

## Serum and Tissue Levels of Zinc, Copper and Metallothionein as Prognostic Factors in Patients with HCV-Related Liver Diseases

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### ABSTRACT

**Background/Aim:** The relationship between chronic hepatitis and trace metals has not been understood clearly. Serum metal levels such as those of zinc and copper have been reported to be highly sensitive in the diagnosis of chronic liver diseases. Metallothioneins (MT) are family of low molecular weight cysteine rich proteins which are widely distributed in various species. They are thought to be involved in heavy metal detoxification, intracellular trace elements storage and scavenging free radicals. The aim of this work was to study the serum and liver tissue levels of zinc, copper and metallothionein in patients with chronic hepatitis, liver cirrhosis and HCC, and to clarify their role in the progression of liver disease.

**Patients & Methods:** This study was carried out on 40 patients with chronic liver disease, divided into three groups: Group I: Included 15 patients with chronic hepatitis C, Group II: Included 10 patients with liver cirrhosis (histologically diagnosed cirrhosis from patients subjected to biopsy for grading of chronic hepatitis or diagnosis of suspicious nodule), and Group III: Included 15 patients with hepatocellular carcinoma. All patients were subjected to liver function tests, viral hepatitis markers, alpha-fetoprotein, abdominal ultrasonography, and estimation of zinc, copper and metallothionein in serum and liver tissue.

**Results:** The results of the present study revealed that serum and liver tissue levels of zinc were significantly less in patients with HCC than patients with chronic hepatitis and cirrhosis. On the other hand, serum and tissue levels of copper and metallothionein were significantly more in patients with HCC than patients with chronic hepatitis and cirrhosis. Also, the present study showed a positive correlation between serum and liver tissue levels of zinc, copper and metallothionein. So, monitoring the serum level of Zn, Cu and MT can limit the need for liver biopsy for detection of their tissue level.

**Conclusion:** We can conclude that serum levels of zinc, copper and metallothionein may be useful as prognostic markers for chronic liver diseases especially with the development of HCC. Future studies are encouraged to evaluate potential therapeutic role of these findings to delay the progression of chronic liver disease in HCV patients.

### INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide<sup>1</sup>. A growing body of evidence indicates that many trace elements play important roles in a number of carcinogenic processes that proceed

through various mechanisms. Copper (Cu) and zinc (Zn) are essential trace elements for several metabolic processes. Overload or deficiency of these elements can lead to metabolic disorders and some other diseases<sup>2,3</sup>.

Serum metal levels, such as copper (Cu), zinc (Zn) and metallothionein (MT) have been reported to be highly sensitive in the diagnosis of some diseases; including chronic liver diseases<sup>4</sup>.

The role of tissue trace elements in liver fibrosis, carcinogenesis and progression of HCC has not been yet clarified, however, it is widely accepted that measurement of tissue trace elements (copper, zinc) and metallothionein have a strong association with the progression of chronic liver diseases, including HCC<sup>5</sup>.

Metallothioneins (MTs) are a family of low-molecular weight cysteine rich proteins which are widely distributed in various species. MTs are thought to be involved in heavy-metal detoxification; intracellular trace elements storage and scavenging free radicals<sup>6</sup>.

The aim of this work was to study the serum and liver tissue levels of zinc, copper and metallothionein in patients with chronic hepatitis, liver cirrhosis and HCC, and to clarify their role in the progression of liver disease.

## PATIENTS AND METHODS

This study was carried out on 40 patients with liver diseases admitted to Tropical Medicine Department, Tanta University. They were divided into 3 groups:

- **Group I:** 15 patients with chronic hepatitis C; their ages ranged between 19 and 48 years with a mean age of  $36.3 \pm 8.3$  years.
- **Group II:** 10 patients with post-hepatitis C cirrhosis; their ages ranged between 22-51 years with a mean age of  $44.7 \pm 5.2$  years.
- **Group III:** 15 patients with post-hepatitis C HCC; their ages ranged between 25-36 years with a mean age of  $38.9 \pm 9.2$  years.

Patients with hepatic decompensation, diuretic therapy, zinc supplementation or nutritional support, and those suffering concurrent infections, in addition to those subjected to surgery or chemotherapy had been excluded.

After verbal consent, all patients were subjected to full history taking, clinical exam, and routine lab testing, in addition to viral hepatitis

markers, alpha-fetoprotein, abdominal ultrasonography, estimation of serum and tissue levels of zinc, copper and metallothionein.

Measurements of serum and tissue zinc, copper and metallothionein were done according to the methods described by Pekarek et al., Cousins, Nakajima et al., Sakurai et al, and Huang et al. respectively<sup>7,8,9,10,11</sup>.

Statistical Analysis was done using SPSS for Windows version 10.

## RESULTS

All patient groups were matched as regards age and sex; all were positive for HCV-RNA but negative for all other viral hepatitis. All have past but not recent exposure to schistosomiasis. All have compensated liver functions with prothrombin time >60% and platelet count  $>100 \times 10^3$ .

The results of our study showed a significant decrease in serum and tissue zinc levels in patients with post-hepatitis C HCC when compared to the other two groups ( $p < 0.05$ ). These levels were less in patients with cirrhosis than patients with chronic hepatitis C, but the difference was statistically significant ( $p < 0.05$ ) only for tissue levels, but not statistically significant for serum levels ( $p > 0.05$ ) (Table 1).

On the other hand, the results of our study showed a significant increase in serum and tissue copper in patients with HCC when compared to the other two groups ( $p < 0.05$ ). These levels were more in patients with cirrhosis than patients with chronic hepatitis C, but the difference was not statistically significant ( $p > 0.05$ ) (Table 2).

Also, the results of our study showed a significant increase in serum and tissue metallothionein in patients with HCC when compared to the other two groups ( $p < 0.05$ ). These levels were more in patients with cirrhosis than patients with chronic hepatitis C, but the difference was not statistically significant ( $p > 0.05$ ) (Table 3).

Moreover, a positive correlation was found between the serum and tissue levels of zinc, copper, and metallothionein (Fig. 1, 2, and 3).

**Table (1) Serum and tissue zinc in our patients with post-hepatitis C liver diseases ( $\mu\text{g}/\text{dl}$  &  $\mu\text{g}/\text{gm}$  wet wt. liver tissue respectively)**

|                    | Group I           | Group II          | Group III          |
|--------------------|-------------------|-------------------|--------------------|
| <b>Serum zinc</b>  | 95.4 $\pm$ 6.08   | 86.27 $\pm$ 6.8   | 62.67 $\pm$ 16.92* |
| <b>Tissue zinc</b> | 103.76 $\pm$ 8.82 | 90.75 $\pm$ 4.92* | 72.07 $\pm$ 3.9*   |

\* Significant

**Table (2) Serum and tissue copper in patients with post-hepatitis C liver diseases ( $\mu\text{g}/\text{dl}$  &  $\mu\text{g}/\text{gm}$  wet wt. liver tissue respectively)**

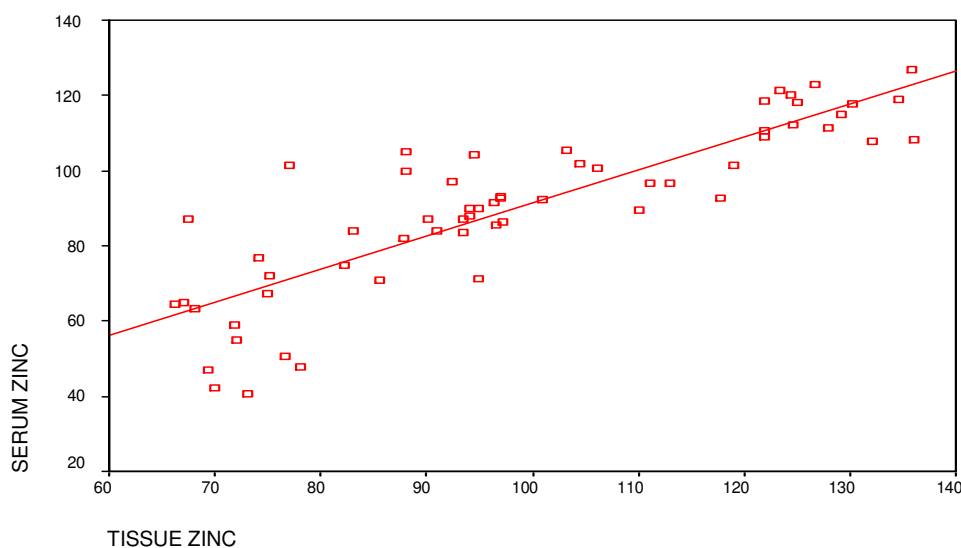
|                      | Group I          | Group II          | Group III           |
|----------------------|------------------|-------------------|---------------------|
| <b>Serum copper</b>  | 65.04 $\pm$ 5.6  | 76.75 $\pm$ 11.89 | 108.77 $\pm$ 15.33* |
| <b>Tissue copper</b> | 29.77 $\pm$ 2.82 | 45.7 $\pm$ 3      | 64.81 $\pm$ 11.99*  |

\* Significant

**Table (3): Serum and tissue metallothionein in patients with post-hepatitis C liver diseases ( $\mu\text{g}/\text{dl}$  &  $\mu\text{g}/\text{gm}$  wet wt. liver tissue respectively)**

|                  | Group I          | Group II         | Group III         |
|------------------|------------------|------------------|-------------------|
| <b>Serum MT</b>  | 7.25 $\pm$ 0.55  | 9.39 $\pm$ 1.52  | 12.31 $\pm$ 1.14* |
| <b>Tissue MT</b> | 20.17 $\pm$ 2.81 | 25.96 $\pm$ 3.19 | 39.15 $\pm$ 7.39* |

\* Significant



**Fig. 1: Correlation between serum and tissue zinc in the studied groups**

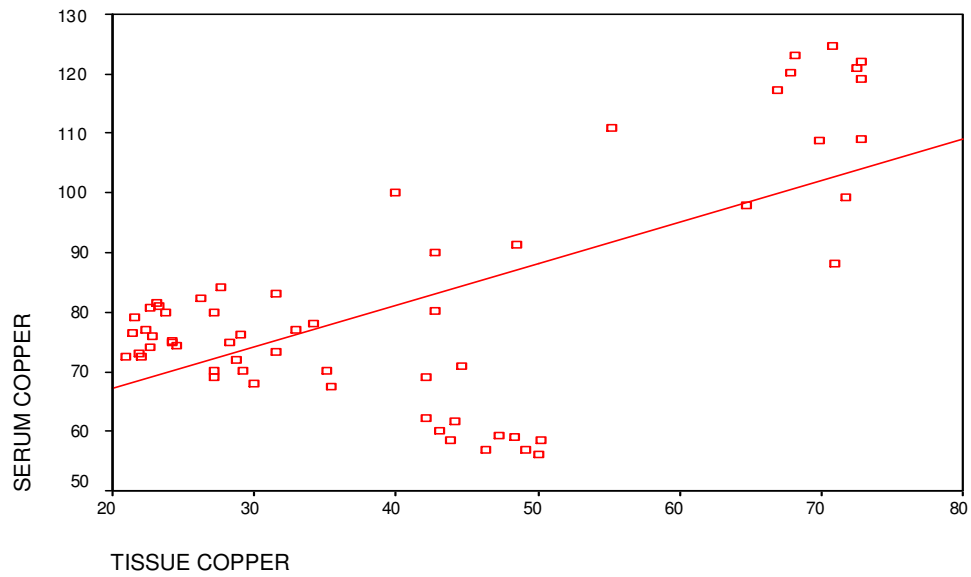


Fig. 2: Correlation between serum and tissue copper in the studied groups

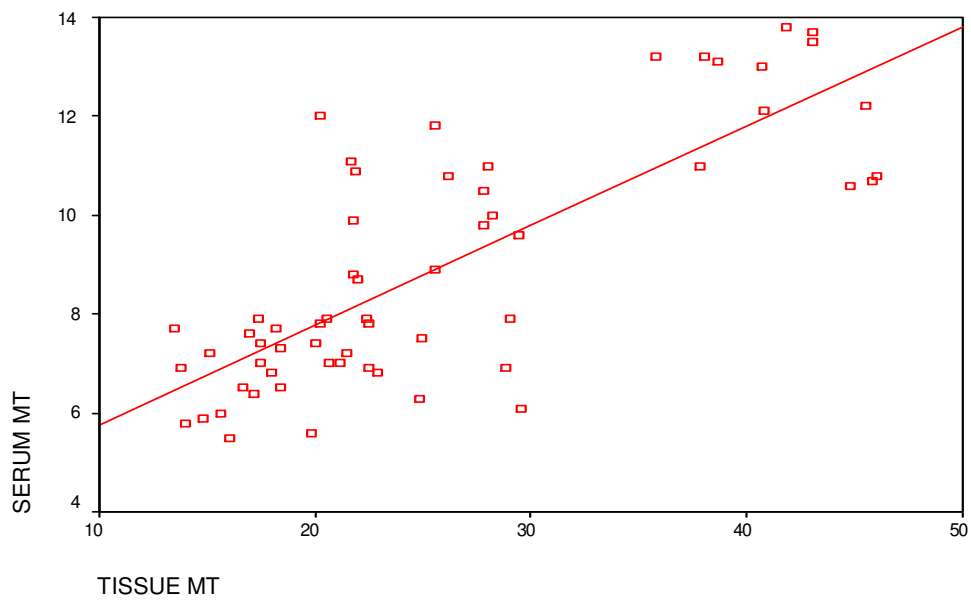
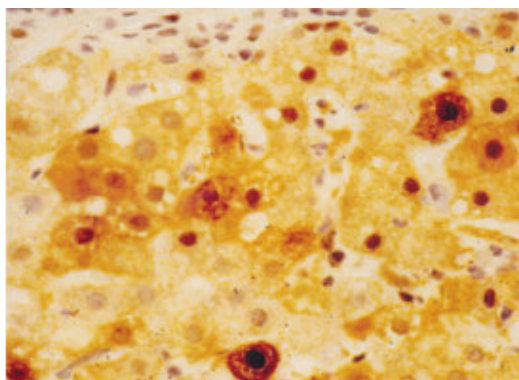
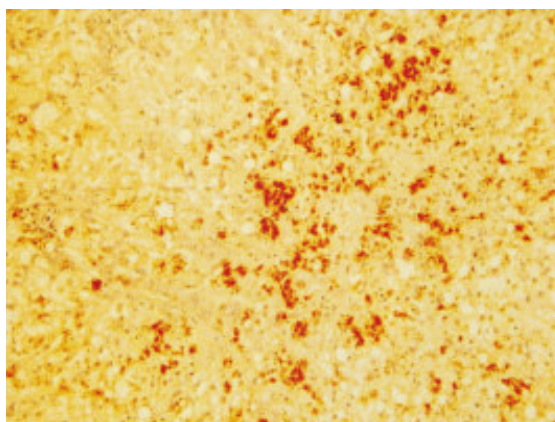


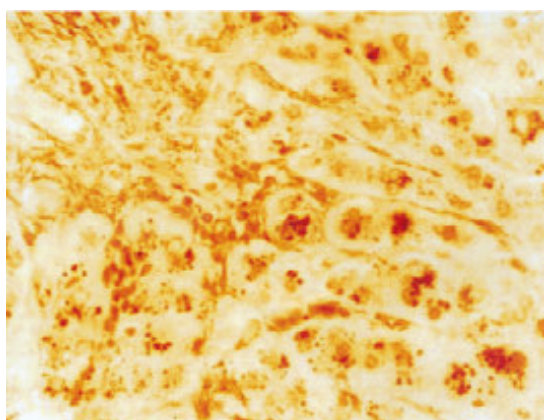
Fig. 3: Correlation between serum and tissue MT in the studied groups



**Fig. 4:** Showed immunohistochemical staining of MT in patients with chronic hepatitis.



**Fig. 5:** Showed immunohistochemical staining of MT in patients with liver cirrhosis



**Fig. 6:** Showed immunohistochemical staining of MT in patients with HCC

## DISCUSSION

Measurement of serum and tissue zinc emerges as a recent biochemical studies in patients with chronic liver diseases<sup>12</sup>.

Our study revealed that serum and tissue zinc levels were significantly decreased in patients with HCC when compared to patients with chronic hepatitis and liver cirrhosis.

This was in agreement with many studies that reported a decrease in serum zinc (Zn) in patients with liver diseases<sup>13,14</sup>.

Also, Ko et al. reported that serum zinc levels were significantly lower in chronic hepatitis patients than in healthy controls<sup>15</sup>.

They explained this finding by decreased intake, disturbed intestinal absorption, and decreased serum albumin as a carrier of zinc, decreased storage in the liver and increased urinary excretion<sup>14</sup>.

Our results were also in agreement with Gur et al. who recorded progressive significant decrease in liver tissue zinc from patients with viral hepatitis to cirrhosis to HCC in parallel to the severity of liver damage<sup>16</sup>.

Some reports reported a difference in trace elements levels between tumor and non-tumorous tissues in patients with hepatic malignancy. Tashiro et al. and Maeda et al. reported that hepatic zinc was significantly lower in HCC tissue compared to non-tumorous liver parenchyma of patients with different hepatic malignancies<sup>12,6</sup>.

The results of this study showed that serum and liver tissue copper was higher in patient with liver cirrhosis and HCC than patients with chronic hepatitis C. This increase was statistically significant only for patients with HCC.

In agreement with our results, Pramoolsinsap et al. reported a significantly higher serum copper in patients with HCC. They explained their findings by suggesting that changes in liver cell pathology accompanied by functional impairment which may alter the metabolism of zinc and copper<sup>13</sup>.

Also, Jorge et al. and Tashiro et al. reported that serum levels of copper in patients with HCC were significantly higher than those in patients with either liver cirrhosis or chronic viral hepatitis<sup>4,6</sup>.

In accordance with this result, Ebara et al. also reported that tissue copper was significantly higher in patients with chronic viral C hepatitis, liver cirrhosis and HCC compared to normal liver<sup>17</sup>.

Also, Masaaki et al. found that copper level in liver parenchyma was significantly higher in HCV positive patients with HCC than those with normal liver<sup>18</sup>.

Recently, much attention was paid to the association between MTs and chronic liver diseases including HCC. Available information suggested that MTs might play important roles in carcinogenic and apoptotic processes of some tumors. Increases of hepatic copper and zinc are able to induce MTs<sup>19</sup>.

In our patients, serum and liver tissue MT was significantly higher in patients with HCC than patients with liver cirrhosis and chronic viral hepatitis.

This was in agreement with Ebara et al. who found that serum MT was significantly higher in patients with HCC when compared to patients with liver cirrhosis and chronic viral hepatitis<sup>17</sup>.

In agreement, Ebara et al. and Alscher et al. reported that hepatic tissue MT was significantly higher in patients with HCC when compared to patients with liver cirrhosis, chronic viral hepatitis and normal liver<sup>17,20</sup>.

But, on the contrary, Nakayama et al. reported that MT levels in patients with liver cirrhosis and HCC were significantly lower than those of patients with chronic hepatitis and controls<sup>21</sup>.

In the present study, all patients with increased hepatic copper also had an increase in hepatic metallothionein. This could be explained by the induction of MT by some transition metals like copper resulting in up regulation of its synthesis.

In agreement, Sakurai et al. have found that copper excess was associated with induction of MT in rat liver. They expected that oxidative

stress induced by dietary copper overload have caused abnormal function of hepatic mitochondria<sup>10</sup>.

We may conclude that zinc may have a therapeutic potential and may be a complementary therapy in chronic hepatitis C patients. Also, serum levels of metallothionein, copper and zinc may help the diagnosis and predict the prognosis of post hepatitis C chronic liver disease. Finally, serum levels of metallothionein, copper and zinc are correlating very well with tissue levels excluding the need for invasive measurement of their levels.

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## دراسة مستوى الزنك والنحاس والميتالوثيونين في مصل ونسيج كبد مرضى التهاب الكبدى الفيروسي (سى) كمؤشر لتطور المرض

تعتبر أمراض الكبد المزمنة من الأمراض الأكثر شيوعا بين المصريين و التي تتسبب في كثير من الوفيات بين مرضى الكبد ولذلك فان هناك بحث مستمر في طرق تشخيص و علاج هذه الأمراض.

ومن أمراض الكبد التي تحظى بعدد كبير من الأبحاث التهاب الكبدى الفيروسي المزمن والتشمع الكبدى و أورام الكبد السرطانية.

ومن الأبحاث الجديدة في هذا المجال قياس مستوى العناصر النادرة وأنواع خاصة من البروتينات في مصل ونسيج كبد مرضى الكبد المزمنة.

**الهدف من البحث:** دراسة مستوى الزنك و النحاس و الميتالوثيونين في مصل و أنسجة كبد مرضى الكبد و علاقتها بتطور هذه الأمراض.

المرضى و طرق البحث: اشتملت خطة البحث على ثلاث مجموعات من مرضى الكبد :-

**المجموعة الأولى:** شملت خمسة عشر مريضا من مرضى التهاب الكبدى الفيروسي المزمن (سى).

**المجموعة الثانية:** شملت عشرة مرضى بالتشمع الكبدى.

**المجموعة الثالثة:** شملت خمسة عشر مريضا من مرضى سرطان الكبد.

و قد تم اخذ التاريخ المرضى و عمل فحص اكلينيكي و معلمي كامل للمجموعات مع أشعة تليفزيونية على البطن و الحوض و قياس لمستوى الزنك و النحاس و الميتالوثيونين في مصل و نسيج الكبد لهذه المجموعات..

**وكانت النتائج كما يلى:**

- هناك نقص ذو دلالة إحصائية في مستوى الزنك في مصل و نسيج كبد مرضى سرطان الكبد عنها في مرضى التهاب الكبدى الفيروسي المزمن و مرضى التشمع الكبدى.. كما وجد نقص في مستوى الزنك في مرضى التشمع الكبدى عنها في مرضى التهاب الكبدى الفيروسي المزمن ولكنها غير ذات دلالة إحصائية.

- هناك زيادة ذات دلالة إحصائية في مستوى النحاس في مصل و نسيج كبد مرضى سرطان الكبد عنها في مرضى التهاب الكبدى الفيروسي المزمن و مرضى التشمع الكبدى.. كما وجدت زيادة في مستوى النحاس في مرضى التشمع الكبدى عنها في مرضى التهاب الكبدى الفيروسي المزمن.

- هناك زيادة ذات دلالة إحصائية في مستوى الميتالوثيونين في مصل و نسيج كبد مرضى سرطان الكبد عنها في مرضى التهاب الكبدى الفيروسي المزمن و مرضى التشمع الكبدى.. كما وجد زيادة في مستوى النحاس في مرضى التشمع الكبدى عنها في مرضى التهاب الكبدى الفيروسي المزمن ولكنها غير ذات دلالة إحصائية.

- وجدت علاقة طردية بين مستوى الزنك و النحاس و الميتالوثيونين في مصل المجموعات المرضية و بين مستواها في نسيج كبد نفس المجموعات.

أوضحت هذه الدراسة أن مستوى الزنك و النحاس و الميتالوثيونين في مصل المجموعات المرضية يتماشى مع مستواها في نسيج كبد هذه المجموعات مما يدل على أهمية استخدام هذه المواد الكيميائية في تشخيص تطور أمراض الكبد المزمنة بداية من التهاب الكبدى الفيروسي المزمن و مرورا بالتشمع الكبدى و خصوصا في حالة التطور لسرطان الكبد.