RESEARCH ARTICLE

Estimation of Ceruloplasmin Activities and Some Biochemical Parameters in Iraqi Patients with Cholelithiasis

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ABSTRACT

Cholelithiasis is known as a presence or formation of gallstones in the bile duct, which is one of the most prevalent gastrointestinal tract diseases. Three times more likely to develop this disease was in female than male. The objective of the present study was to evaluate the changes in Cp activities (oxidase and ferroxidase) and lipid profile in sera of cholelithiasis patients compared to healthy subjects matched in age and gender. The study was conducted on 113 individuals divided into two groups: 63 patients with Cholelithiasis (PM 26 male and PF 37 female), and 50 healthy persons as a control group (CM 25 male and CF 25 female). The Cp oxidase and ferroxidase activities were measured by spectrophotometry; serum total protein (TP), copper (Cu) and lipid profile were measured by available commercial kits. The results were indicated no significant difference in TP levels in all studied groups. A high significant increase in Cp oxidase and ferroxidase activities in patients groups (PF & PM) compared with controls (CF & CM), while no significant differences between PF and PM in Cp oxidase activity with significant differences in Cp ferroxidase activity. Furthermore, high significant increase found in Cu concentration in PF than CF and significant increase in PM than CM as well as no significant differences between PF and PM. High significant increase found in Technetium (TC), triglycerides (TG), low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL in patients groups (PF and PM) as compared to their corresponding control groups (CF and CM) with significant decrease in HDL. No significant differences reported in TC, TG, HDL, LDL & VLDL between PF and PM groups. In conclusion, cholelithiasis was associated with abnormal lipid profile as well as the high oxidase and ferroxidase activity of Cp may be considered as a risk factor and development the gallstone.

Keywords: Ceruloplasmin, Cholelithiasis, Copper, Lipid profile.

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INTRODUCTION

Cholelithiasis or gallstones are hardened stones that form in the gallbladder or bile ducts. Gallstones can be as small as a grain of sand or as large as a golf ball. On the other hand, small stones can cause the most problems since these stones can leave the gallbladder and become lodged, whereas larger stones prefer to remain quietly in the gallbladder.[1] Because of the effect of female sex hormones on hepatic function, women are significantly more likely than males to have gallstones; likewise, roughly 1 in 5 older persons has gallstones.[2,3] Gallstone is classified into different types based on their chemical composition, including cholesterol stones (CS) {which is the most common type that results from the presence of too much cholesterol in the bile}, pigment stones (PS), and mixed stones (MS).[4,5] The majority, around two-thirds, of patients with gallstones are asymptomatic throughout their life.[6] However, pain in the belly and back that is uncommon but intense, followed by an increase in abdominal pain after eating a fatty meal, fever, and pain are all indications of cholelithiasis.[7]

Ceruloplasmin {CP; EC: 1.16.3.1} is glycoprotein also known as blue oxidases with a molecular weight (132 KDa) that binds six copper (Cu) ions; thus, it contains about 90–95% of Cu in serum. In addition, Cp is produced and secreted mainly in the liver as an apo-form with 1046 amino acids protein. Its expression was also found in numerous organs such as the heart, lung, kidney, and brain.[8] Ceruloplasmin is a multifunctional copper-containing enzyme that possesses many functions including (1) transport, mobilization, and homeostasis of Cu, (2) ferroxidase activity, (3) oxidase activity, and (4) serum antioxidant, as well as (5) endogenous modulator of the inflammatory response.[9] Previous studies reported that high Cp accumulation is found in several conditions, like infections, malignancy, inflammatory reactions, and tissue damage.[10] As presented in the literature, over 90% of Cu in humans is bound to Cp as a non-dialyzable fraction, while the

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rest (5–10%) of plasma Cu is thought to be weakly bound to albumin and histidine, with only traces of Cu present as free Cu^{+2}. Cp is also known as Cu oxidase because of its oxidase activity, which can determine Cp. Cp also performs ferroxidase activity at the cell surface by binding iron to transferrin, which is the initial step in converting Fe^{+2} to Fe^{+3}.[11]

The lipid profile is a group of tests that are often requested together, which includes: Triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL). Hyperlipidemia is defined by high TC, TG and LDL, levels in the blood, and low HDL levels. Some investigations have discovered a link between hyperlipidemia and gallstones,[12,13] while on the contrary others have shown no link between hyperlipidemia and gallstones.[14,15]

Thus, the objective of the current study was to evaluate the changes in Cp oxidase and ferroxidase activities and compare the abnormalities of lipid in the gallstones patients with the healthy controls.

MATERIALS AND METHODS

Subject

A total of 113 individuals were included in the present study, 63 patients with cholelithiasis and 50 apparently healthy individuals as controls. The patients' samples were collected from September to November 2020 among patients attending the Baghdad Medical City, Baghdad, Iraq. The patient groups consist of 63 patients with cholelithiasis divided into two subgroups, 26 male (PM) group with age (36.39±9.52 years) and BMI (25.16 ± 3.69 kg/m^2), and 37 female (PF) group with age (35.00± 10.20 years) and BMI (25.26 ± 3.93 kg/m^2). The control groups consist of 50 healthy individuals classified into two subgroups, 25 male (CM) group with age (30.4±8.6 years) and BMI (23.8 ± 3.9 kg/m^2), and 25 female (CF) group with age (33.24 ± 7.31 years) and BMI (24.12 ± 3.08 kg/m^2).

Blood Samples

The venous blood samples about (8–10) mL from patients and control groups was drawn and collected in the plane tube then left for 5 minutes at room temperature for clotting. Blood was centrifuged at 3000 rpm for 5 minutes at room temperature to separate the sera. The obtained serum was aliquot and stored at −20°C until use.

Determination of the Activities of Cp and Total Protein (TP) Level

The Erel assay[16] was used to determine serum Cp ferroxidase activity, while the modified Rice method[17] was used to measure serum Cp oxidase activity.

Serum total protein concentrations (g/dL) were determined by observing a Biuret reaction using a total protein reagent kit (Human, Germany). To calculate the Cp oxidase and ferroxidase specific activities, the following equation was used:

$$\text{Specific activity (U / g of protein)} = \frac{\text{Enzyme activity (U / L)}}{\text{TP concentration (g / L)}}$$

Determination of Copper Concentration

The serum copper level was measured by a direct colorimetric assay kit (Centronic, Germany). Briefly, the chromogen {3,5-DiBr-PAESA} react with cupric ions {$Cu^{2+} \rightarrow Cu^{+}$} to form a blue-violet compound, then the absorbance (A) was measured at 580 nm. The Cu concentration in the sample is proportional to the color intensity.

Determination of Lipid Profiles

The lipid profile assay in serum was including TC, TG, and HDL, which measured spectrophotometrically using enzymatic colorimetric method,[18,19] according to Human kits

Table 1: The age and BMI of all studies groups

	Groups			
Parameters	*CF (n = 25)*	*PF (n = 37)*	*CM (n = 25)*	*PM (n = 26)*
Age (years)	33.24 ± 7.31	39.41 ± 10.20	30.40 ± 8.60	36.39 ± 9.52
BMI (Kg/m^2)	24.12 ± 3.08	25.26 ± 3.93	23.80 ± 3.90	27.27 ± 2.75^{b*}

*p<0.05, **p<0.001
(a): significant difference between (CF) and (PF).
(b): significant difference between (CM) and (PM).
(c): significant difference between (PF) and (PM).

Table 2: The Cp oxidase and ferroxidase activities with their specific activities and TP concentration of all studied groups

	Groups			
Parameters	*CF (n=25)*	*PF (n=37)*	*CM (n=25)*	*PM (n=26)*
TP (g/dl)	7.057 ± 0.939	7.032 ± 0.479	7.490 ± 0.802	7.179 ± 0.565
Cp oxidase activity (U/L)	81.504 ± 10.99	98.290 ± 18.37^{a**}	84.189 ± 10.47	95.352 ± 10.07^{b*}
Cp oxidase specific activity (U/g)	1.171 ± 0.20	1.408 ± 0.27^{a**}	1.139 ± 0.21	1.338 ± 0.20^{b*}
Cp ferroxidase activity (U/L)	941.2 ± 195.4	1884.7 ± 228.1^{a**}	951.6 ± 128.4	1733.5 ± 201.9^{bc**}
Cp ferroxidase specific activity (U/g)	13.46 ± 2.73	26.97 ± 4.05^{a**}	12.88 ± 2.42	24.34 ± 3.82^{bc**}

*p<0.05, **p<0.001
(a): significant difference between (CF) and (PF).
(b): significant difference between (CM) and (PM).
(c): significant difference between (PF) and (PM).

(Germany). In contrast, LDL and very VLDL were calculated using the Friedewald formula.[20]

Statistical Analysis

The statistical analysis was performed using the SPSS software (version 19). The data were presented as mean ± standard deviation (SD) and the statistical comparison between the studied groups by using one-way ANOVA, which was considered significant at ($p<0.05$), highly significant at ($p<0.001$), and non-significant at ($p>0.05$).

RESULTS

The mean ± SD of ages and BMI for all studied groups who enrolled in this study are presented in Table 1. The results show that there was no significant difference in age between patients (PF&PM) and control groups (CF&CM) at ($p>0.05$). In addition, there were no significant differences in BMI in all studied groups ($p>0.05$) except between (CM and PM) groups were significant differences at ($p<0.05$).

In Table 2, there were no significant differences in TP concentration in all studied groups. A high significant increase was found in Cp oxidase and ferroxidase activities with them specific activities in PF group compared with CF. Also, a significant increase was indicated in PM than CM groups in Cp oxidase and specific activities with a significant increase in Cp ferroxidase and specific activities. When comparing PF and PM, there were significant differences in Cp ferroxidase activity with its specific activity while no significant differences in Cp oxidase activity with its specific activity (Table 2).

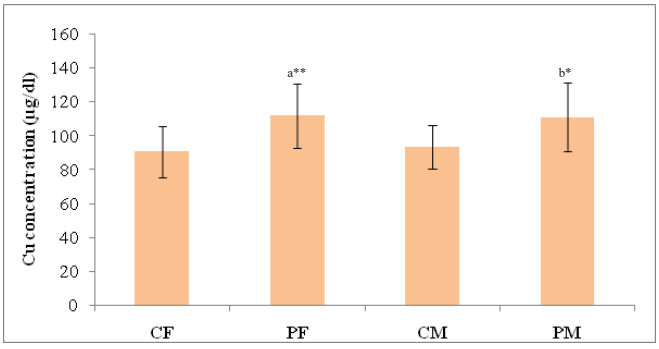

Figure 1: The Cu concentration in serum of all studied groups* $p<0.05$, ** $p<0.001$
(a): significant difference between (CF) & (PF).
(b): significant difference between (CM) & (PM).
(c): significant difference between (PF) & (PM).

As shown in Figure 1, a highly significant increase indicated in serum Cu concentration in PF group compared with CF group ($p<0.001$), and a significant increase in PM compared with CM ($p<0.05$). Additionally, no significant differences were found between PF and PM.

Lipid profiles of patients and control groups are presented in Table 3. The results of serum TC were showed a highly significant increase in patients groups ($p<0.001$) as compared to their corresponding control groups, while no significant differences between (CF and CM) groups were found ($p>0.05$) with a highly significant increase in PF than PM.

Moreover, there was a highly significant increase ($p<0.001$) in TG levels in PF and PM comparing to CF and CM, respectively. While no significant differences were reported between PF and PM groups.

The result of HDL levels found a significant decrease ($p<0.05$) in PF than CF, and PM than CM, but no significant difference between PF and PM.

The LDL levels showed significant differences between (CF and PF), (CM and PM) ($p<0.001$), while no significant differences between PF and PM ($p>0.05$).

In addition, very-low-density lipoproteins (VLDL) levels increased in PF and PM compared to their corresponding controls CF and CM, respectively. However, no significant difference ($p>0.05$) was found between PF and PM.

DISCUSSION

Cholelithiasis or gallstone diseases are among the most prevalent gastrointestinal disorders and very common diseases; hard stone-like substance, round or oval faceted, usually occurs in the gallbladder or bill duct.[3] The basis for which is the weakened metabolism of cholesterol, bilirubin, and bile acids, characterized by the formation of gallstones in the hepatic bile duct, common bile duct.[21]

Table 3: The lipid profile for all studied groups

	Groups			
Parameters	*CF (n=25)*	*PF (n=37)*	*CM (n=25)*	*PM (n=26)*
TC (mg/dL)	136.35 ± 14.44	181.28 ± 9.68^{a**}	141.22 ± 15.84	171.98 ± 7.87^{bc**}
TG (mg/dL)	113.81 ± 9.11	171.11 ± 4.81^{a**}	111.42 ± 12.50	170.08 ± 3.78^{b**}
HDL (mg/dL)	44.30 ± 5.74	39.84 ± 6.19^{a*}	46.82 ± 5.75	41.69 ± 4.87^{b*}
LDL (mg/dL)	69.36 ± 14.21	103.63 ± 10.97^{a**}	71.67 ± 13.87	96.28 ± 8.99^{b**}
VLDL (mg/dL)	22.75 ± 1.82	34.22 ± 0.96^{a**}	22.31 ± 2.50	34.03 ± 0.76^{b**}

* $p<0.05$, **$p<0.001$
(a): significant difference between (CF) and (PF).
(b): significant difference between (CM) and (PM).
(c): significant difference between (PF) and (PM).

Ceruloplasmin is a glycoprotein that contains Cu, and its physiological role involves redox reactions in the plasma. It is the major protein that carrying Cu in the blood and is involved in iron metabolism. Also, Cp is a liver enzyme that contains 6 atoms of copper in its structure and carries around 70% of the total Cu in human plasma, whereas albumin only carries around 15%.[22]

Our present study revealed that there were no significant differences in total serum protein between all studied groups. According to the literature, this is the first study deal with the association between the activities of Cp and cholelithiasis, which indicated that the activities of Cp (oxidase and ferroxidase) were significantly elevated in patients groups (PF and PM) in comparison with control groups (CF and CM). The previous study shows a significant increase of the serum Cp concentration in the group pre and each of post {short and long terms} cholecystectomy compared with the healthy control group.[23] The possible reason for this increase was the increase of the hepatic protein synthesis resulting in endogenous mediator stimulants [cytokine and leukocyte] released during an acute phase response to tissue damage. In addition, our study indicated an elevated level of Cu in gallstone patients than healthy control. This finding agreed with other researchers who reported a significantly high serum Cu level in gallstone patients compared to healthy individuals.[24,25]

Gallstones are categorized into three kinds: cholesterol stones, pigment stones, and mixed stones. Cholesterol stones include between 51 and 99% pure cholesterol, whereas mixed stones comprise cholesterol, calcium salts, bile acids, phospholipids, and bile pigments in addition to cholesterol.[26] When there is too much cholesterol in the bile generated by the liver, cholesterol stones can form. Bile dissolves or breaks down cholesterol, while bilirubin is a substance created when the liver destroys old red blood cells. The stones form when the gallbladder is unable to break down the extra bilirubin. As a result, gallstone disease, a prevalent illness, may be linked to lipid abnormalities. So, the initial medical screening tool for lipids abnormalities is lipid profile tests in the blood such as TC, TG, and HDL.[27,28]

In this study, serum TC, TG, LDL, and VLDL levels in the patient's groups were significantly higher than in the control group, whereas HDL levels were significantly lower. This result was in conformation with the previous study found by Al-Saadi *et al*.,[29] a study done by Hayat *et al*.,[30] who reported a significant TG increase and HDL decrease in gallstone patients compared to the controls increase and LDL decreased in patients than controls but not statistically significant. Another study reported a statistically significant increase in TG and VLDL serum levels while no significant differences in serum levels of TC, HDL, and LDL in gallstone patients compared to controls.[31] In addition, a previous study reported significantly higher mean levels of TC, TG, LDL, and HDL in gallstone patients comparing with control.[32] However, Jindal *et al.*[33] found that serum TC, TG, and LDL levels were higher, and levels of serum HDL were lower in gallstone patients than of the control group, though not statistically significant. Therefore, the saturation of bile with cholesterol and lipid abnormalities (hyperlipidemia) plays a significant role in the pathogenesis of gallstones, and the link between gallstones and high levels of serum cholesterol in individuals can be explained by a variety of factors, including geography, social status, heredity, and dietary habits, all of which are linked to the pathogenesis of different types of gallstones.

Our results also revealed that the TC levels in the female patients (PF) group were significantly higher than the male patients (PM) group, which indicated that gallstones are more common in women than in men. These findings were in concordance with studies presented by Jindal *et al.*[33] and Kim *et al.*[34] So, the majority of the patients with gallstone belonged to the female gender may be attributing that estrogen increases biliary cholesterol secretion, causing cholesterol super-saturation of bile.

CONCLUSION

It was concluded that high oxidase and ferroxidase activities of Cp and serum lipid abnormalities (hyperlipidemia) play an important role in gallstone disease, which may be considered risk factors and necessary to establish the gallstones formation and development.

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