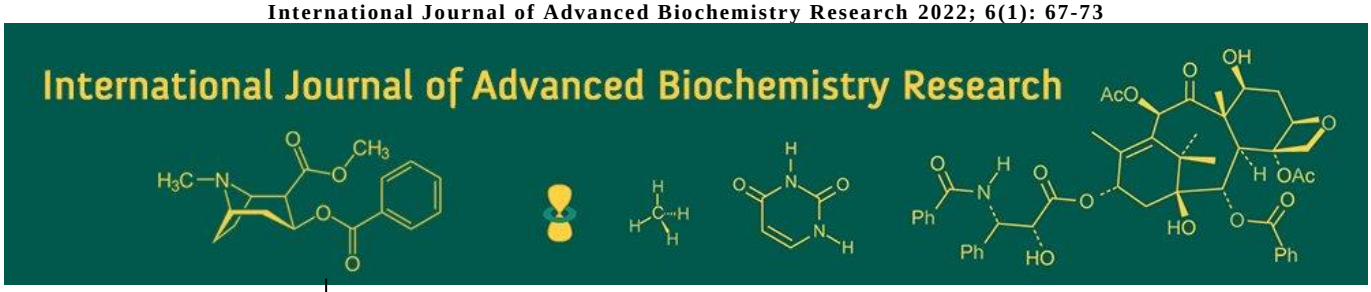

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2022; 6(1): 67-73
www.biochemjournal.com
Received: 19-01-2022
Accepted: 23-03-2022

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Study of serum levels of trace elements in chronic liver disease

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DOI: https://doi.org/10.33545/26174693.2022.v6.i1a.91

Abstract
Introduction: Trace elements play crucial roles in the maintenance of health since they are involved in many metabolic pathways. The liver largely regulates most of the metabolism of trace elements. A deficiency or an excess of some trace elements, including zinc, copper and magnesium frequently causes some metabolic disorders. Thus, the administration or depletion of these trace elements can improve metabolic disorders and liver dysfunction.
Objective: The purpose of this study was to evaluate the relationship between serum trace elements with liver function tests among chronic liver disease (CLD) patients.
Materials and Methods: The study was carried out from January to December 2021 in the Department of Biochemistry, Teerthanker Mahaveer Hospital, and Moradabad. 100 subjects, 50 patients with chronic liver disease and 50 healthy controls were included. Serum copper (Cu), zinc (Zn), and magnesium (Mg) concentrations were measured by using an automated method.
Results: Serum Cu was significantly higher in the CLD group than in the control group (p<0.001), whereas serum Zn and Mg were significantly lower in the CLD than that in the control group (p<0.001 and p<0.05) respectively. Serum Cu was significantly positively correlated with serum total, direct & indirect bilirubin, SGOT and GGT. However, serum Zn and Mg were correlated negatively with SGPT and ALP respectively (p<0.001 for all).
Conclusion: Increasing liver dysfunction alters the metabolism of trace elements towards an excess of copper and deficiency of zinc and magnesium. Supplementation of zinc, magnesium and reduction of copper intake would delay the progression of chronic liver disease and can improve the survival of patients.

Keywords: Chronic liver disease, copper, magnesium, trace elements, zinc

Introduction
Liver is a vital organ that contributes to the metabolic process, immune responses as well as the production of complex compounds. It also regulates the transportation and distribution of trace elements [1]. Chronic liver disorders occur due to the slow breakdown through time of liver tissue (CLD) [2]. Infectious, autoimmune, metabolic, vascular, medicines and carcinogens as well as undiscovered etiologies, can all cause them. Cirrhosis and eventually, liver failure are common complications of CLDs. Moreover, the liver is important in the regulation of trace element bioavailability, tissue distribution and toxicity as well as the metabolic pathway & transport. It also plays role crucial in the bile production process that is how trace elements are excreted [3]. Chemical elements that are required in minute amounts for appropriate growth, development and physiological activities of the organism are known as trace element. By binding to plasma proteins the duodenum or jejunum absorbs trace elements which then pass out through the portal circulation. These trace elements are distributed to tissues and organs based on their needs. The liver is in process of producing proteins that are linked to a variety of elements such as copper and zinc in order to transport or distribute them. Alteration in trace element levels, whether due to deficiency or excess have a significant impact on several biological systems. In a few studies, trace elements such as Zn, Cu & Mg also were shown to have important protective or stimulating effects on the course of CLD [4].
Zinc is a trace substance which plays a function in a variety of physiological activities. It is the body second most prevalent trace element and has a major antioxidant and anti-inflammatory effects.

It is necessary for tissue formation as well as tissue healing mechanism [5]. Zinc decreases the risk of carcinogenesis and it protects cells against the effects of oxidative stress. Also, it is a component of superoxide dismutase, a free radical-removing enzyme [6]. Zn is well known for its ability to protect cells against oxidative damage, apoptosis and inflammation [7]. As a result, Zn deficiency can cause the generation of reactive oxygen species which can lead to liver inflammation. Zn has been suggested as being the most efficient chemical antagonist for propyl hydroxylase a collagen-forming activator. Zn through activating the collagenase enzyme, a Zn metalloenzyme promotes in the degradation of collagen and the prevention of liver fibrosis [8].

Another critical trace element, copper is involved in a variety of enzymatic and redox processes. Ceruloplasmin is a copper transporter that is produced in the body and is involved in a rapidly progressive reaction. It has an effective role in hemoglobin synthesis increased iron absorption and phospholipids synthesis. However, in excessive doses it is extremely toxic and can cause cellular damage [9]. Copper insufficiency can cause a change in mitochondrial structure, resulting in a reduction in fatty acid beta-oxidation. In patients with NAFLD, these occurrences can have an impact on hepatic lipid deposition. The increased copper level was thought to affect the liver parenchyma through lipid peroxidation which could lead to cell membrane malfunction, decreased fluidity, enzyme deactivation & ion permeability alterations [10].

Magnesium, a trace element is the body's commonest intracellular ion and fourth most abundant cation. It may be found in the nucleus, cytoplasm, mitochondria & endoplasmic reticulum and is detected in almost every organ of the body [11]. Magnesium is found to be linked to over 300 enzymatic activities including immunoglobin formation, adhesion of resistant cell, cytolysis of antibody & others. Low magnesium levels in serum and liver tissue on the other hand can prolong the progression of these disorders by disrupting mitochondrial function, inflammatory responses or metabolic abnormalities, according to findings from a study. Magnesium supplementation can also improve liver function in patients with certain liver disorders [12]. Alcohol has been shown to disrupt brain, skeletal muscle, heart and liver all have Mg transport and homeostasis. It was found that every 100 mg increase in magnesium consumption is linked to a 49% reduction in the chance of death from all liver disorders [13]. Patients with liver cirrhosis had considerably lower muscle strength and Mg, suggesting that it could play a role in hepatic encephalopathy and muscle cramps [14].

The role of copper, magnesium & Zinc in various liver diseases is less known, despite of our knowledge about the role of Zn and Mg in humans. The data that are available from previous studies is insufficient to reach a definitive conclusion on the relationship among serum copper, magnesium and zinc with chronic liver disease patients. Thus, as per evidences from above statements trace elements might also be changed as a consequences of the liver enzymes. Therefore, the current study is directed to investigate the levels of trace elements (Zn, Cu and Mg) in serum & also compare the results with that of normal healthy controls.

Materials and Methods

The study was done in the Department of Biochemistry, Teerthanker Mahaveer College & Research Center General Medicine OPD in Moradabad, Uttar Pradesh from January 2021 to December 2021. Total 100 subjects were selected and divided into two groups. Group I included 50 diagnosed cases with chronic liver disease. The subjects were in the age group of 20 to 60 years, including both males and females [15]. Group II included 50 age sex-matched healthy control subjects. Informed written consent was obtained from all the participants under study.

The study was approved by the Institutional Ethical Committee of the university TMU/IEC/20-21/100.

After an overnight fasting 12hrs, a blood sample (8 ml) was obtained from all subjects & dispensed into plain vials under proper aseptic conditions. After centrifugation 3000 RPM for 5 min, a serum sample was used for analysis of serum copper, serum zinc, serum magnesium and serum liver profile by different methods.

Exclusion criteria

Malignancy, Diabetes mellitus, Pregnancy, Renal failure, Drugs affecting levels of trace elements e.g. corticosteroids, digoxin thiazide, diuretics & others and past history of severe liver disorders were exclusion criteria [15].

Trace elements (serum copper, zinc & magnesium) was estimated by following methods:

- Copper and zinc serum levels were estimated by Colorimetric Method [16].
- Calmagite technique was used to estimate serum magnesium [17].
- The Diazo technique was used to calculate total and direct bilirubin [18].
- Serum glutamate pyruvate transaminase (SGPT/ALT) & Serum glutamate-oxaloacetate transaminase (SGOT/AST) were estimated by IFCC method [19].
- Serum ALP and GGT estimated by the Kinetic method [20].

Statistical analysis

Statistical Package for the Social Science (SPSS) version 28.0 for data analysis and Microsoft Word and Excel for graph generation. Mean and standard deviation was assessed & comparison was done using independent student's t-test. Correlation were done with Pearson's coefficient (r-value). p-value of <0.05 was considered as significant [S]. p-value of <0.001 was considered as highly significant [H.S].

Results

The results of the measured by different biochemical parameters in the research were listed below for chronic liver disease patients and controls.

Table 1: Showing distribution of cases according to age (in percentage)

Age in year	Number of patients (n=50)	Percentage (%)
20-30	8	16
31- 40	7	14
41-50	14	28
51-60	21	42

We found in our study that the largest number of chronic liver disease patients belongs to 51-60 years age group.

Table 2: Showing distribution of cases according to gender

Gender	Number of patients (n=50)	Percentage (%)
Male	32	64
Female	18	36

In this study, we found that 64% of patients were males and 36% were females with chronic liver disease patients. This shows that males are more susceptible to chronic liver disease than females.

Table 3: Comparison of various measured parameters between chronic liver disease patients and healthy controls

Parameters measured	Cases Mean ± S.D (n= 50)	Controls Mean ± S.D (n= 50)	p-value
Age (years)	44.88±11.39	43.32±10.16	0.472
Total bilirubin (mg/dl)	7.60±3.42	0.74±0.14	<0.001***
Direct bilirubin (mg/dl)	3.36±1.74	0.21±0.08	<0.001***
Indirect bilirubin (mg/dl)	4.22±2.08	0.53±0.10	<0.001***
SGOT (IU/L)	107.12±36.49	27.25±5.58	<0.001***
SGPT (IU/L)	123.74±30.66	30.66 ±5.20	<0.001***
ALP (U/L)	133.77±18.66	84.52±20.51	<0.001***
GGT (U/L)	114.15±34.87	49.38±9.33	<0.001***

****p*<0.001.Highly significant, >0.05. Not Significant

Table 4: Comparison of copper, zinc and magnesium between cases and controls

Trace elements	Patients Mean ± S.D (n= 50)	Controls Mean ± S.D (n= 50)	p-value
Copper (µg/dl)	126.68±29.51	87.45 ± 16.68	<0.001***
Zinc (µg/dl)	57.80 ± 12.21	68.87± 5.87	<0.001***
Magnesium (mEq/L)	1.25±0.61	1.48 ± 0.40	<0.05*

**p*<0.05. Significant, ****p*<0.001.Highly significant

The copper levels in chronic liver disease (126.68±29.51 µg/dl) were statistically significantly higher than found in healthy controls (87.45 ±16.68 µg/dl), *p*<0.001. When compared to the mean values measured in controls (68.87±5.87 µg/dl), the mean levels of serum zinc (57.80 ±12.21 µg/dl) were statistically consistently lower in chronic liver disease (*p*<0.001). The magnesium serum levels in chronic liver disease (1.25±0.61) mEq/L were significantly lower than in healthy individuals (1.48±0.40) mEq/L, *p*<0.05.

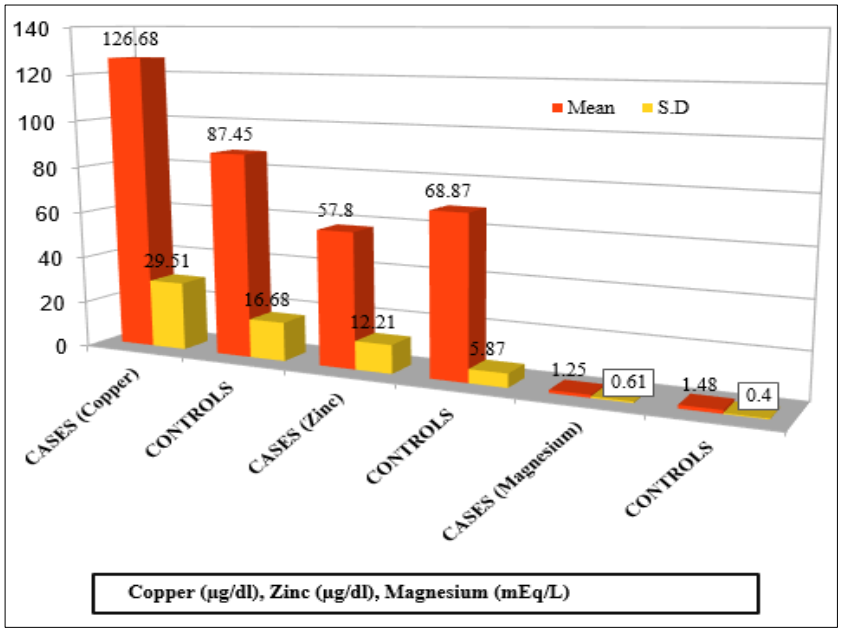

Fig 1: Trace elements in cases and controls

Table 5: Correlation between different trace elements (copper, zinc & magnesium) with serum levels of total bilirubin, direct bilirubin, indirect bilirubin, SGOT, SGPT, ALP and GGT in cases

Trace Elements		Total bil.	Direct bil.	Indirect bil.	SGOT	SGPT	ALP	GGT
Copper	Pearson Correlation	0.858	0.748	0.796	0.841	0.023	0.009	0.809
	p-value	<0.001***	<0.001***	<0.001***	<0.001***	0.874	0.949	<0.001***
Zinc	Pearson Correlation	0.103	0.061	0.110	-0.147	-0.829	0.150	0.089
	p-value	0.475	0.672	0.448	0.308	<0.001***	0.299	0.537
Magnesium	Pearson Correlation	-0.174	0.109	0.203	-0.026	0.102	-0.445	-0.068
	p-value	0.227	0.452	0.157	0.857	0.480	<0.001***	0.638

**p*<0.05. Significant, ****p*<0.001.Highly significant, >0.05. Not Significant

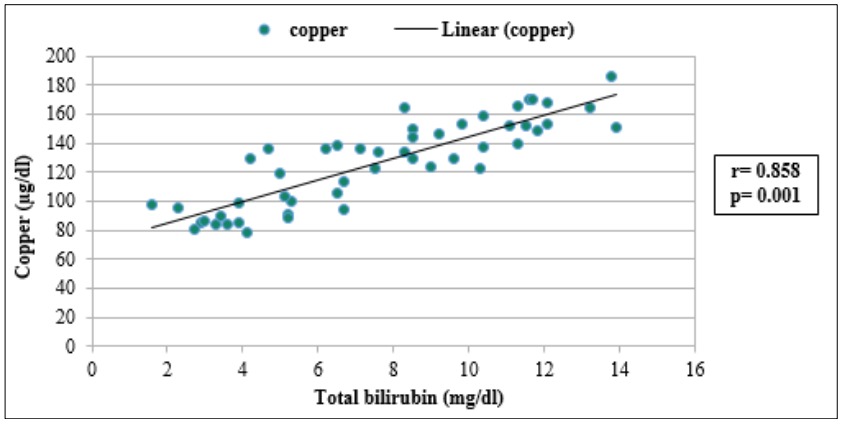

Fig 2: Correlation of serum copper level with serum total bilirubin in chronic liver disease patients

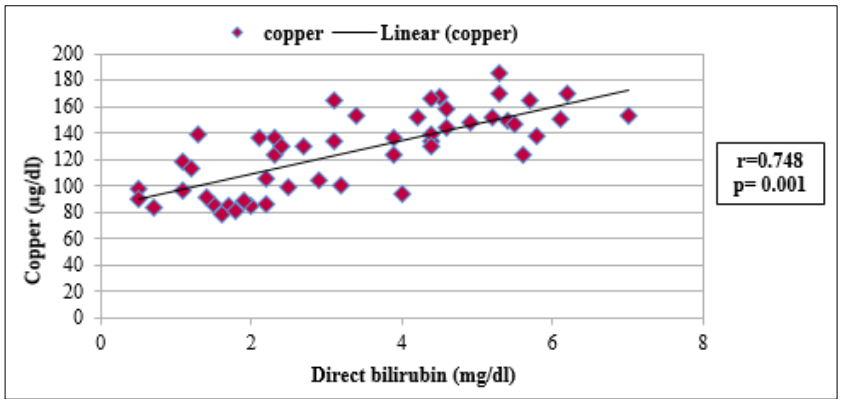

Fig 3: Relationship between serum copper level and serum direct bilirubin in chronic liver disease patients

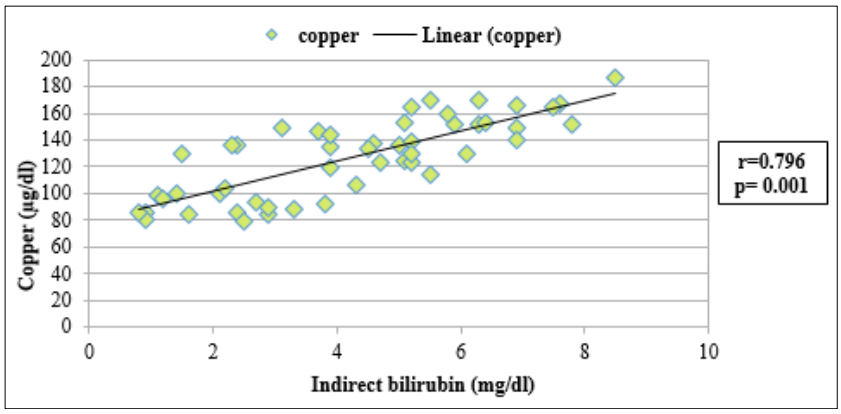

Fig 4: Correlation of serum copper level with serum indirect bilirubin in chronic liver disease patients

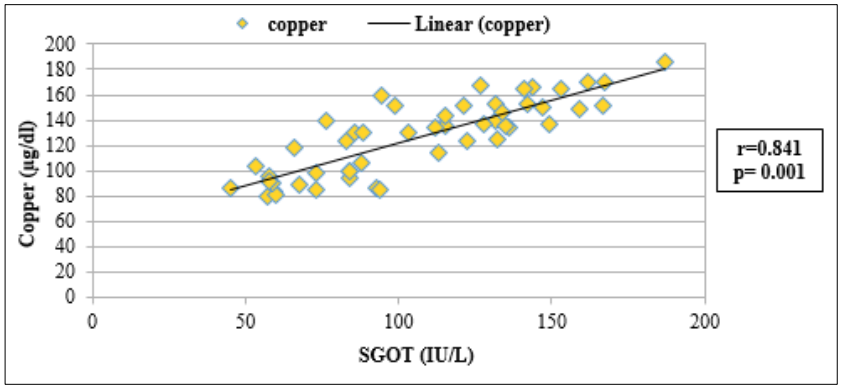

Fig 5: Correlation of serum copper level with serum glutamate-oxaloacetate transaminase (SGOT) in chronic liver disease patients

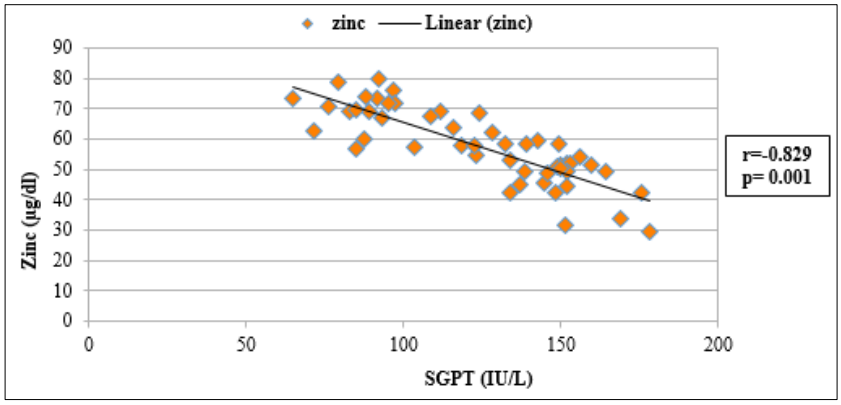

Fig 6: Relationship between serum zinc level and serum glutamate– pyruvate transaminase (SGPT) in cases

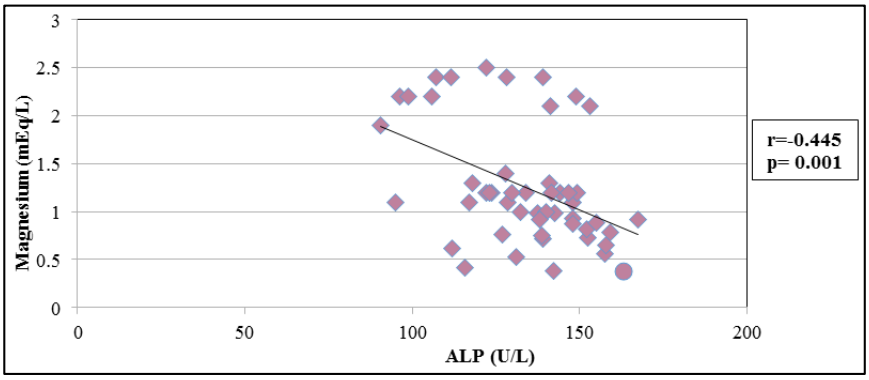

Fig 7: Correlation of serum magnesium level with serum alkaline phosphatase (ALP) in chronic liver disease patients

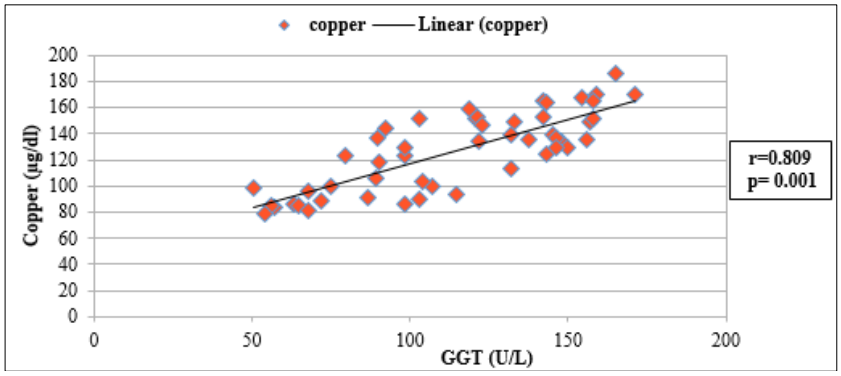

Fig 8: Correlation of serum copper level with serum gamma glutamyl transferase (GGT) in chronic liver disease patients

Discussion

Infectious, metabolic, autoimmune, genetic, and drug-induced etiologies all have a role in chronic liver illnesses. Many of these disorders have similar symptoms and early laboratory findings, but only specialist laboratory testing and a thorough inspection of the liver tissue may provide a definitive diagnosis. For hepatic disorders, a variety of biochemical measures are frequently monitored, including TB, DB, INB, SGOT, SGPT, GGT & ALP [21].

Trace elements, particularly those with antioxidant system, such as Zn, and those with redox properties, like Cu and Mg. Copper has a vital function in pathological development of CLD and Mg balance can be distributed in numerous physiological and pathological processes can be caused by a variety of conditions and differences in magnesium content. Variation in trace elements level either by deficiency or excess greatly affects different body systems. Trace element such as magnesium, copper and zinc has essential protective or promoting effects of CLDs [22]. In present study, we included various measured parameters and found that the serum total bilirubin, serum direct bilirubin and serum indirect bilirubin was 7.60±3.42 mg/dl, 3.36±1.74 mg/dl and 4.22±2.08 mg/dl respectively in chronic liver disease patients, which is found to be statistically higher than the mean values of their respective controls (0.74±0.14) mg/dl, (0.21±0.08) mg/dl and (0.53±0.10) mg/dl (p<0.001).

Also, we found that the serum levels of SGOT, SGPT, ALP and GGT was 107.12±36.49IU/L, 123.74±30.66IU/L, 133.77±18.66U/L and 114.15±34.87U/L in CLDs patients 27.25±5.58IU/L, 30.66±5.20IU/L, 84.52±20.51U/L and 49.38±9.33U/L were in the healthy controls respectively. This signifies increased in the SGOT, SGPT, ALP and GGT in CLDs cases as comparison to individuals (p<0.001).

The main prospective of our study was to compare the levels of Cu, Zn & Mg in patients of chronic liver diseases to healthy controls. The serum concentration of copper between the cases and controls was 126.68±29.51 µg/dl and 87.45 ± 16.68 µg/dl. It has been evident that raised levels of serum copper was observed in chronic liver disease patients as compared to their controls and their statistically, the relationship was shown to be statistically significant (p<0.001).

The same observation were also witnessed by Ali NM *et al*. who found that elevated level of serum copper was found in cases than healthy controls. This can be explained as copper serve as a cofactor in the production of collagen, which helps to prevent hepatic fibrosis in chronic liver disease [23]. In corresponding based on our findings, Attia AM *et al*. 2018 [24] it was determined that as liver disease progressed, serum copper levels increased significantly. Nangliya *et al*. 2015 [25] showed an increasing concentration of copper with liver disease severity when compared to early stage cirrhosis, which is consistent with evidence. In accordance Kar K 2014 *et al*. [26], one probable reason for increased copper concentration may occur by increased uptake from the gut, Copper reserves are released as a result of decreased liver excretion and tissue breakdown.

The next parameter of our study, serum zinc has shown the mean values of serum Zn as 57.80 ± 12.21 µg/dl in patients of CLDs and in controls were 68.87± 5.87 µg/dl and signifies statistically lower levels as compared their healthy subjects (p<0.001). These also matched with study of Atia F *et al*. 2012 [27] they determined that healthy patient's levels of zinc were slightly reduced in healthy patients. They suggested that Zn supplementation could reduce inflammation and contribute to faster illness resolution in cirrhotics due to lower serum Zn levels.

Similar observations were also integrated with the studies carried out by, Suneel *et al*. 2012 [28] and Reddy *et al*. 2012 [29] who noted it as well, serum Zn levels were found in cases as compared to controls. They went on to suggest that when liver cells are damaged or inflammation, they take up more Zn in order to synthesise Zn-related enzymes, nucleic acid, protein, and nucleic acid-related enzymes. As the damage to the liver progresses, Zn intake and absorption decreases as a result of reduced appetite, impaired high portal vein flow, as well as intestinal and gastric function. As a result, there is less association with Zn, and it is quickly removed through renal excretion due to the diffusion characteristic of blood Zn. Moreover, the serum magnesium levels were measured in the present investigations in CLDs patients was found to be 1.25±0.61 mEq/L and 1.48 ± 0.40 mEq/L in healthy controls which is statistically significantly lower in the CLDs as comparisons to healthy controls (p<0.05) [28, 29]. In the same direction low serum magnesium levels were shown to be frequent in CLD & cirrhosis. So they further determined that magnesium treatment might be helpful to improve hepatic enzyme levels [30]. Also, Ahad E *et al*. 2018 [31] Low serum magnesium levels were found to be due to lowered magnesium nutritional intake and elevated magnesium excretion due to decreased plasma albumin levels, administration of magnesiuric thiazides (furosemide), poor magnesium absorption in the distal jejunum, and the indirect effect of alcohol on renal tubules. According to Saxena *et al*. 2012 [32] blood Mg concentration was substantially (p<0.001) lower patients in liver cirrhosis (0.85±0.17mg/dl) than in healthy controls (1.36±0.40mg/dl) in comparison to healthy controls. Magnesium may be useful as a sensitive indication of liver disorders such as cirrhosis.

Rahelic *et al*. 2006 [33] found similar results in their investigation, with Mg serum levels in patients of liver cirrhosis rising as compared to healthy subjects (p=0.132). Magnesium concentrations was higher in individuals with severe liver disease, according to the researchers, probably due to extensive intrahepatic and portosystemic shunting. As a result, in light of the preceding remark, to prevent magnesium deficiency, Mg is among the elements that should be given special attention in cirrhosis treatment and it could be employed as a sensitive biomarker of cirrhosis. A daily basis magnesium measurement in liver cirrhosis patients may be useful in developing a care routine to slow disease progression and avoid serious health problems.

Moreover, in our study, we also correlated the different liver enzymes and trace elements (serum copper, serum zinc and serum magnesium) respectively among chronic liver disease patients by using Pearson's correlation coefficient. (In Table: 5, Figure 2-8).

In the present study, we observed that there is no correlation found between the serum total bilirubin, direct bilirubin, indirect bilirubin with zinc and serum magnesium levels in each case of chronic liver disease.

Interestingly, in the present study, we found that the serum copper with serum SGOT (r=0.841) and serum Cu with serum GGT (r=0.809) are both strongly positively correlated with biochemical indicators of liver damage. Furthermore, there is a strongly negative connection between serum zinc and serum SGPT (r=-0.829, p<0.001). Additionally, serum magnesium has a negative relationship with serum ALP (r=-0.494, p<0.001).

In the present study, serum Cu was significantly positively correlated with Total bilirubin, direct bilirubin, indirect bilirubin, SGOT, GGT. (P<0.001 for all). In addition, there was a negatively correlation of serum zinc and the biochemical parameter of liver damage (SGPT, p<0.001) the metabolism of excess copper is highly poisonous and causes cellular damage. It can attach to proteins and nucleic acids, causing lipid and protein oxidation by increasing the generation of harmful oxidative stress.

Our results for serum Cu and markers of liver damage, such as liver enzymes, were similar to those of other researchers. It was also reported that serum Cu was positively associated with SGOT, SGPT, GGT and total and direct bilirubin (p<0.01 for all) and the serum Zn was linked negatively with SGOT and SGPT (P<0.01 for both) [34]. However, Ibrahim NL *et al*. found that the following measures had a positive connection with serum copper: serum GGT, SGOT, and SGPT, total and direct bilirubin (p<0.01). Serum Zn, on the other hand, had a negative connection with the following parameters: SGOT and SGPT (p<0.01) [35].

According to the findings of the current investigation, trace element abnormalities may indicate liver disease. As a result of our findings, we suggest that liver dysfunction can affect trace element metabolism and that supplementing trace elements can help to delay or prevent the complications of chronic liver disease.

Conclusion

The elevation of Cu in chronic liver disease may cause liver dysfunctions which alters the metabolism of trace elements. Therefore, dietary or therapeutic Cu intake, should be sufficient with chronic liver disease patients while lower levels of Zn & Mg levels have been linked to an increase in the severity of cirrhosis.

As a result, supplementation with Zn & Mg should be included among the micronutrients that are given special attention in cirrhotic therapy to avoid Zn & Mg shortage and could be utilized as a sensitive marker of cirrhosis of the liver. Screening of serum Cu, Zn & Mg in individuals with liver disease may be useful in the development of a treatment strategy to slow disease development and prevent serious health problems.

Acknowledgement

I am grateful to all the faculty members and technical staff of Biochemistry department and laboratory for their kind

support.

Conflict of interests: No conflict of interests declared.

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