OXIDATIVE STRESS AND LEVEL OF IRON INDICES IN CORONARY HEART DISEASE PATIENTS

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Objective: Oxidative stress is characterized by an increased concentration of oxygen free radicals which can cause a critical, or even an irreversible, cell injury. The study was designed to determine and compare the levels of oxidative stress and iron indices in Coronary Heart Disease and healthy individuals. **Material and Methods:** Blood malondialdehyde, iron, total iron-binding capacity, transferrin saturation and ferretin levels were determined in 140 Coronary Heart Disease and 100 healthy subjects. **Results:** Values of blood malondialdehyde, iron, transferrin saturation and ferretin were observed to be significantly increased with exception of total iron-binding capacity, which was significantly decreased ($p<0.005$) in Coronary Heart Disease patients when compared with normal healthy controls. **Conclusion:** Elevated serum malondialdehyde, iron concentration and body iron stores in patients reveal a possible role of iron indices in the development of coronary atherosclerosis. Therefore, it is suggested by this study that levels of malondialdehyde and biochemical markers of body iron stores can be used as an early investigative tool for assessing the oxidative stress in coronary heart disease.

Keywords: Coronary Heart Disease, Oxidative Stress, Malondialdehyde, Iron, Total iron-binding capacity, Transferrin Saturation, Ferritin

INTRODUCTION

Oxidative stress is characterized by an increased concentration of oxygen derived products that provoke critical, even irreversible, cell injury. Oxygen reduction leads to the synthesis of reactive intermediate compounds such as the superoxide anion, hydroxyl radical, hydrogen peroxide and peroxidative derivatives of polyunsaturated fatty acids (PUFA) such as conjugated dienes, lipid hydroperoxides and malonyldialdehyde (MDA).[1] Oxidation of circulating low density lipoprotein (LDL) has been linked to the initiation and pathogenesis of atherosclerosis and ultimately to the pathogenesis of cardiovascular disease.[2]

Iron is an essential element for mammalian cell growth. It is a required constituent of numerous enzymes, including iron-sulphur and haem proteins of the respiratory chain, as well as ribonucleotide reductase, which catalyses the rate-limiting step in DNA synthesis.[3,4] Iron ions circulate bound to plasma transferrin, and accumulate within cells in the form of ferritin. Under normal circumstances, only trace amounts of iron exist outside these physiologic sinks. In the healthy state there is never an appreciable concentration of 'free iron' (or iron chelated by low molecular weight compounds).[5] Transferrin does not release its iron at a normal *p*H, while at a low *p*H, which may be the case in arterial wall, iron can be released from transferrin and induce oxidation of low density lipoprotein.[6]

The transition property of iron from ferrous ion to ferric ion and vice versa is essential for energy generation in mitochondria. Because the free radicals generated by transition element iron have high potential, all cell organelles are protected by a variety of anti-oxidative systems. Under pathological conditions, however, potential iron, even though small in amount, generates free radicals, resulting in peroxidation of membranous organelles known as oxidative stress. Iron-induced oxidative stress may be negligible in healthy subjects, but can be a trigger of organ damage in some sensitive hosts.[7]

Iron catalyses the formation of reactive oxygen species through the Fenton and Haber-Weiss reactions.[8] The generally accepted view is that the main biological actor in damaged tissues and the one causing oxidative damage to all kinds of molecules, for example, in lipid peroxidation, is mainly caused by the highly toxic hydroxyl radical generated by the transition metal-catalyzed Haber-Weiss reaction.[9,10]

The hydroxyl radical is capable of abstracting a hydrogen atom from polyunsaturated fatty acids to initiate lipid peroxidation.[5] Lipid peroxidation is normally limited by a variety of antioxidant mechanisms including some enzymes such as superoxide dismutase and catalase and some vitamins such as alpha-tocopherol, ascorbic acid, retinol, etc.[11] Once lipid hydroperoxides accumulate, free iron may directly initiate additional lipid peroxidation. The resulting accumulation of lipid hydroperoxides destroys membrane structure and function. That explains the threat of 'free iron' or 'labile iron' or 'chelatable iron' during life. It can produce an oxidative stress, involved in many pathological manifestations (arteriosclerosis, diabetes, ageing, etc.).[5]

Oxidative stress alters the plasma lipoprotein profile (particularly LDL), the coagulative parameter

(with an increased thrombotic risk), the endothelium (with a decrease in prostacyclin synthesis and an increase of thromboxane production) and the cell membranes (which undergo peroxidation).[1]

Oxidative stress is involved in the pathogenesis of atherosclerosis.[12] High blood pressure and increased heart rate can increase consumption of oxygen by the heart, causing increased production of free radicals. If the body's antioxidant system does not respond to quench these increased levels of free radicals, oxidative damage to the heart muscle can result. This is one of the mechanisms whereby obesity can promote lipid peroxidation of the myocardium, the middle layer of the heart composed of heart muscle. Oxidative stress can also impair the ability of the endothelium, the inner layer of cells that line the blood vessels, to expand and dilate in response to blood flow. Based on this scenario, an accumulation of reactive oxygen species has been linked to 'cardiac contractile dysfunction', potentially leading to arrhythmia and heart attack.[13] Lipid peroxidation plays a key role in rendering low density lipoprotein atherogenic.[14] Oxidation, particularly oxidative modification of low density lipoproteins within the artery wall and its subsequent unregulated uptake by macrophages, has been postulated to be important in disease development.[15]

The present study has been designed to evaluate and compare the level of serum malondialdehyde (an oxidative stress marker), and biochemical markers of body iron stores (serum ferritin and transferrin saturation) in control healthy subjects, and in patients suffering from coronary heart disease.

MATERIAL AND METHODS

One hundred and forty patients diagnosed with coronary heart disease, and hundred control healthy subjects having no history of diabetes mellitus, coronary heart disease, hypertension or any other disease participated in this study. They study was conducted at Department of Biochemistry, Institute of Chemical Sciences, University of Peshawar. All patients having coronary heart disease and control subjects taking lipid lowering drugs, oral contraceptive pills, and those on multivitamins, especially antioxidant vitamins were not included.

Blood (8 ml) samples were taken after an overnight fast of 12–14 hours, from the antecubital vein of the patients as well as control individuals. Blood was allowed to clot and serum was separated by centrifugation at 2500 rpm for 15 minutes. Serum was stored at -20 ºC until analyzed for malondialdehyde and of iron profile. Malondialdehyde (MDA) was measured as thiobarbituric acid (TBA) activity by using the colorimetric method recommended by Buege and Aust cited by Valenzuela.[16]

Serum iron and Serum total iron binding capacity (TIBC) were analyzed by enzymatic colorimetric method by spectrophotometer R&M, using kits supplied by Randox Laboratories Ltd. Ardmore, United Kingdom. Transferrin Saturation was calculated from the ratio of serum iron concentration to serum total iron binding capacity expressed as a percentage.[17] Serum Ferritin was estimated by enzyme linked immunoassay method by ELISA Reader using kit supplied by Clinotech® Diagnostics & Pharmaceuticals, Inc. Horseshoe Way Richmond, Canada.

The results were expressed as Mean±SD. Statistical significance was evaluated by student's *t*-test. Differences were considered significant at $p<0.05$.

RESULTS

Coronary heart disease patients 140 in number and control group having 100 healthy individuals had mean ages (46±7.69 years) and (46.04±11.61 years) respectively. Statistically no difference in mean ages of both groups was observed. Body Mass Index (BMI) of coronary heart disease group (26.89±3.06 Kg/m^2) was significantly higher ($p<0.001$) as compared to control group (24.60±1.63 Kg/m^2). Serum malondialdehyde and iron indices of coronary heart disease patients and control individuals are shown in Table-1.

The levels of serum malondialdehyde, iron, and biochemical markers of body iron stores (serum ferritin and transferrin saturation) were showing significant increase in coronary heart disease patients as compared to control group whereas the levels of total iron binding capacity showed a significant decrease in coronary heart disease patients in comparison to control subjects.

Table-1: Blood levels of malondialdehyde and iron indices in patient group and control subjects.
(Mean±SD)

Groups	MDA (µmol/l)	Iron (µg/dl)	TIBC (µg/dl)	Transferrin Saturation (%)	Ferritin (ηg/ml)
Control (n=100)	2.28±0.47	79.45±10.86	372.09±51.43	22.08±5.78	83.51±14.97
CHD, Patient group (n=140)	3.87±0.49$^{\clubsuit}$	85.38±17.39‡	354.51±38.10$^{\phi}$	24.91±7.87$^{\clubsuit}$	105.26±11.78$^{\clubsuit}$

$^{\ddagger}p<0.01$ as compared to Control subjects. $^{\Phi}p<0.005$ as compared to Control subjects, $^{\clubsuit}p<0.001$ as compared to Control subjects

DISSCUSSION

Chronic diseases are the largest cause of death in the world. The global prevalence of all the leading chronic diseases is increasing with the majority occurring in developing countries and projected to increase substantially over the next 2 decades.[18] Coronary Heart disease is one of the leading causes of death in most industrialised countries of the world and it is now also considered as a prominent health problem in developing countries.[19] Between 1990 and 2020, mortality from ischemic heart disease in developing countries is expected to increase by 120% for women and 137% for men.[18]

Free iron is capable of participating in the production of free radical mediated tissue injury and thus play an important role in reactive oxygen metabolite and lipid peroxidation.[20,21] The general effect of catalytic iron is to convert poorly reactive free radicals, such as hydrogen peroxide, into highly reactive ones, such as the hydroxyl radical.[22] One of the most important mechanisms of antioxidant defence is thus the sequestration of iron in a redox-inactive form by transferring.[20]

Oxygen free radicals have been implicated in cardiac ischemic injury. These free radicals (super oxide anions and hydroxyl radicals) are produced in the body by reduction of oxygen. In normal circumstances they are removed by the different scavenger systems present in blood and tissues. In case of myocardial ischemia which can lead, to myocardial infarction, excessive free radicals may be generated.[23]

Oen and co-workers[24] showed significantly increased levels of lipid peroxides in patients suffering from coronary heart disease as compared to the control subjects. Belch and associates[23] showed evidence of increased free radical activity in patients with myocardial ischemia than the control subjects. This study also shows significantly increased malondialdehyde levels a marker of lipid peroxidation in coronary heart disease patients as compared to the control subjects, which are in agreement with the work of aforementioned groups.

Iron is essential for many physiological processes; excess iron can lead to tissue damage by producing the generation of reactive oxygen species. Iron is capable of supporting lipid peroxidation, acceleration of atherosclerosis development has been postulated as a potential mechanism by which iron overload may increase the risk of ischemic cardiovascular events. Iron may also have deleterious effects on vascular function. Locally enhanced vascular production of reactive oxygen species decreases the bioavailability of nitric oxide, impairing vasorelaxation and promoting platelet adhesion and aggregation. Therefore, a dynamic interaction exists between endothelial function and thrombosis, both of which may be influenced by oxidative stress.[25] Free iron serves as a catalyst for lipid and protein oxidation and the formation of reactive oxygen species.[26]

Stadler and colleagues[15] used the minimally invasive technique of electron paramagnetic resonance (ERP) spectroscopy and inductively coupled plasma mass spectroscopy (ICPMS) to quantify iron in ex vivo healthy human arteries and carotid lesions. They detected statistically elevated levels of iron in the intima of lesions compared with healthy controls. Metwalli *et al*[27] showed serum iron non-significantly decreased in patients with acute myocardial infarction. In a study conducted by Baer and his group[28] failed to show iron deficiency as defined by low transferrin saturation to be protective against acute myocardial infarction. They concluded by saying that their observations do not support the hypothesis that coronary artery disease risk is related to iron stores. Baykan *et al*[29] reported after a 45 day follow-up study, serum iron concentration to reach there maximum on the 1st post myocardial infarction day, serum ferritin level started to increase from the admission day of the patients and showed a mean peak value on the 3rd day and decreased to a minimum level on the 45th day. On the contrary, the transferrin level started to decrease on the 1st day, decreased to minimum level on the 3rd day and continued approximately the same level until the 45th day. Ahmed and associates[30] found significantly increased levels of serum iron, transferrin saturation and serum ferritin in both groups that is smokers having coronary heart disease and in the subjects without any major risk factor. The total iron binding capacity was found to be significantly low in these groups reflecting large volume of iron. The high levels of stored iron found in the subjects of coronary heart disease with smoking risk factor, as compared with controls suggest that there is a cumulative risk of high stored iron in the development of coronary heart disease. They also observed that in the coronary heart disease without any major risk factor, stored iron was also significantly increased than in controls, suggesting that stored iron may be independent risk for coronary heart disease. Both research groups Haidari *et al*[8] and Bozzini *et al*[31] reported significantly increased levels of serum ferritin in coronary heart disease patients as compared to normal control subjects. Haidari and his group[8] also observed a significantly increased level of serum ferritin in men with coronary heart disease than normal healthy men; on the other hand they could not find a significant difference in serum ferritin concentration when women with coronary heart disease were compared with healthy women with no coronary heart disease. Our results are also in

agreement with afore-mentioned workers, showing serum iron and transferrin saturation significantly high, whereas total iron binding capacity was found to be significantly low in coronary heart disease patients as compared to control subjects.

CONCLUSION

Elevated serum malondialdehyde, iron concentration and body iron stores (serum ferritin and transferrin saturation) in patients reveal a possible role of iron indices in the development of coronary atherosclerosis. Therefore, it is suggested by this study that levels of malondialdehyde and biochemical markers of body iron stores (serum ferritin and transferrin saturation) can be used as an early investigative tool for assessing the oxidative stress in coronary heart disease.

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