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Research Article

Study of trace elements and malondialdehyde levels in cardiovascular disease patients

Ramprasad Nagarajrao *

*Assistant Professor of Medical Biochemistry, Department of Medical Laboratory Science, College of Applied Medical Sciences, Shaqra University, Al- Quwayiyah, Kingdom of Saudi Arabia.
*Corresponding author: *ramprasad7u@gmail.com*

Abstract

Cardiovascular disease (CVD) is a leading cause of global morbidity and mortality covers any disease of the circulatory system. Oxidative stress occurs as a result of increase level of lipid profile, malondialdehyde (MDA) as well as the decreased in the trace elements levels in CVD. Trace elements are those found in such small amounts in the living tissues. Trace elements are being increasingly recognized as essential mediators of the development and progression of heart diseases. On theoretical grounds, trace elements may be protective against oxygen free radicals in the development of CVD. In the present study, our aim was to investigate the lipid peroxidation product i.e. malondialdehyde, lipid profile and trace elements levels i.e. Selenium (Se), Zinc (Zn), Copper (Cu), Magnesium (Mg) and Iron (Fe) in CVD patients. This study was carried out in 40 CVD patients and 51 healthy controls was randomly selected and compared. Increased levels of cholesterol, triglycerides, LDL-C, MDA, Cu and Fe ($p<0.001$), whereas significant decreased of HDL-C, Se, Zn and Mg ($p<0.001$) levels were observed in CVD patients compared to controls. The hypothesis of the current study indicates that increased concentration of lipids and lipid peroxides may be contribute to decreased levels of trace elements that are associated with increased consequences of CVD. Therefore, assessing of these above biomarkers may be useful in diagnosis of cardiac patients.

Keywords: Cardiovascular disease, lipid profile, malondialdehyde and trace elements

Introduction

Cardiovascular Diseases (CVD) is one of the major health problems responsible for increasing mortality, morbidity and disability in the worldwide. The kingdom of Saudi Arabia (KSA) is experiencing an alarming rising in incidence and death rates from CVD. The global rise in CVD is driven by both urbanization and its related lifestyle modifications. According to the latest census, the mortality rate dropped from 17.9 % in 2008, 16.3 % in 2009 and 16.7 % in 2010 published by the Saudi ministry of health (Ibrahim et al., 2014).

Coronary Heart Disease (CHD) is the commonest cause of death from CVD in KSA. It is expected that the burden of CVDs will continue to grow in KSA due to continuous exposure to risk factors like smoking, hypertension, diabetes mellitus, high dietary fat intake, lack of physical exercise, body mass index and in addition to the traditional lipid panel have been documented as independent risk factors for the development of CVD (Lloyd- Jones., 2010).

Oxidative stress is defined as the interruption of balance between oxidants and reductants within the body due to excess production of peroxides and free radicals. Several researchers have indicated that the development of CVD is related to free radical

processes, lipid peroxidation and oxidative modification of LDL (Pavlova et al., 2007). 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. 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 (Mahbood and Rahman., 2005). Thus lipid peroxidation in the blood provides useful information for the prognosis of CVD 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.

Trace elements play a vital role in the body to perform its function properly especially in cardiac disease. Trace elements play an important role in the structure of proteins, enzymes and complex carbohydrates to participate in biochemical reactions. Essential trace elements are involved in a number of metabolic activities, including neuroconduction, transport, excretory processes and serving as cofactors for enzymes (Victory et al., 1986). Some of the trace elements like Selenium, Zinc, Copper, Magnesium and Iron are cofactors or structural components of antioxidant enzymes. Morever, Selenium and glutathione peroxidase play an important role in the protecting cell membrane from oxidative damage. Zinc and Copper are essential for protein synthesis and metabolically important trace metal for nutrients (Boosalis., 2008). Magnesium serves as a cofactor in approximately three hundred enzyme systems, important nutrient for body functions; it plays a vital role in normal cardiac function (Chakraborti et al., 2002).

In recent years, awareness has created on the very important role of trace elements that play either beneficial or harmful in human health and disease. The search for its etiology has led to some theories that dietary intake of minerals and in particular trace elements may have a role in the progress of atherosclerosis. In this regard, deficiency or excessive intake and uncontrolled homeostasis of some of these elements may lead to cardiovascular mortality (Toohey et al., 1998). Trace elements determinations in blood serum have become important to investigate their vital role in human metabolism, as well as obtain information regarding the health status of individuals (Singh et al., 1998).

Therefore, the aim of the present study was to investigate the Lipid peroxidation marker i.e MDA and serum trace elements like Selenium (Se), Zinc (Zn), Copper (Cu), Magnesium (Mg) and Iron (Fe) levels were measured in patients with CVD and compared with normal healthy subjects.

Materials and Methods

Study design and area

The study was case controlled in design. We have selected the patients as they are presented. The study patients were all admitted in the Intensive Coronary care Unit (ICCU) or attending the Outpatient department (OPD) of medicine of the Al- Quwayiyah Government General Hospital, Shaqra University, Kingdom of Saudi Arabia.

Study subjects

The study group consisted of 40 patients with CVD and they are admitted to hospital and they are between 40 –60 years of both the sexes (22 males and 18 females). The criteria for the diagnosis of CVD was made on the basis of clinical history, chest pain, history of myocardial infarction, 12 leads electrocardiogram (ECG) and coronary angiography findings. Smoking was defined as regular smoking of cigarettes / Beedies (local type of tobacco). Those patients whose body mass index (BMI) whose >25 we considered as an obese. Controls had 51 healthy volunteers age and gender matched non diabetic and non myocardial infarction healthy individuals. 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 and type 1 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 specimens and Biochemical Analysis

Fasting venous blood sample was drawn from all the subjects, 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 automated analyzer (Cobas Integra 800 from ROCHE Diagnostics, Germany). The concentration of serum total 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 by enzymatic methods (Pissani et al., 1995). Serum levels of MDA, a marker of lipid peroxidation were measured by thiobarbituric acid (TBA) method (Yagi., 1987). We measured important five trace element levels; Selenium (Se), Zinc (Zn), Copper (Cu), Magnesium (Mg) and Iron (Fe) in serum were determined by flame atomic absorption spectrophotometer with deuterium background correction (Perkin- Elmer model 5000) (Elmer and Conn., 1975).

Statistical evaluation

All values are expressed as mean ±SD. Student t- test was used to estimate the significant difference between the groups. The level of statistical significant was set at $p<0.001$. SPSS for windows 17.0 was used for statistical analysis.

Results

The clinical characteristic of the CVD patients and controls are presented in Table 1. In the present study number of BMI, smokers, and hypertensive were more in the CVD patients compared controls.

Serum total cholesterol, triglycerides, LDL- C and MDA levels were significantly increased, whereas decreased levels of HDL-C ($p<0.001$) was observed in CVD patients as compared to controls as shown in table 2. However, serum trace elements like Se, Zn and Mg levels were significantly decreased ($p<0.001$) in CVD patients. Whereas the mean serum Cu and Fe levels showed a significant elevation in CVD group when compared to control group ($p<0.001$) respectively as shown in Table 3.

Table 1: The demographic features of Controls and CVD patients

Particulars	Controls (n=51) Mean ±SD	CVD (n=40) Mean ±SD
Age (yrs)	50.2 ± 5.2	51.1 ± 4.8 *
Sex (male / female)	27 / 24	22 / 18 *
BMI (kg/m^2)	22.3 ± 3.9	24.5 ± 5.3 *
HTN (%)	7 %	40 % *
Smokers (%)	12 %	51 % *
Family history of CVD	---	35 % *

* $P<0.001$, Highly significantly compared to controls. CVD= Cardiovascular disease, BMI= Body mass Index, HTN= Hypertension.

Table 2: Various Biochemical parameters of the study subjects

Parameters	Controls (n=51) Mean ±SD	CVD (n=40) Mean ±SD
Total Cholesterol (mg/dl)	153.9 ± 15.2	232.0 ± 20.1*
Triglycerides (mg/dl)	99.2 ± 11.1	218.0 ± 14.5*
HDL-C (mg/dl)	51.2 ± 4.5	33.2 ± 5.7*
LDL-C (mg/dl)	89.3 ± 8.8	198.8 ± 13.3*
MDA (nmoles/ml)	3.3 ± 1.4	7.4 ± 25*

*P<0.001, Highly statistically significantly vs controls. CVD= Cardiovascular disease, HDL-C= High density lipoprotein cholesterol, LDL-C= Low density lipoprotein cholesterol, MDA= Malondialdehyde.

Table 3: Serum Trace elements levels in patients and controls.

Parameters	Controls (n=51) Mean ±SD	CVD (n=40) Mean ±SD
Se (µg / dl)	98.1 ± 10.2	67.1 ± 8.2 *
Zn (µg / dl)	112.8 ± 8.2	70.5 ± 9.8 *
Cu (µg / dl)	79.2 ± 13.1	123.3 ± 12.2 *
Mg (mg / L)	28.1 ± 3.1	17.8 ± 2.9 *
Fe (µg / dl)	92.5 ± 12.3	128.8 ± 11.4 *

*P<0.001, Highly statistically significantly compared to controls. CVD= Cardiovascular disease, Se = Selenium, Zn= Zinc, Cu= Copper, Mg= Magnesium, Fe= Iron

Discussion

Cardiovascular disease (CVD) is a process for which there is substantial evidence of a role for oxidative stress. The prevalence of CVD and relatively mortality risk associated with several risk factors. 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 CVD (Singh et al., 1998). Our data showed that prevalence of smoking was significantly higher in CVD patients as compared to controls. The lower incidence of CVD as seen in females probably due to the protective effect of estrogens. In the present study we observed, hypertension and obesity was found to be high in CVD 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 (Ibrahim et al., 2014).

Hypercholesterolemia and triglyceridemia are independent risk factor that alone or together can accelerate the development of CVD and progression of atherosclerotic lesions. HDL may be protective by reversible cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL (Kaviarasan et al., 2005). 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. Increased oxidative stress and the generation of free oxygen radicals can result in modification of LDL to oxidized LDL that could lead to cardiac diseases. Recent studies demonstrated that disturbed lipid profile is one of the important and potent risk factors in CVD (Varbo et al., 2013). In our study also, increased levels of cholesterol, triglycerides, LDL and decreased HDL in CVD patients.

Malondialdehyde (MDA) is a natural product of lipid peroxidation (LPO) and reflects the oxidant status of the biological systems. LPO is a free radical mediated chain reaction ant it is self perpetuating and it plays an important role in ageing, diabetes and atherosclerosis (Kesavulu et al., 2001). Several authors have reported that increased levels of MDA in CVD patients (Ramprasad., 2014, Sharma Anitha et al., 2012). In our study also, significant increased level of MDA in CVD patients compared to controls, because due to greater degree of oxidative stress and poor enzymatic and non- enzymatic antioxidant defense system. The estimation of MDA along with lipid profile in the CVD patients is very useful as it may serve as a useful monitor to judge the prognosis of the patient. Trace elements are being increasingly recognized as essential mediators of the development and

progression of heart diseases. On theoretical grounds, trace elements may be protective against oxygen free radicals in the development of CVD. Dietary factors such as inadequate intake of vitamins, minerals and trace elements may contribute to the development of heart diseases (Ramakrishnan et al., 2010). Selenium (Se) is one of the essential trace elements in human plasma. Se is a part of glutathione peroxidase in the cytosol and mitochondria, which protects biomembranes against destruction. Se is also a central enzyme for eliminating oxygen free radical and peroxidase (Purnima Dey Sarkar et al., 2007). Moreover, it boosts the immune system by increasing the activity of white blood cells and prevents premature aging, degenerative diseases, inflammatory diseases, stroke, cataracts, rheumatoid arthritis and CVD. Deficiency of the element can cause Keshan disease, characterized by an enlarged heart and poor heart function (Cuparigova and Stafilov., 2011). Mena Yahya et al., (2014) and Purnima dey Sarkar et al., (2007), revealed that there is a decreased level of Se in CVD patients. In the present study we found significant decreased levels of Se in CVD patients when compared to controls. These data showed that Se deficiency not only increases the risk of CVD, but also increase the risk of acute events of myocardial infarction.

Zinc (Zn) is the essential trace element present in the body. It takes part in various important body functions including protein synthesis, DNA synthesis and cellular growth. It is found almost in every cell and plays a vital role in body's immunes system affecting innate and acquired immunity. Zn also has significant antioxidant properties thereby protecting the cells from damage due to free radicals (Shazia et al., 2012). Various experimental models, clinically and statistically, have shown that the nutritional status of element such as Zn and Cu may have some effect on pathogenesis of CVD (Koo and Ramlet., 1983). In this study, we found significantly decreased levels of Zn in CVD patients compared to controls. Our results are in accordance with previous studies in which they documented lower levels of Zn in CVD patients (Kromhout et al., 1985). A decrease in the levels of Zn and HDL strongly suggested the role of Zn in cholesterol and lipid metabolism as observed by Wen et al., (1993).

Copper (Cu) plays an essential role in maintaining the structure and functions of some protein and antioxidants, and seems to be involved in the progress of atherosclerosis. Although deficiency or marginal intake of Cu has been proposed as a risk factor for CVD, serum Cu concentration was found to be increased in atherosclerosis obliterans. Severe loss of Zn and a significant increase in Cu were observed during inflammation and after cardiopulmonary bypass by Akcil et al., (2003) and Al-Bader et al., (1998). In the present study, serum Cu levels showed

significantly increase in patients with CVD compared to controls. Increased levels of Cu may be rise in the copper- binding capacity of ceruloplasmin. The increase in Cu may also be due to injury and subsequent necrosis of myocardial cells (Alfthan et al., 1992). Similar observation was made by Ramakrishnan et al., (2010). Among the cationic, ligands, Cu deserve particular consideration because it acts as a transition metal; it is very potent to generate ROS after a reaction with oxygen. Free Cu (II) ion can interact with hydrogen peroxide (H_2O_2) leading to the formation of the deleterious hydroxyl radical via the Fenton reaction. Bound to proteins, Cu is generally less susceptible to participate in the Fenton reaction (Marjolaine et al., 2008).

Magnesium (Mg) is essential for maintaining proper body function. It is vital for body's immune system, CVD and musculoskeletal systems. Deficiency of this element will lead to HTN, diabetes, kidney disease and CVDs (Al- Samarrai et al., 2008). The modulatory effect of Mg on immune cell function has observed in vitro and on reperfusion injury in patients with acute myocardial infarction pretreated with Mg sulfate (Mooren et al., 2003). Several epidemiological studies had shown that decrease levels of Mg in CVD in human organism (Ahmad et al., 2011 and Munoz et al., 2000). Similarly in our study also, we observed decreased levels of Mg in CVD patients compare to controls values. Lower Mg levels may influence numerous regulatory functions of Mg^{2+} ions in hormonal, cardiovascular and immune systems; depletion of Mg in plasma induces higher susceptibility of the lipoproteins to the oxidative stress, and a possible pro-oxidant effect (Rayssiguier et al., 2001).

Human body requires iron (Fe) for the synthesis of oxygen carrying protein called haemoglobin found in red blood cells, and myoglobin which is also a protein found in muscles. The free Fe will cause the formation of very harmful compounds, such as hydroxyl radical (OH). The hydroxyl radicals are highly reactive and attacks lipids to form lipid peroxides which contribute to oxidative stress. Fe load is serious complication of long term blood transfusion (Raghuveer et al., 2009). Several epidemiological studies had shown that the level of body Fe stores is positively correlated with the incidence of CHD in human populations (Salonen., 1993). In the present study, an increased level of Fe was observed in CVD patients compare to control cases. Similar observation was seen by Ramakrishnan et al., (2010). There was a higher risk of myocardial infarction in those with serum Fe high concentrations and the risk related to Fe may be increased in those with elevated cholesterol (Griffiths., 1985).

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.

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. It is very clear from this study that there are abnormalities in lipid profile, lipid peroxidation (MDA) and trace element levels in CVD patients. The assessment of these biomarkers in the cardiac patients is very useful as it may serve as a useful monitor to judge the prognosis of the patient. In view of these findings, the present study illustrates that reduced consumption of alcohol, smoking, animal saturated fat and increased consumption of n- 3fatty acids, intake of antioxidant rich fruits, vegetables, tree nuts, whole grains, supplementation of trace elements, physical activity and maintenance of health body weight and secondary measures like control of hyperglycemia.

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