Evaluation of Ceruloplasmin ferroxidase activity and lipid profiles in patients with Valvular heart diseases

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Abstract. One of the major health problems causing defects or damage to one or more of the four heart valves [aortic, mitral, pulmonary, and tricuspid] is valvular heart disease [VHD]; it occurs due to congenital abnormalities or acquired pathology. It is a defect that results in weak heart valves and is therefore unable to function as precise pathways of the blood. The aim of the current study was to evaluate the ferroxidase activity of ceruloplasmin (Cp) and the lipid profile of valvular heart disease patients in sera. Ninety subjects were included in this study and 60 patients with HDV were divided into two subgroups according to the affected valve: 33 patients with aortic valve disease (AV) and 27 patients with mitral valve disease (MV group). In addition, 30 healthy individuals were registered in all groups as control(C) group Serum copper (Cu), total protein (TP), activity and specific activity of ferroxidase Cp, and lipid profile were measured. The results showed that there was a highly significant increase in patient (AV & MV) groups compared to the C group in activity, specific Cp and Cu activity levels. In addition, compared to the C group, triglyceride (TG) and very low density lipoprotein (VLDL) levels showed a highly significant increase in the AV and MV groups. No significant differences were found between patients and control groups in TP, Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C) & High Density Lipoprotein Cholesterol (HDL-C) levels. Likewise, for all parameters, there were no significant differences between the AV and MV groups. In conclusion, the high serum level of Cu and the activity of Cp ferroxidase may be considered to be a risk factor for VHD.

Keywords: Heart valve disease, Ceruloplasmin, copper, lipid profile

1. Introduction
Valvular heart disease (VHD) is a pathological situation correlated with heart valves. The affected valve may be inefficient to close fully, led to not opening and closing of the heart properly, this allows an amount of blood to leak backward. Also, this may happen due to the stiffened, thickened or fused leaflets causing the improper opening of the valve [1]. Valves that are involved in VHD, including: on the left (aortic and mitral) and the right (pulmonary and tricuspid) [2]. VHD can evolve before birth or acquired during the life due to some infection such as: a rheumatic fever; while acquired VHD is more common. Sometimes the reason is unknown, but often the valves structure changes as a result of mineral deposits, either all over it or its surrounding tissues [3]. Unfortunately, all functional disorders of heart valves may not lead directly to specific symptoms. The injured may remain undetected the disease for a long period of time that may extend to years. Because the disease is not discovered and neglected, the heart will suffer from real damage and thus all fuse disorders are a rotational burden on the heart and eventually lead to heart failure [4].
Ceruloplasmin (Cp) [EC 1.16.3.1, Ferroxidase; Iron (II): O2 oxidoreductase] can be defined as the major blue copper containing glycoprotein which has a molecular weight of 132 kDa. It is consists of a single polypeptide chain of (1046) amino acid residues with carbohydrate content (between 7% and 8%). Essentially, Cp is synthesized and secreted by the liver as well as its expression was also found in numerous organs such as: heart, lung, kidney, brain and lymphocytes [5].

Ceruloplasmin, the multifunctional copper containing enzyme, possesses significant functions such ferroxidase activity, oxidase activity, transport, mobilization, and homeostasis of copper, serum antioxidant, and endogenous modulator of the inflammatory response [6,7]. Interestingly, the copper (Cu) in Cp plays an important role in iron oxidation before it is transported to the plasma. In recent years, it has become increasingly evident that the essential metal plays critical roles in a wide range of physiological processes, Fe, Zn, Mn, and Cu. Remarkably, Cu importance is apparent in human physiology [8].

Lipid profile is about tests group which are often requested together to determine the heart disease risk. Therefore, these tests are considered good indicators which someone is possible to have a heart attack or stroke due to blockage of blood vessels or arteries hardening. It is typically comprises: [9] (i) total cholesterol [TC] that strongly associated with progression of heart disease [10]; (ii) High density lipoprotein-cholesterol [HDL-C] which is also known as good cholesterol, and its function to protect from atherosclerosis [11]; (iii) Triglycerides [TG] are a form of bloodstream fat that derived from glycerol and fatty acids [12]. It has several functions, such: an excellent insulation material which forms a layer around the body that conserves heat and converts toxic excess of the sugars required for brain functions [13]; (iv) Low density lipoprotein-cholesterol [LDL-C] which is also called bad cholesterol, and its Cholesterol-rich lipoproteins that result from the breakdown and removal of TG from intermediate-density lipoproteins. However, elevated LDL-C levels in blood increase the risk of artery and heart disease [14]. Thus, the current study aims to determine the Cp ferroxidase activity, Cu level and evaluate the lipid profile parameters in sera of Iraqi patients with VHD.

2. Materials and Methods

2.1 Topics for Research

This research enrolled participants who attended the Baghdad, Iraq, Ibn Al-Bitar Center for Cardiac Surgery. Group 1 consists of 33 aortic valve (AV) patients (17 male and 16 female) with an age (55.5±13.3 years) and BMI (28.73±6.26) kg/m2. Ninety individuals were classified into three classes. Group 2: consists of 27 patients with mitral valve (MV) (10 male and 17 female) aged (53.81±10.00 years) and BMI (28.99±4.01) kg/m2 respectively. Group 3: a control group (C) composed of 30 healthy individuals (16 male and 14 female) with age (46.63 ± 8.79 years) and BMI (28.35 ± 3.79) kg/m2. Patients with diabetes, kidney and liver disorders were the criterion for exclusion. The body mass index (BMI) was measured in kilograms by weight, divided into square meters by length. The study protocol was accepted by the College of Sciences/ University of Baghdad Ethical Committee.

2.2. Samples

In the plane tube, blood samples (10 ml) were obtained from all participant (patient and control) groups. The tubes were left at room temperature for coagulation for 10 minutes. The blood samples were then centrifuged at 4000 rpm for 10 min. The serum was isolated, aliquoted and processed for analysis at (-20 °C).

2.3 Cp Activity of ferroxidase and Total protein levels

The Cp (U/L) ferroxidase activity was calculated using the Erel[15] process. Results at a wavelength of 600 nm were measured using the Colorimetric method and absorbance. Using a total protein reagent kit (AGAPPE, Switzerland), serum total protein levels (g/dl) were calculated by observing a Biuret reaction to determine the basic activity of Cp ferroxidase. The basic Cp activity was expressed in protein U per g[6].

2.4 Copper concentration determination in serum
A direct colorimetric assay kit (LTA, Milano, Italy) without sample deproteinization[16] was used to evaluate copper in serum. The 3,5-DiBr-PAESA chromogen reacts with cupric ions (Cu2+/Cu+) and forms a blue-violet compound. The absorbance was then measured at a wavelength of 580 nm. The strength of the color is proportional to the sample concentration of Cu.

2.5 Lipid Profile Determination
For quantitative in vitro diagnostic measurements, serum total cholesterol (TC) was calculated using the colorimetric method Cholesterol Oxidase Phenol 4-Amino antipyrine peroxidase {CHOD-PAP} method [17], whereas triglycerides (TG) levels were assessed using the GPO-PAP enzyme assay [17,18]. A two-step assay was used to estimate HDL-C: (1) precipitation and then (2) enzymatic determination [17]. Using Friedwald's formula[19], serum LDL-C and VLDL levels were calculated.

2.6 Review of Statistics
SPSS version 21 (One-Way ANOVA) performed data analysis in this study, where the difference is considered to be highly significant at P<0.001, meaningful at P<0.05 and non-significant at P>0.05. The data is expressed as mean ± norm (mean ± SD) variance.

3. Results
The mean ± SD of age, gender and BMI for all AV, MV & C groups is shown in Table 1. There was a large difference in age between patients (AV & MV) and control (p<0.05), as shown in Table (1). In addition, there were no substantial variations (P>0.05) in BMI between the patients and the control groups.

Table 1. Age, gender and BMI of all studied groups.

| Parameters | Groups | C (n=30) | AV (n=33) | MV (n=27) |
|------------|--------|----------|-----------|-----------|
| Age (years) |        | 46.63±8.79 | 55.50±13.3* | 53.81±10.0* |
| Gender | Male (%) | 16 (53.3%) | 17 (51.5%) | 10 (37.0%) |
| | Female (%) | 14 (46.6%) | 16 (48.4%) | 17 (70.8%) |
| BMI (Kg/m²) | | 28.35±3.79 | 28.73±6.26 | 28.99±4.01 |

* p<0.05, ** p<0.001

Table 2 presents the Cp ferroxidase function, TP and Cp specific activity in both patients (AV & MV) and C groups. Cp activity and specific activity were found to be highly significant changes in the AV and MV groups relative to the C group (p<0.001), although no significant differences between AV and MV were observed. The findings, meanwhile, showed no major differences in TP levels in all the groups examined.

Table 2. Mean values ± SD of total protein, Cp ferroxidase and specific activities in the serum of patients and control groups.

| Parameters | Groups | C (n=30) | AV (n=33) | MV (n=27) |
|------------|--------|----------|-----------|-----------|
| Cp ferroxidase activity (U/L) | 836.53±50.11 | 1735.59±101.61** | 1735.33±75.95** |
In addition, the serum Cu concentration of both patient groups (AV & MV) showed a highly significant increase \((p<0.001)\) relative to the C group, while there were no significant differences between AV and MV. Comparing the two patient groups (AV&MV) with the C group in Table 3, the findings showed that there was a highly significant increase in TG and VLDL levels \((p<0.001)\) in the patient group relative to the C group. Furthermore, the findings in Table 3 showed no substantial differences \((p>0.05)\) in the levels of TC, LDL-C and HDL-C in the AV and MV groups with respect to the C group. The findings also showed no major variations in all parameters between the AV and MV classes (TC, TG, HDL-C, LDL-C and VLDL).

**Table 3.** Mean values ± SD of lipid profiles parameters levels in the serum of patients and control groups.

| Parameters     | Groups                  |
|----------------|-------------------------|
|                | C \(n=30\)              | AV \(n=33\)             | MV \(n=27\)             |
| TC (mg/dl)     | 176.33±17.91            | 178.74±16.77            | 185.44±15.83            |
| TG (mg/dl)     | 118.6±19.13             | 178.9±16.44**           | 185.44±12.87**          |
| HDL-C (mg/dl)  | 46.57±6.35              | 47.82±6.35              | 48.96±4.71              |
| LDL-C (mg/dl)  | 106.0±17.29             | 97.72±13.14             | 99.41±18.43             |
| VLDL (mg/dl)   | 23.72±3.83              | 35.79±3.21**            | 37.07±2.57**            |

* \(p<0.05\), ** \(p<0.001\)

4. Discussion

Ceruloplasmin (Cp) is a human serum protein that holds about 95% of the total Cu plasma in healthy individuals. It is synthesized in the liver [95% in the hepatocyte], but other forms of cells, such as astrocytes, monocytes, and sertoli, are also formed [20]. A number of studies on several forms of cardiovascular diseases [CVD] have reported a near link between increasing serum Cp and an increased risk of myocardial infarction (MI), arteriosclerosis, angina and coronary artery disease (CAD) [21-23], while there are few studies on Cp in rheumatic patients and VHD [20]. This is the first research, to the best of our knowledge, to deal with the relationship between Cp and VHD activity, which showed that Cp ferroxidase activity was significantly elevated in patients with VHD. A previous study carried out in children with acute rheumatic fever documented elevated levels of Cp at the time of diagnosis [24]. Similarly, Petelenz et al. also revealed that Cp levels were significantly increased relative to safe controls in acquired VHD patients [25].

Ceruloplasmin (Cp) is the most enzymatic contributor to the antioxidant protection of human plasma, which acts as an antioxidant through various mechanisms, such as: inhibition of iron-dependent lipid peroxidation and formation of hydroxyl radical [OH] from hydrogen peroxide through its involvement in
ferroxidase, reaction and scavenging of H2O2 and superoxide anion, and inhibition of copper-induced lipid peroxidation

Unfortunately, there is no clear explanation of the function and status of Cp in cardiovascular pathophysiology. One of the mechanisms shown by Cp to have major oxidase activities, including the ability to catalytically consume NO through NO oxidase activity, resulting in decreased bioavailability of NO plasma as well as enhancing oxidation of low-density lipoprotein [22, 27]. NO also plays an important role in heart contraction and alteration in the secretion of endogenous NO may lead to heart failure (HF)[22,28]. The key proatherogenic role of oxidized low-density lipoprotein in the arterial wall, however, is that reduced NO bioavailability promotes endothelial dysfunction, which also leads to atherosclerosis[22]. In vitro and in vivo studies in humans with aceruloplasminemia showed a decrease in the activity of plasma NO oxidase following Cp immunodepletion [27,29]. Thus, via increased NO oxidase activity, high Cp levels can decrease the available NO in the heart; then leading to increased oxidative stress (OS) causes further dysfunction, which would explain the correlation between higher Cp levels and heart disease incidents.

Essentially, Cu, which plays a vital role in the oxidant/antioxidant, is one of the significant trace elements for humans. Cu dyshomeostasis can result in cardiovascular disease (CVD). Several studies therefore support the possibility that high Cu levels can increase the risk of CVD [30]. The current study showed a higher than safe control level of Cu in VHD. In particular, there are no studies in the literature to examine the degree of Cu in VHD; we compared our findings with other forms of CVD, however. In a study conducted in patients with HF, serum Cu levels were found to be higher than in healthy individuals, finding an important correlation between high serum Cu and HF [31]. It is also increasingly recognized that Cu is an important mediator in MI, CAD and atherosclerosis development and progression [30,32]. In addition, the high serum Cu in VHD is likely to represent a highly significant increase in Cp, which binds approximately 95% of the serum's circulating Cu [33]. Cp has also been shown to have many functions in the transportation of Cu, coagulation, angiogenesis, oxidant stress defense, and iron homeostasis. In addition, Cp oxidizes the {ferroxidase function} of Fe2+ to Fe3+ to be connected to transferrin, exerts the activity of antioxidant glutathione peroxidase, and scavenges reactive oxygen species {ROS}. It is also likely to be mainly involved in iron-mediated free radical injury protection [34,35].

Several studies have shown several parallels between atherosclerosis and calcific aortic stenosis (CAS) histopathologic features [36-38]. Atherosclerosis risk factors and CAS also interfere; one of them is dyslipidemia [39,40]. In AV and MV situations, as well as controls, the lipid profile was carried out in the present analysis. The findings showed a highly significant rise in the amount of TG in patients as opposed to controls. This result is in line with Peltier et al.[41], who documented higher levels of TG in patients with CAS. Another study carried out in patients with VHD showed that the amount of TG in patients was substantially higher compared to normal individuals. Hypertriglyceridemia is therefore caused by excess TG in the blood, and elevated TG in the blood has been linked to atherosclerosis[14]. Mean TC, HDL-C and LDL-C levels in this sample, meanwhile, showed no substantial difference in both patients and control groups. Our findings are consistent with the results of previous studies by Afaq et al. and Ortlepp et al. [42,43]. In comparison to the other studies reported by Peltier et al. who found that there was a rise in TC level (hypercholesteremia) in patients with CAS[41] and Hasan et al. who found that there were significantly higher LDL-C and TC levels, while significantly lower HDL-C levels were observed in patients with VHD compared with normal individuals [14]. Regarding the level of LDL-C, our research was in line with the study which showed insignificant levels of LDL-C between patients with calcific valvular heart disease and controls [44].

5. Conclusion
There is no research that discusses the enzymatic behavior of Cp in patients with VHD to date by reviewing the literature. The current findings concluded that changes in Cp ferroxidase activity and Cu levels were found to be highly important in groups of VHD patients and could be used as risk factors for disease prognosis and diagnosis. The Cp value can also be used as an independent biomarker related to
the level of VHD. For all factors, except for triglycerides (TG), there were no variations in the lipid profile that could be used as a predictor for predicting the early occurrence of VHD.

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