Oxidative stress and inflammation in ischemic heart disease: role of trace elements, oxidants and antioxidants

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Objective Clinical evidence suggests oxidative stress and inflammation linked to reactive oxygen species (ROS) over generation may be a key in the pathogenesis of certain disease. Cardiovascular disease is one example of this process. ROS have been proposed to be the mediators of inflammation in experimental cardiovascular pathology. Till now, areas with regard to the effects of ROS in the ischemic heart disease (IHD) are not completely understood. The present study was conducted to investigate the involvement of inflammation as a consequence of oxidative stress in the pathology of IHD.

Methods Serum lipid profile, trace element, antioxidant enzyme activities [catalase (CAT), superoxide dismutase (SOD)], hydrogen peroxide (H_2O_2) and the inflammation marker (TNF-α and IL-6) were measured in 50 patients with IHD and 50 healthy subjects as control group. All results were statistically analysed.

Results A highly significant decrease was found in the serum level of both zinc (Zn) and copper (Cu) in patients with IHD compared to control ($P < 0.05$). Serum levels of antioxidants (SOD, CAT) were also significantly decreased in the patient group ($P < 0.05$) compared to control. However, H_2O_2 and cytokines (TNF-α and IL-6) levels demonstrated a significant increase in IHD patients ($P < 0.01$).

Conclusion The results of the present study provide evidences that oxidative stress, inflammation and trace element are closely linked and involved in IHD that may have future role in developing new therapeutic strategies.

Keywords oxidative stress, inflammation, ischemic heart disease, antioxidants, trace elements

Introduction

Heart disease is one of the major health problems of developing countries of the world. Recent research has shown that free radicals, particularly, reactive oxygen species (ROS) play an important role in the pathogenesis of oxidative myocardial damage with consequential cardiac malfunction.[1] Oxidative stress describes the condition where an excessive production of ROS overwhelms endogenous anti-oxidant defense mechanisms. The resultant elevation in ROS levels has a detrimental effect on cellular function, a consequence of ROS-induced damage to lipid membranes, enzymes and nucleic acids.

Generation of ROS has been involved in various cardiovascular disorders, including ischemia/reperfusion (I/R), atherosclerosis and cardiotoxicity induced by drugs.[2] These ROS caused an injury to vascular cells and cardiac myocytes directly, and can initiate a series of local chemical reactions that result in an amplification of the initial ROS-mediated cardiomyocyte dysfunction.[3] Oxidative stress is now thought to play an important role in the pathogenesis of coronary heart disease through oxidation of low-density lipoprotein (LDL)-cholesterol and free radical formation.[4] Oxidised LDL-cholesterol aids the evolution of early arterial wall lesions into atherosclerotic plaques by promoting the formation of foam cells from macrophages as well as the recruitment and retention of monocytes in the arterial wall.[5] ROS may contribute to atherogenesis by damaging the arterial endothelium, promoting thrombosis and interfering with the normal vasomotor regulation.

It is assumed that antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) constitute a first line of defense against oxidative stress by removing ROS.[6] SOD, catalyses the dismutation of the superoxide anion ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) within cells and in the extracellular matrix, while CAT and GSH-Px remove H_2O_2, and GSH-Px can also convert lipid peroxyl radicals to nontoxic alcohols.[6] Low levels of activity of these antioxidant enzymes have been shown to associate with increased risk of CHD.[3] Recently, inflammation has been linked, both experimentally and clinically, to cardiovascular disease.[7] During inflammation ROS are generated, which can be due to immune cells, such as [dendritic cells (DCs), lymphocytes, neutrophils and macrophages] or interleukins and other inflammatory cytokines, such as tumor necrosis factor (TNF-α).[8,9] Because metals can cause oxidative modification of LDL-cholesterol and the synthesis of ROS, the effect of several prooxidant metals, including Cu on cardiovascular disease has come under investigation. Recent researches demonstrate the importance of certain elements in the pathogenesis of cardiovascular disorders. Statistically significant positive correlations have been found between trace element concentrations (Cu, Zn, Se) of heart tissue with physiological parameters (CO: cardiac output, EF: ejection fraction) of the heart. It is probable that free oxygen radicals and oxidatively modified particles of LDL participate in the development of atherosclerotic lesions and the potential role of natural antioxidants (Vit. C - ascorbic acid; Vit. E - tocopherol) is inhibition of this process.[10] However, understanding of inflammation and ROS, especially with their pathophysiological role in cardiovascular dysfunction, is still unclear and further investigations will facilitate the development and/or delivery of selective

anti-inflammatory agents (antioxidants) that provide better management of cardiovascular diseases.

Materials and Methods

Subjects

Serum Zn and Cu, antioxidant enzymes and inflammatory marker levels were measured in 50 (30 males and 20 females) patients with ischemic heart disease (IHD), and 50 healthy subjects (32 males and 18 females) as a control group. The mean age of the control group (58 ± 19.4 y) and in the patient group (60 ± 20.1 y) were randomly selected from patients IHD from March to August 2014. Information regarding the medical history of each subject was obtained, including age, sex, diseases suffered and duration of illness with their daily diet and occupation. None of the patients had consumed alcohol, nor was there any history of surgery.

Methods

All groups were subjected to thorough clinical history, examination and specific cardiac investigation. Venous blood samples (5 mL) were collected from the patient and control groups. Serum was separated by centrifugation (Gallenkamp, Germany) at 3000 RPM for 10 min and stored in capped plastic tubes at −20°C until analysis. Total cholesterol, LDL-cholesterol, high-density lipoprotein (HDL)-cholesterol and triglyceride were measured by commercial kits (RANDOX Laboratories, San Francisco, CA, USA). Trace elements like Cu and Zn were determined by using a Pye Unicam Model SP9 atomic absorption spectrophotometer. Activities of antioxidant enzymes like CAT and SOD in the serum were determined by the method of Mccord and Fridovich[11] and Mueller et al.,[12] respectively. Horseradish peroxidase (HRP)-linked assay using homovanillic acid was used to measure the production of H_2O_2.[13] TNF-α and IL-6 were measured by ELISA.

Statistical Analysis

Data are expressed as means ± SEM. Statistical analysis was carried out using Statgraphics Centurion XVI (StatPoint Technologies, Inc., VA, USA). The significant difference between control and experimental subjects were determined by Student's t-test. In order to explore significant differences between control and different patient groups, one-way ANOVA or a non-parametric ranking (Kruskal-Wallis) were carried out as appropriate. After ANOVA, post hoc analysis using Tukey's test was carried out. A P value of <0.05 is considered significant throughout.

Results

The levels of total cholesterol, LDL-cholesterol and triglyceride were significantly higher and that of HDL-cholesterol was significantly lower than those of controls (P < 0.05, Table 1). There was a clear relationship between serum trace element (Cu and Zn) concentrations and IHD. Serum Cu and Zn levels were found to be significantly lower in IHD patients compared to control (P < 0.05; Table 2). Female patient results showed a significant difference in serum Cu, HDL-cholesterol and triglyceride levels (P < 0.05) than those found in males (Table 3). Table 4 shows serum Cu and Zn levels from patients grouped according to the type of disease with IHD. There was a significant change in Cu and Zn concentrations in patients with IHD only or IHD and hypertension compared to healthy controls (P < 0.05). A significant decrease was found in the activities of antioxidant enzymes CAT and SOD (P < 0.01, Figs. 1 & 2, respectively) in the IHD patients compared to the control, whereas H_2O_2 levels were significantly increased in patients compared to control (P < 0.01, Fig. 3). Serum levels of TNF-α and IL-6 were measured in patients with IHD using ELISA. TNF-α and IL-6 were significantly increased in the serum of IHD patients compared to control (P < 0.01, Fig. 4).

Discussion

It is well known that ROS is of great importance in a number of biological processes, mostly through their action as signalling molecules that mediate different intracellular pathways, by influencing the permeability of cell membranes or through other mechanisms. Therefore, it is reasonable to assume that ROS would also exert an action, either directly or indirectly, on the cardiac cell or on other systems related to cardiovascular function e.g. the lipid and carbohydrate metabolism.

IHD patients in this study showed a significant decrease in serum Cu (Table 2), which is consistent with the findings observed by other investigators.[14,15] Also lipid profile was significantly different from control lipid profile (Table 1). Our findings support the argument that decreased serum levels of Cu was associated with increased oxidative stress and develop IHD risk factors due to Cu is an antioxidant nutrient. Cu plays an important role in the regulation of oxidative free radicals and its deficiency lead to increase the ability for peroxidation of lipoprotein,[16] oxidation and free radical formation are two components of atherogenesis. In addition, Cu

Table 1. **Serum total cholesterol, LDL, HDL and triglyceride levels in both IHD patients and healthy control groups.**

Parameter	Patient group N = 50	Control group N = 50	P value
Total cholesterol (mmol/L)	16.22 ± 0.66	5.45 ± 1.17	<0.05*
LDL-cholesterol (mmol/L)	14.93 ± 1.81	9.63 ± 0.87	<0.05*
HDL-cholesterol (mmol/L)	0.73 ± 0.19	1.05 ± 0.39	<0.05*
Triglycerides (mmol/L)	1.72 ± 1.68		<0.05*

***The mean difference is significant at P < 0.05.

Table 2. **Serum Cu and Zn levels in both IHD patients and healthy control groups.**

Parameter	Patient group N = 50	Control group N = 50	P value
Serum copper (μ mol/L)	9.49 ± 1.01	15.49 ± 1.29	<0.05*
Serum zinc (μ mol/L)	7.59 ± 0.66	13.35 ± 1.14	<0.05*

*The mean difference is significant at P < 0.05.

Table 3. **Serum Cu, Zn levels and lipid profile in IHD patients and healthy controls classified by sex.**

Parameter	Female, n = 20	Male, n = 30	P < 0.05
Copper (µmol/L)	15.74 ± 0.98	12.2 ± 1.08	<0.05*
Zinc (µmol/L)	10.3 ± 0.51	10.75 ± 0.69	NS
Total cholesterol (mmol/L)	14.49 ± 0.59	15.93 ± 0.69	NS
LDL- cholesterol (mmol/L)	10.5 ± 0.73	12.48 ± 0.62	NS
HDL-cholesterol (mmol/L)	0.86 ± 0.18	0.66 ± 0.15	<0.05*
Triglycerides (mmol/L)	1.92 ± 0.41	2.3 ± 0.58	<0.05*

*The mean difference is significant at $P < 0.05$. NS (non-significant) $P > 0.05$.

Table 4. **Serum Cu, Zn levels in IHD patients and healthy control classified depending on the disease.**

Parameter	No	Zn (µmol/L)	P value	Cu (µmol/L)	P value
IHD with Diabetes mellitus	11	10.35 ± 1.63	NS	13.96 ± 2.8	NS
IHD with Hypertension	9	10.95 ± 0.83	<0.05	11.82 ± 0.94	< 0.05*
IHD with asthma	4	14.73 ± 1.13	NS	15.44 ± 2.26	NS
IHD only	26	10.56 ± 0.57	<0.05	11.12 ± 1.05	<0.05*
Control	50	13.35 ± 1.14		15.49 ± 1.29	

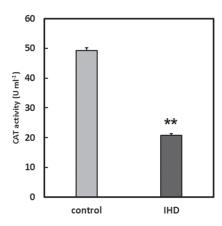*****The mean difference is significant at $P < 0.05$.

Fig. 1 **Serum CAT activity in patients with IHD. Serum samples were isolated from the blood of patients with IHD. CAT activity was assessed in the presence of Amplex Red and HRP. Data are expressed as means ± SEM, for 50 patients, $n = 50$ with triplicate measurements. **indicates significant differences compared to the control (Student's t-test, $P < 0.01$).**

Fig. 2 **Serum SOD activity in patients with IHD. Serum samples were isolated from the blood of patients with IHD. SOD activity was assessed in the presence of cytochrome c and XO. Data are expressed as means ± SEM, for 50 patients, $n = 50$ with triplicate measurements. **indicates significant differences compared to the control (Student's t-test, $P < 0.01$).**

Fig. 3 **H_2O_2 production in patients with IHD. Serum samples were isolated from the blood of patients with IHD and control. H_2O_2 levels in the serum were determined using HRP/HVA. Data are expressed as means ± SEM, for 50 patients, $n = 50$ with triplicate measurements. **indicates significant differences compared to the control (Student's t-test, $P < 0.01$).**

Fig. 4 **Serum pro-inflammatory cytokine levels in the patients with IHD. Plasma serums were isolated from blood of patients with IHD and control. TNF-α and IL-6 levels were analysed by ELISA. Data are expressed as the means ± SEM, for 50 patients, $n = 50$ with triplicate measurements. **indicates significant differences compared to the control (Student's t-test, $P < 0.01$).**

deficiency leads to an increase in plasma cholesterol and oxidises LDL-cholesterol, increasing its atherogenicity.[17] Alternatively, copper may be a risk marker for inflammation rather than a risk factor for coronary heart disease directly involved in the pathogenesis of atherosclerosis. There is more than one explanation for the mechanism of copper
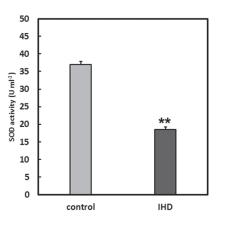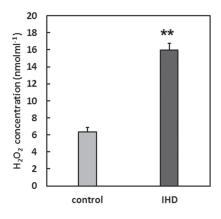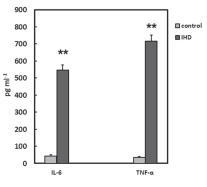
deficiency in enhancing heart diseases. Copper decreased cardiac myocyte functional capacity with subsequent impaired pumping capacity of the heart and finally heart failure. The abnormal levels of copper in patient with CHD are probably due to changes in the concentration of ceruloplasmin in the plasma, an acute phase protein that was

synthesised by the liver in response to tissue damage and inflammation.[18] The present study demonstrates a significant difference in serum Cu between females and males with IHD (Table 3). These findings were consistent with the findings by another researcher.[19] However, a number of factors influence circulating concentrations of copper. Circulating concentrations of copper are positively associated with age, white blood cell count, cigarette smoking, serum LDL-cholesterol, systolic blood pressure, body mass index and oral contraceptive use or estrogen replacement

therapy.[20,21] In a couple of studies, copper was inversely associated with HDL-cholesterol.[20,22]

The results of the present study provide additional evidence of the importance of zinc in IHD. As expected, serum zinc levels were significantly decreased in patients with IHD (Table 2). These results which are consistent with recent studies suggest that low serum concentrations of zinc are associated with coronary artery disease.[23,24] Low serum Zn levels in the IHD patient group may be related to excess release of steroids due to the release of leukocyte endogenous mediators which redistributes the body Zn from serum and may cause a drop in serum Zn and also due to elevated levels of α2-macroglobulin which is a transport protein containing large amounts of Zn.[25] Recent characterization, suggest that zinc may share absorptive mechanisms with a variety of divalent cations, including cadmium, copper, iron and lead.[26] Metallothionein has a greater binding capacity for Cu than for Zn[27] thus causing elevation of serum Cu level and lowering serum Zn level. Recent studies also indicate that deficiency in Zn is also a risk factor for the development of atherosclerosis caused by lipid peroxidation by oxidative stress,[28] also Zn may modulate atherosclerosis through its effects on gene stabilization, transcription factor levels and apoptosis.[29,30] Only a significant decrease was found in Cu and Zn levels in patients with IHD only or IHD and hypertension patients (Table 4). This may be contributing to a pathological condition associated with this disease.

In the present study, CAT and SOD activities were significantly decreased in IHD patients (Figs. 1 & 2). The results of this study are in agreement with those reported by Kayyum et al.[31] Thus, low levels of antioxidant enzyme activity could reflect either a possible accumulation of H_2O_2 (an ROS) which has the potential to bring about oxidative damage of the bio-macromolecules and the consequential tissue damage or low levels of defense against it, a high oxidative stress status has been reported in IHD patients, even in stable cases.[32,33] For instance, circulating oxidised LDL level is positively associated with severity of acute coronary syndromes[33] and with subclinical CHD.[34] In contrast, the serum H_2O_2 level was significantly increased in IHD patients (Fig. 3), this increase indicates an intensification of ROS production due to neutrophil activation and/or derangement within the mitochondrial electron transfer.[35]

Our findings also have established that TNF-α and IL-6 significantly increase in patients with IHD (Fig. 4). This reveals that IHD enhances the immune system and releases pro-inflammatory modulators that result in IHD necrosis. It has been demonstrated that increased secretion of TNF-α and IFN-gamma by circulating mononuclear cells from patients with IHD may relate to the propagation of atherosclerosis by promoting intravascular coagulation and cell adhesion. Therefore, TNF-α may be involved in the evolution of atheroma after the initial ischemic event.[36] Our findings of increased TNF-α and IL-6 levels in patients with IHD are consistent with previous studies.[37,38] Increased plasma concentration of TNF-α has been found in patients with coronary artery disease, stressed myocardium activates pro-inflammatory cytokines, such as TNF-α, which produce abnormalities in the myocyte contractile function and soluble TNF receptors that bind to TNF-α which may prevent and even reverse TNF-α damage. Pre-treatment with TNF-α antibodies reduces myocardial infarct size. TNF-α is a key molecule among the proinflammatory cytokines, which activates a transcriptional factor (NF-κB) in the nucleus[39] which triggers and mediates the inflammatory response such as activation of neutrophil migration, production of proinflammatory cytokines and activation of metalloproteinase. Furthermore, oxidative stress can activate a variety of transcription factors such as NF-κB.[40]

Conclusion

The findings of the present study provide evidence that oxidative stress and inflammation are intimately linked with IHD. It is likely that IHD pathology is mediated by the generation of ROS and induction of inflammation. Therefore, future studies are needed to understand the interaction of inflammation, immune cells and ROS with cardiovascular disease which may prove useful in developing a therapeutic approach to the resolution of inflammation in IHD. ■

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