

STUDY OF C-REACTIVE PROTEIN AND SOME SMALL ANTIOXIDANT MOLECULE LEVELS IN CORONARY ARTERY DISEASE (CAD)

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Abstract

Coronary artery disease (CAD) is the most common type of heart disease. It's the leading cause of death in the developed countries and other parts of the world. The aim of this study was to evaluate antioxidant status (vitamin E, vitamin C and bilirubin) and CRP in 50 patients with (CAD) (Age range: 40 -55 years) and in (40) healthy subjects.

The status of antioxidants in serum as represented by bilirubin levels decreased significantly ($p < 0.01$) in the conditions of coronary artery disease (CAD), than their controls, also we evaluated in this study the concentration of vitamin E and vitamin C in coronary artery disease. Patients with (CAD) showed significantly ($p < 0.05$) lower vitamins concentrations than controls.

Furthermore, we assessed the levels of CRP in patients with coronary artery disease compared with healthy. CRP levels were significantly higher ($p < 0.05$) in patients compared with healthy subjects.

Introduction

Coronary artery disease (also called coronary heart disease or heart disease) is a narrowing or blockage of the arteries and vessels that provide oxygen and nutrients to the heart. It is caused by atherosclerosis, an accumulation of fatty materials on the inner linings of arteries. The resulting blockage restricts blood flow to the heart. When the blood flow is completely cut off, the result is a heart attack (Zoler & Michael, 2003). The world is poised for the tidal wave of coronary diseases. It is responsible for 10% of DALYs (disability-adjusted life years) lost in low and middle-income countries and 18% in high income countries (MacKay and Menash, 2004).

Coronary artery disease is a chronic process that begins during adolescence and slowly progresses throughout life. Independent risk factors include a family history of premature coronary artery disease, cigarette smoking, diabetes mellitus, hypertension, hyperlipidemia, a sedentary lifestyle, and obesity. These risk factors accelerate or modify a complex and chronic inflammatory process that ultimately manifests as fibrous atherosclerotic plaque (Ariyo et al, 2003).

Over the past two decades, considerable evidence has been gathered in support of the hypothesis that free-radical-mediated oxidative processes and specific products arising therefore play a key role in

atherogenesis (Steinberg, 1997). Normally these free radicals (ROS) are effectively kept in check by the various levels of antioxidant defenses. Imbalance of this reaction either due to excess free radical formation or insufficient removal by antioxidants leads to oxidative stress (Maritim et al., 2003). These ROS can stimulate oxidation of low-density lipoprotein (LDL), cholesterol, cholesterol derived species, protein modifications which can lead to foam cell formation and atherosclerotic plaques (van der et al., 2002). It is therefore, logical to presume that antioxidants should help to prevent the coronary diseases (Mc Kechnie et al., 2002). Oxidation of LDL lipids leads to the production of a diverse array of biologically active compounds, including some that influence the functional integrity of vascular cells (Navab et al, 1996).

Oxidants are products of normal aerobic metabolism and the inflammatory response. They constitute a chemically and compartmentally diverse group, and it is presently unknown which, if any, are critical to the disease process. Like oxidants, antioxidants constitute a diverse group of compounds with different properties. They operate by inhibiting oxidant formation, intercepting oxidants once they have formed, and repairing oxidant-induced injury (Diaz et al, 1997). Numerous excellent reviews related to cardiovascular disease and antioxidants have been published in the last

ten years (Manson et al., 2003). In terms of the coronary heart disease process, several points of antioxidant intervention have been proposed, as reviewed in detail (Diaz et al, 1997). Inhibition of LDL oxidation is the best characterized of these and includes effects on the concentration or reactivity of oxidants capable of modifying LDL and on the susceptibility or resistance of LDL to these oxidants. although the antioxidant defense system includes both endogenously and exogenously (diet) derived compounds, dietary antioxidants including vitamin C (ascorbic acid), vitamin E (eg, α -tocopherol), and β -carotene (provitamin A) have received the greatest attention with regard to coronary heart disease prevention. α -Tocopherol and β -carotene have been of particular interest because both are carried within LDL particles. Enrichment with α -tocopherol increases LDL oxidative resistance in vitro (Reaven et al, 1993). There is supportive evidence that vitamin C and E exert protective effect against Coronary artery diseases by reducing oxidative stress (Schnackenberg, 2002). Thus, epidemiological studies have clearly documented the benefit of α -tocopherol and vitamin C and vitamin C plus vitamin E on the risk of coronary artery diseases (Winklhofer-boof et al., 2004). Vitamin E is a group of about 8 naturally occurring tocopherols (Marcus & Coulstom, 1996). This vitamin is essential in many species for normal reproduction, development of muscles, resistance of RBC's to hemolysis and a number of other physiological and biochemical functions (Mohsun, 1995). Vitamin E acts as a potent anti-oxidant, as it is capable of donating electron to free radicals and thereby quenching it. It is, therefore, capable of breaking free radical chain reaction by scavenging free radicals and thus protecting unsaturated fatty acids, especially of cell membranes, from oxidative damage (Fitzgerald et al, 1986).

The association of bilirubin with atherosclerosis might be explained by findings showing antioxidative and cytoprotective properties (Amit & Boneh, 1993) Bilirubin scavenges peroxy radicals and suppresses the oxidation in liposomes more efficiently than alpha-tocopherol (Stocker et al, 1987). The

antioxidative properties may be independent of whether bilirubin is unconjugated, conjugated, free, or albumin-bound (Wu et al, 1996). Other investigations proposed bilirubin to be an anti-atherogenic end product in the heme-oxygenase-carbon monoxide signaling pathway (Paganga et al, 1992).

There is increasing evidence that inflammation is an important determinant of the development of atherosclerosis. Inflammation is associated with the activation and proliferation of macrophages, smooth-muscle cells, and endothelial cells (Alexander, 1994 & Ernest, 1994). The levels of C-reactive protein (CRP) are correlated with the presence and severity of coronary, cerebral, and peripheral atherosclerosis (Heinrich et al, 1995) in addition; elevated CRP levels are strongly related to the occurrence of cardiovascular complications such as sudden cardiac death and acute myocardial infarction (Thompson et al, 1995).

Materials and Methods

This study was conducted on 50 cases of coronary artery disease (CAD) and 40 healthy subjects as control. The subjects were in the age group of 40 to 55 years, they were followed at Al-diwaniya hospital. The diagnosis of (CAD) in this study was established according to clinical examination: chest pain, ECG changes, lipoprotein tests and CPK elevation. Patients with any kidney, liver, diabetes diseases and pregnancy were excluded from the study. Ten milliliters of blood was drawn by venipuncture and collected in a vial without EDTA in the morning (exact at the 8:00 a.m). Serum was separated and the samples stored at 4 °C till being processed.

* Serum vitamin E levels were determined by method of Baker and Frank, 1988. serum vitamin E reduce ferric to ferrous ions, which then form a red coloured complex with α , α' -dipyridyl.

* Vitamin C was estimated by 2,6-dichlorophenolindophenol titration method. 2, 6-dichlorophenolindophenol is red in acid solution and on titration with a solution of ascorbic acid is reduced to the colourless, the ascorbic acid being oxidized to

dehydroascorbic acid (Rose and Nahrwold, 1981).

*C-reactive was determined by agglutination of latex particles on slide. Latex particles allow visual observation of the antigen – antibody reaction. (Smith et al, 1977).

*Serum bilirubin was determined with spectrometry using a reaction with p-diazobenzenosulphonic acid (Doumas & Wu, 1991).

Statistical Analysis

The data from patients and controls were compared using Student's t-test. Values were expressed as mean \pm standard deviation (SD). SPSS program version 10 was used for statistical analysis. P-values of less than 0.05 was considered to indicate significance.

Results

This study is an attempt to look for the diagnostic and prognostic importance of serum CRP and antioxidants levels in patients of CAD. In the present study serum vitamin C and vitamin E levels were found to be significantly decreased in patients group (0.65 ± 0.13 mg/dl and 0.7 ± 0.14 mg/dl, $P < 0.01$ respectively) as compared to control (1.19 ± 0.13 mg/dl and 1.29 ± 0.15 mg/dl, respectively) (table 1). Also (table 1) showed low serum levels of bilirubin (0.46 ± 0.22 mg/l, $p < 0.05$) and high levels of serum CRP (8.2 ± 0.19 mg/l, $p < 0.001$) in CAD patients in comparison to the control group, (0.84 ± 0.34 mg/dl,) bilirubin and (2.1 ± 0.11 mg/l) CRP.

Discussion

The role of free radicals through heightened oxidative stress, and averred role of antioxidants in coronary artery diseases is based on the premise that free radicals can injure arteries, can induce atherosclerosis by inducing fatty streaks resulting in atheroma by oxidation of LDL or possibly HDL also, can injure myocardium during reperfusion in MI (Kris-Etherton and West, 2005). Also some studies have indicated that oxidative stress increases the susceptibility of LDL to lipid peroxidation whereas vitamin E and \ or vitamin C supplementation significantly decreases LDL oxidation (Bougeios, 2003).

In this study Table (1) shows significantly lower serum vitamin antioxidant (vitamin E) levels in patients group as compared to control subjects. The results of this study confirm previous observations that there is low plasma vitamin E in cardiovascular disease patients compared with controls. This is in accordance with studies of Singh et al, 1994 who demonstrated that there was a significant drop in vitamins C, E, A and β -carotene, whereas lipid peroxides were significantly higher in patients, compared with controls. This indicates severe damage to antioxidant system, which is unable to combat oxidative stress and inflammation (Neela, 2007).

In our study the decrease in the levels of antioxidants vitamin C Table (1) occurs with the progression of disease. The lowered values of vitamin C are because it functions as an important component of cellular defense against oxygen toxicity and lipid peroxidation caused by free radical mechanism (Mezzetti et al, 1990), while elevated CRP levels (Table (1)) have been known to be associated with CAD risk as pointed by Liao, 2002). CRP is a protein released into the bloodstream any time there is active inflammation in the body. Our findings are consistent with a variety of experimental observations that suggest a direct role for CRP in the pathogenesis of atherosclerosis. CRP renders oxidized LDL more susceptible to uptake by macrophages, induces the expression of vascular-cell adhesion molecules, stimulates the production of tissue factor, and impairs the production of nitric oxide (Verma et al, 2002).

Previous epidemiological studies described an association between low bilirubin levels and coronary heart disease (Djousse et al, 2001). It was found that plasma bilirubin levels correlated inversely with known CAD risk factors, such as smoking, LDL cholesterol, diabetes, and obesity, and correlated directly with HDL cholesterol, the relation between low bilirubin levels and CAD patient, however, remained significant after adjustment for known CAD risk factors (Schwertner, 1998). This association was further confirmed in the prospective British Regional Heart Study that observed 737 major ischemic heart disease events in 7685 middle-aged men during 11.5 years of follow-up

(Breimer et al, 1995). A small case-control study found decreased values of bilirubin indicative of the presence of atherosclerosis in smokers (Cerne et al, 2000). The Family Heart Study pointed to a gender-specific effect of bilirubin on CAD (Hunt et al, 2001). This is in accordance with our results showing only significantly lower bilirubin levels in patients (Table (1)). The cytoprotective properties of bilirubin may be explained by its inhibition of protein kinase C (PKC) (Mietus-Snyder et al, 1997). Haeme oxygenase activity is related to a faster resolution of inflammation, whereas the inhibition of this enzyme seems to increase the inflammatory response (Willis et al, 1996).

Table (1)
Serum antioxidants levels and CRP in the control group and patients with CAD.

Parameter	Controls (n=40)	CAD (n=50)	P value
Vitamin C (mg/dl)	1.19±0.13	0.65± 0.13	P<0.01
Vitamin E (mg/dl)	1.29±0.15	0.7± 0.14	P<0.01
CRP (mg/l)	2.1±0.11	8.2±0.19	P<0.001
Bilirubin (mg/dl)	0.84±0.34	0.46±0.22	P<0.05

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الخلاصة

يعد مرض الشريان التاجي CAD من اغلب الأمراض القلبية المنتشرة حيث يعتبر من الأسباب الرئيسية للوفيات في الدول المتقدمة ومناطق أخرى من العالم. هذه الدراسة أجريت لتقييم مستويات مضادات الأكسدة (فيتامين E وفيتامين C و البروتين) والبروتين الفعال C في مصل 50 من مرضى الشريان التاجي تتراوح أعمارهم (40 إلى 55) سنة و40 من الأصحاء.

حيث وجد نقصانا معنويا ($p < 0.01$) في مستوى البروتين كمضاد للأكسدة في مرضى الشريان التاجي مقارنة بالأصحاء، من جهة أخرى تم تقييم مستويات كل من فيتامين (C, E) في مرضى الشريان التاجي مقارنة بالأصحاء إذ وجد نقصانا معنويا في مستوى فيتامين E وفيتامين C في المرضى ($P < 0.05$).

كذلك تم تقييم مستوى البروتين C- الفعال في مرضى الشريان التاجي مقارنة بالأصحاء، حيث وجد ارتفاعا معنويا في مستواه ($P < 0.05$).

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