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Ceruloplasmin Concentration in Patients with Chronic Liver Disease

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ABSTRACT

Ceruloplasmin (CP) is a glycoprotein containing copper located in the globulin part of human blood serum α2. More than 90 percent of the copper in the circulation of healthy human is carried by Ceruloplasmin secreted from hepatocytes that give powerful antioxidant and avoids lipid peroxidation through removing oxygen radicals. Metabolism of CP and function disorders occur in a number of liver diseases. This study was conducted in Hilla city, from October 2020- April 2021. The samples were collected from Babylon Center of Internal Medicine and Cardiology in Marjan Teaching Hospital in Hilla city Babylon Province.This study was included 86 (44 males, 42 females) subjects, which were divided into two groups 50 (26 males, 24 females) patients with chronic liver disease, and 36 (18 males, 18 females) apparently healthy persons as a control group. Ceruloplasmin concentration were determined by use enzyme-linked immunosorbent assay (ELISA) technique, The results of the present study were shown CP concentration not differ significantly between these groups .The present study advises to carry out genetic study for ceruloplasmin gene and CP concentration in patient with chronic liver disease as well as control.

Introduction:

Ceruloplasmin (CP) is a glycoprotein containing copper located in the globulin part of human blood serum α2. It has 132 kDa molecular mass [1] . More than 90 percent of the copper in the circulation of healthy human is carried by a Glycoprotein secreted with hepatocytes called ceruloplasmin. Researches have shown that CP give powerful antioxidant and avoids lipid peroxidation through removing oxygen radicals. [2] Ceruloplasmin is a serum ferroxidase, the rate of oxidation of ferrous iron by molecular oxygen is incredibly quickened in vitro by the plasma copper protein, ceruloplasmin. Based on this enzymatic activity, ceruloplasmin has been delegated a ferro-02-oxidoreductase and designated ferroxidase. It has been suggested that the enzymatic oxidation of ferrous iron is a fundamental step in the production of transferrin [3].

It is best understood for its role in Wilson's pathogenesis and effect on the metabolism of iron. Ceruloplasmin is also consider as a positive acute-phase agent, thus increasing its levels in acute inflammatory conditions or cell damage [4]. the liver parenchymal cells synthesized it primarily ,with a little quantities by

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macrophages and lymphocytes .[5] A copper is placed by ATPase inside the cell to the protein chain, after formation it by ribosome. Typical folding of ceruloplasmin need Copper and it's significant additionally oligosaccharide attachment. The intracellular degradation occurs at high quantities of apoceruloplasm, that contains no copper or ATPase, whereas a small proportion enter circulating blood but these quantities has short half-life few days about four to five [4] . metabolism of CP and function disorders occur in a number of liver diseases [6] . Except for Wilson's disease patients with severe hepatitis show fundamentally lower levels of Ceruloplasmin concentration comparing with patients have other liver diseases [7,8] detailed that inflammation state and fibrosis stage contrarily relate and indirectly with ceruloplasmin in chronic viral hepatitis B patients. Routine lab factor is used in the APPCI model include CP to predict liver fibrosis accurately. In any case, Connections among inflammation of liver and CP and fibrosis remain unknown and It is essential that we investigate whether low serum CP amounts show prolonged hepatic damage or chronic hepatic disease repair. In this study, serum concentration of CP was analyzed and activity in chronic liver disease patients

Literature Review

Structure of ceruloplasmin (CP):

CP (in brain ,milk,spinal fluid and plasma) is mainly formed by 1046 sequence of amino acids and it has an overall mass around 130 kDa, 120 kDa of protein and 10 kDa of carbohydrate N linked to it [7]. The structure of human serum ceruloplasmin illustrate that it consists of six domains organized in a triangular array of plastocyanine forms [8]. The whole mononuclear coppers are connected to a cysteine residue, but also in domains 4 and 6 copper attached to methionine residue while in domain 2 methionine residues changed with leucine residue which can also form van der Waals contacts with the copper atom. In domain 6, the trinuclear centre and copper creates a group generally similar to those of ascorbate oxidase, which mainly indicates an oxidase activity of ceruloplasm in the plasma [9].

There are two forms of its CP one of them soluble types circulat in blood and other form found in glial (CNS as well as retina) and also Sertoli (testis) cells which is not free (GPI) anchoring ceruloplasmin [10] . Ceruloplasmin is already an ancient oxidase multicopper, developed to ensure safe oxygen holding in certain metabolic vertebrate pathways. The information now available on its structure provided a hint of its plasticity that reveals a number of binding sites illustrating a detailed multifunctional activity component mechanism [11] serum CP is biosynthesized as Apo-CP (Cu-free) from the liver cell endoplasmic reticulum (ER) and combine with Cu into the trans-Golgi system after the "chaperone" ATOX1 has been delivered to the Cu "pump" ATP7B (this is the same Wilson Disease Subunits), which results in the Cu conjucated form of CP called holo-CP (Cu) excretion from the cell by Exocytosis secretion. Cells in many other secretory areas of the body also produce CP, including the renal, mammary cells, placenta and the brain choroid

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plexus. [12] also CP produced during inflammation in the macrophages and mononuclear cells in blood [15,16] . In any event two different types of CP can express by cells. Firstly,CP linked to glycophosphatidyl inositol connects to the cell surface with the membrane (GPI). Now that differ by the alternative splice of exons ninteen and twinty only at transcript C end results in inserting 30 alternative amino acids replacing the last 5 amino acid of the plasma CP form, which mark it for the GPI connections. [7] A number of cells, including glial cells, pancreas cells, the hepatocytes, macrophage and the retinal epithelium cells, generate that latter isoform and expressing it. Thus the cellular egress of iron plays a key role. CP participates with the novel transmembrane protein called ferroportin which ready to send out ferrous iron (Fe+2) from the cell. This reaction should be finished by oxidation to ferric iron (Fe+3), to proper binding of extracellular iron to transferrin.

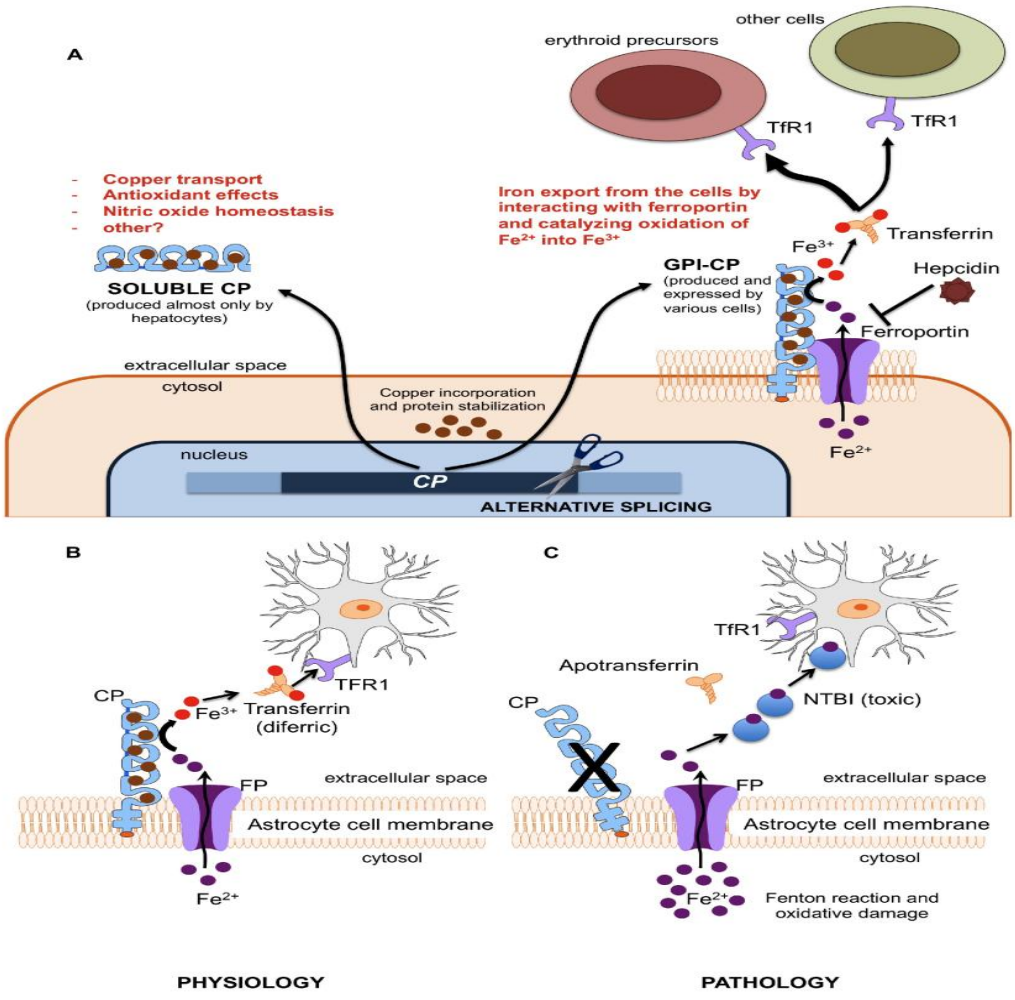

Figure (1-2): (A) Different elements of ceruloplasmine which is dissolvable and membrane attached. Membrane CP ferroxidase action is important, as only Fe+3 can be bonded into plasma transferrin and subsequently transmitted via Transferrin Receptor to the different cells (essentially erythroid precursors) . (B,C) The iron-talk pattern between astrocytes & neuronal cells. (B) Ceruloplasmin is required to oxidize Fe+2 to Fe+3, a single iron structure that could be transferred to neurons via the

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transferrin receptor type 1 for proper iron reception (TfR1). (C) Lack of ceruloplasm causes astrocytes to accumulate iron and neurons hunger. This later facilitates the neuronal absorption of additionally dangerous non-transferrin-bound iron sources (NTBI)

CP cojucated with GPI has also been known to interfere by hepcidin, a systemic iron homeostasis controller, with the modulation of ferroportin activity (figure 1)[12,17,18]. Other type, the soluble isoform is synthesized only by hepatic cells and constitutes approximately 94% of the plasma copper [15]**.** CP enzymatic Activity and levels affected by a variety of important factors, such copper deficiency, inflammatory cytokines such as interleukins, estrogen and progestogen levels. [16] CP function disorders and metabolism in several hepatic diseases are involved [17]

Methods

The target population of present study was 70 patients who have been diagnosed previously that having chronic liver disease specially chronic HBV and HCV which referred form Center of Internal Medicine and Cardiology in Marjan Teaching Hospital in Hilla city Babylon Province ,Iraq during October 2020- April 2021. In accordance with the background inspection clinical and biochemical investigations, patients are already diagnosed with chronic liver disease by the physician. A second group of generally healthy people was the control group. Each patient's history, including their ages, length of disease, and medical history, was collected. The samples were split into two groups: patients and control group.

Methodology

Ceruloplasmin concentration by ELISA

A- Kites Reagents

- Standard Solution at concentration (2400mg/L)
- Diluent vial for Standard
- streptavidin-HRP
- Substrate A Solution
- Substrate B Solution
- Concentrated Washing Buffer for (25X)
- Biotinylated human CP/CER Antibody

Bring all these reagents kit to room temperature before using. Ordinary To make a 1200mg/L standard stock solution, mix 120µl of the concetrated standard (2400mg/L) with 120µl of standard diluent. Allow 15 minutes

Before making dilutions, it is customary to sit with gentle agitation. Dilute the standard stock solution to make duplicate standard points. (1200mg/L) 1:2 with standard water in a series of steps steps to the end result numbered from 5 to 1: 1200mg/L, 600mg/L, 300mg/L, 150mg/L, 75mg/L. Buffer for Washing 20 mL Wash Buffer, diluted To make 500 mL of one time Wash Buffer, dilute 25 times with

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deionized or distilled water.

B-Assay procedure

1. We prepared Reagent kits, standard solutions, and samples are all included as instructed.
2. Firstly we was Calculate how many strips are needed for the assay. To use the strips, place them in the frames.
3. 50 µl of standard was pipetted and added it into the standard well. Since the standard solution includes biotinylated antibody
4. 40 µl of serum it was Placed in the wells specialized for samples, and then we added 10 µl of anti-CP/CER antibody50 µl of streptavidin-HRP in sample wells and those that contains standard (Not in the blank control well)and then we Mix and seal the plate Using a sealer then putting it about 60 minutes and 37 C of incubation
5. The sealer was removed and rinse with a laundry buffer 5 times for Washing, then all the well removed and washed them 5 times by washing solution buffer. Towels or other absorbent materials are used to blot the plate
6. Then 50 micron of substrate A added and the same volume also 50 micron substrate B to each well. After that a plate Incubated with sealant in the dark at 37°C for 10 min.
7. In each well we added 50µl of stop solution, the blue color will start turning yellow.
8. Directly after addition of a stop solution we used a 450 nm microplate reader, the density of the optics (OD) of each well was calculated.

C-Result Calculation

Create the standard curve by drawing an average optics density OD in the vertical axis (Y) cross the horizontal axis (X) for each standard, and draw the line curve in accordance with the points in the graph. Computer-based curve adjustment software is perfect for these measurements, and regression analysis can help decide the best match line.

Results

3.1. Demographic and Clinical Characteristics of the Study Groups

3.1.1. Difference Levels of CP concentration and activity between patient group and control group

The results are compared by using the analysis of variance (ANOVA). The differences with P-value of less than 0.05 are considered statistically significant

The results presented in **Table (1)** showed that there were no significant differences in Levels of CP concentration between patient group and control group**.**

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Table (1) : Difference Levels of CP concentration between patient group and control group

Parameter	Study group	No.	Mean ± SD	P value
Ceruloplasmin concentration (mg/dl)	Chronic liver disease patients	50	55.1345± 15.9305	0.714
	Healthy Control persons	36	56.4217± 11.0819	

Gender

Eighty six sample were enrolled in the present study as well as fifty patient , were 26 males (52%) and 24 females (48 %), as shown in figure (3-1).

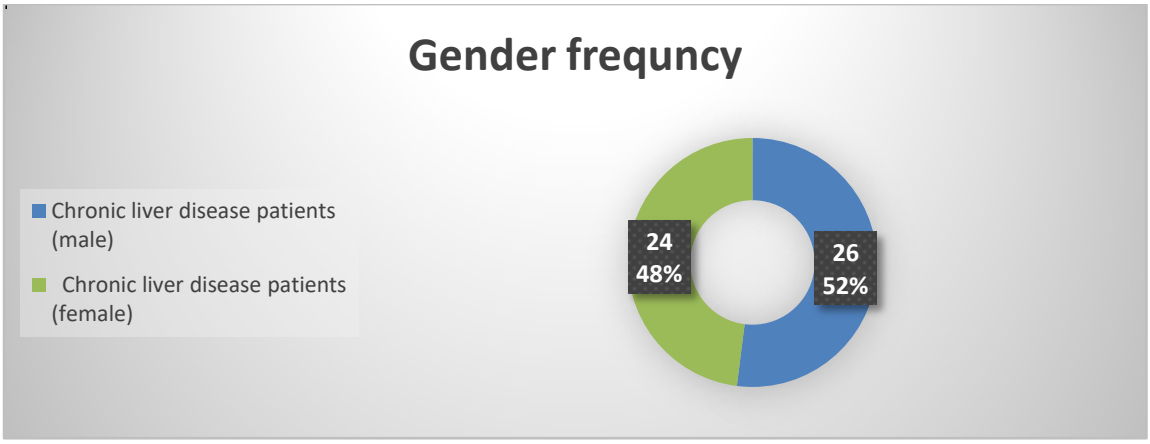

Figure (3-1): Gender frequency of patient group

The results presented in **Table (2)** showed that there were no significant differences in Levels of CP concentration between patient male and female group**.**

Table (2): Difference Levels of CP concentration between patient group male and female

Parameter	Study group	No.	Mean ± SD	P value
Ceruloplasmin concentration (mg/dl)	Chronic liver disease patients (male)	26	55.7259± 15.047	0.788
	Chronic liver disease patients (female)	24	54.4939± 17.1381	

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Type of chronic hepatitis

fifty patients with chronic liver disease were enrolled in the present study were 33 caused by hepatitis C virus (66%) and 17 caused by hepatitis B virus (34 %), as shown in figure (3-2).

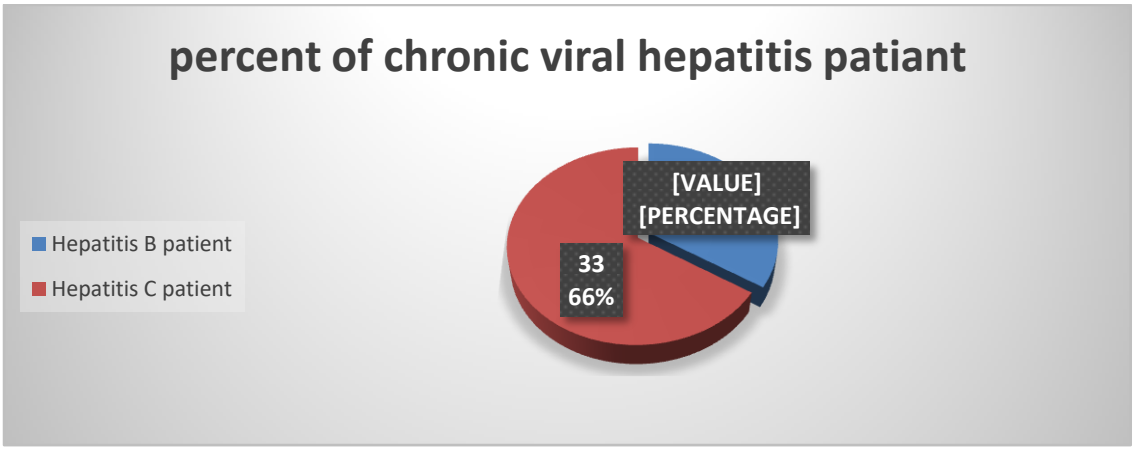

Figure (3.2): frequency of type of chronic hepatitis

The results presented in **Table (3)** showed that there were no significant differences in Levels of CP concentration between HBV and HCV patient group

Table (3): Difference Levels of CP concentration between patient group depend on type of hepatitis

Parameter	Study group	No.	Mean ± SD	P value
Ceruloplasmin concentration (mg/dl)	Hepatitis B patient	17	59.3921± 18.4638	0.306
	Hepatitis C patient	33	52.9412± 14.2672	

Discussions

Ceruloplasmin And Liver Disease:

1. Liver Fibrosis:
   Ceruloplasmin level in patients with chronic viral hepatitis has been shown to predict liver fibrosis, both in chronic hepatitis B (CHB) and chronic hepatitis C (CHC). Liver biopsy is still considered the “gold” standard in assessing liver fibrosis. Nevertheless, the biopsy procedure has some limitations such as invasiveness, sampling variability, and cost. Moreover, liver biopsy has been superseded to some extent by the development of imaging techniques such as transient elastography (TE), acoustic radiation force impulse (ARFI),

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ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI)[[18][19][20] [21]. On the other hand, the high cost of (or poor access to) modern imaging techniques may represent additional limitations in the evaluation of liver fibrosis in developing countries, which can preclude their use. Zeng DW *et al.* showed that CP was independently and negatively associated with liver fibrosis, furthermore; they developed a novel method based on routine serum markers (namely GGT in addition to ceruloplasmin level), for predicting liver fibrosis in CHB patients with normal or minimally raised ALT. They constructed the following CG model:

$$CG= 3.76-0.034 \times CP\ (mg/L) + 0.013 \times GGT\ (IU/L)$$

The optimal value for predicting cirrhosis from this CG model was -1.38.[22]

Similarly Na-Ling Kang *et al.*, concluded that serum CP level had an inverse correlation with liver fibrosis on 75 patients with CHB [23]. They constructed a predictive index including CP [Ceruloplasmin hepatitis B virus (CPHBV)] utilizing CP level, platelets count and HBs Ag. levels, as follows:

$$CPHBV= 37.122-10.072 \times \log CP\ (mg/L) - 4.291 \times \log PLT\ (109/L) - 0.958 \times \log HBsAg\ (IU/mL)$$

The cutoff value of this equation was 0.0304 for F2, 0.496 for F3 and 0.553 for F4 in the training group and 0.174 for F2, 0.176 for F3 and 0.206 for F4 in the validation group. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 81.1%, 71.9%, 76.9%, and 76.7%, respectively, in the training group, and 72.4%, 80.3%, 82.1%, and 70.0% in the validation group.

Da-Wu Zeng et al. showed that CP levels correlate negatively and indirectly with inflammation and fibrosis stages in male CHB patients[21].They utilized 198 samples from patients with CHB, all were evaluated by liver biopsy in addition to the level of ceruloplasmin in their sera.

Mohammed Salah Hussein concluded that ceruloplasmin can be used as a marker for responsiveness to direct acting antiviral drugs (DAAs), he included 100 patients with CHC and he showed that serum Ceruloplasmin levels were slightly higher after receiving DAAS and so it can be used as a marker for responsiveness to direct acting antiviral drugs [24].

2. Hepatitis: Acute viral hepatitis: Ceruloplasmin levels increase in cases of acute viral hepatitis in the first week after the appearance of jaundice and starts to decrease in the following weeks to return to normal in four weeks[6].

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In chronic viral hepatitis, little is known about the alteration in the level of ceruloplasmin level in patients sera.

To our best knowledge, this is the first work to investigate the significance of ceruloplasmin oxidase activity in patients with chronic hepatitis, including CHB and CHC in different stages of therapy .

Conclusion

CP concentration not effected by chronic liver disease according of its levels between chronic liver disease patient and control in this study so we can't depend on it as marker for chronic liver disease

Limitations and Future Studies

The present study advises to carry out Genetic study for ceruloplasmin gene and CP concentration and activity in patient with chronic liver disease as well as control . also Study the effect of the antiviral drug that taken by HBC&HCV patient to the CP activity and concentration

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