GPx in IHD patients compared to controls. This again points out that the cardiac tissue has suffered from ischemic damage due to oxidative imbalance thereby decreasing this biomarker. However hypercholesterolemia is also one of the reasons for the decreased GPx activity in Cardiac patients. Another one of the important antioxidant enzyme in human body is SOD. Some studies reported that increased activity of SOD in IHD patients (Ghosh et al., 2013). On the other hand P D Sarkar et al., (2007) says decreased activity of SOD in IHD patients and Kesavulu et al., (2001) revealed that there is no change in SOD in IHD patients. Whereas in the present study we observed decreased levels of SOD in IHD patients because of superoxide is the main reactive oxygen species which react with nitric oxide radical and forms peroxynitrite thereby causing oxidative stress, cellular damage and also increased levels of lipid profile, LPO and conjugated dienes (Filiz et al., 2005). This would seem unexpected because in a disease with elevated oxidative compounds, a compensatory increase in antioxidant enzymes would be desirable. Catalase is the most efficient enzyme found in our cells. In our study, IHD patients showed a significant decrease in Catalase activity. Debasish et al., (2013) also observed same findings in IHD patients because a possible accumulation of hydrogen peroxide which has the potential to bring about oxidative damage of the bio- macromolecules and the consequential tissue damage.

Serum PON was synthesized in the liver is a HDL associated enzyme that prevents oxidative modification of LDL. Serum PON is responsible for the antioxidant activity of HDL. Mackness et al., (1991) and Leviev et al., (2001) reported that the PON activity was significantly lowered in patients with diabetes and IHD. Low serum PON activity has been associated with increased susceptibility to atherosclerosis, neuropathy, retinopathy and other complications. Some studies revealed that decreased levels of PON in CAD patients (Al- Ghonaim Mohammed et al., 2012). In the present study, we observed decreased PON activity in IHD patients compared to controls. We also observed correlation analysis between TBARS with GPx, SOD, Catalase and PON activity. Our results showed a significant negative correlation between TBARS versus GPx, SOD, Catalase and PON activities, which suggest that the antioxidant enzymes would be desirable.

There were some limitations in the present study, sample size was small and it was a hospital based study, so we can't represent whole population. There is need to perform such studies on larger and community based population.

6. CONCLUSION

This will be the first report to show the knowledge of Cardiac disease among Saudi population in Al-Quwayiyah region of Saudi Arabia. However, they are not very well aware of the diabetes, kidney disease and cardiovascular diseases. We may conclude that our study shows there are significant increase in lipid profile, Lipid peroxide levels and significant decrease in antioxidant enzymes in IHD patients. This indicates an imbalance between oxidant and antioxidant molecules in these patients. Therefore, Assessing of these biomarkers of oxidative stress may be useful in diagnosis of patients with IHD. The present study also illustrates that reduced consumption of alcohol, smoking, animal saturated fat and increased consumption of fruits, vegetables, tree nuts and natural antioxidants, supplementation of trace elements, maintain the physical activity and healthy body weight, HTN and control of hyperglycemia. Further studies are needed to correlate these associations and accordingly future antioxidative therapy may be beneficial or helpful in IHD patients.

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