



# Trace Elements Status among Patients with Hepatocellular Carcinoma: A Case-Control Study

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## Abstract

**Background:** Our research hypothesis is that copper, zinc, and selenium may have a potential role in the development of Hepatocellular Carcinoma (HCC). We carried out this study to determine the serum levels of copper, zinc, and selenium among patients with HCC compared to the normal subjects, and to explore the contribution of their serum levels to the development of both HCC and late HCC.

**Patients and Methods:** A case-control study including 91 patients with HCC and 92 normal subjects was carried out. All the study population were provided clinical evaluation, imaging studies (including triphasic abdominal computed tomography), and laboratory investigations (including estimation of the serum levels of copper, zinc, and selenium).

**Results:** Both groups were matching. All patients with HCC had evidence of liver cirrhosis. Serum levels of zinc ( $76 \pm 20$  mcg/dL vs  $139 \pm 36$ ,  $p$  0.022) and selenium ( $50.6 \pm 6.97$  mcg/dL vs  $93 \pm 5.7$  mcg/dL,  $p$  0.037) were significantly lower among patients with HCC compared to normal subjects, respectively. Serum levels of copper were higher among patients with HCC compared to the normal subjects; however, this was not statistically significant. When comparing patients with late HCC to those with early HCC, serum levels of zinc were significantly lower among patients with late HCC ( $51.84 \pm 19.2$  mcg/dL vs  $103.58 \pm 24.1$  mcg/dL,  $p$  0.016).

**Conclusions:** Lower serum levels of zinc and selenium can be associated with a higher risk of HCC development. In addition, high serum levels of copper may be associated with a higher stage of HCC. Further evaluation for the biologic role of these trace elements in the development of HCC is warranted.

## Introduction

There is a global increase in the incidence of Hepatocellular Carcinoma (HCC) worldwide [1]. The recognized risk-factors of HCC are multifaceted and include chronic viral hepatitis, alcohol, metabolic diseases [2,3]. Accumulating data are now raising the role of some trace elements such as selenium (Se), copper (Cu), and zinc (Zn) in the protective effect or, on the other hand, the harmful effect on oncogenesis process e.g. HCC [4-7]. Reactive copper can participate in liver damage either directly or indirectly, through Kupffer cell's stimulation [8]. Zinc is known as an antioxidant agent that contributes in the stabilization of cellular membrane and cytoskeleton, as an anti-apoptotic agent and as a co-factor in Deoxy-Ribonucleic Acid (DNA) synthesis [9]. Also, selenium exerts a protective antioxidant effect against cellular damage by reactive oxygen species and counteracts free radical production, through the regulation of the GSH-peroxidase activity [10].

So, we aimed to study the serum levels of copper, zinc, and selenium among patients with HCC compared to the normal subjects, and to explore the contribution of their serum levels to the development of both HCC and late HCC.

## Patients and Methods

### Patients' recruitment

Between January 2016 and December 2017, a case-control single center study was designed to enroll all consecutive patients presented at Hepatology Unit, Assiut University Hospital, Egypt, with proven HCC (HCC-group,  $n=91$ ). Another apparently healthy subject from blood donation bank were included as a control group ( $n=92$ ).

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**Table 1:** Demographic and laboratory characteristics of the study population (n=183).

	Control subjects (n=92)	HCC (n=91)	p
Age (years)	49.7 ± 8.1	54.3 ± 11.6	0.314
Gender (male)	52 (56.5)	63 (69.2)	0.079
Residence (rural)	67 (72.8)	57 (62.6)	0.283
Tobacco smoking	36 (39.1)	50 (55)	0.116
Alcohol consumption	4 (4.3)	7 (7.7)	0.052
BMI	28.7 ± 4.3	23.9 ± 2.9	0.294
DM	10 (10.9)	15 (16.5)	0.098
ALT (IU/mL)	23 ± 5.5	58.8±32	0.038 *
AST (IU/mL)	30.4 ± 5.7	104.75 ± 155.6	0.006 *
ALP (IU/mL)	79.2 ± 10.4	136.8 ± 13.7	0.041 *
Albumin (g/L)	40.14 ± 4.9	25.1 ± 6.9	0.011 *
Bilirubin (mg/dL)	0.84 ± 0.15	3.38 ± 1.56	<0.001 *
AFP (IU/mL)	4.6 ± 2.5	460.8±194.1	<0.001 *

HCC: Hepatocellular Carcinoma; BMI: Body Mass Index, DM: Diabetes Mellitus; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; AFP: Alpha-Fetoprotein. Data are expressed as mean ± standard deviation, except for gender, residence, tobacco smoking, alcohol consumption, and DM which are presented as frequency (percentage). \*Statistically significant.

HCC diagnosis and staging was in concordance to Barcelona Clinic Liver Cancer (BCLC) staging system [11-13]. Patients with stage-A and B were considered to have early HCC, while those with stage-C and stage-D were considered to have late HCC.

Patients receiving multi-vitamin supplements were excluded from the study. In addition, patients with previous, recurrent or with any therapeutic maneuvers of HCC were not enrolled.

**Methods**

For all the enrolled patients, clinical evaluation (medical history and physical examination), abdominal ultrasonography, triphasic CT of the abdomen, estimation of the fasting serum levels of glucose, copper, zinc, selenium, and Alpha-Fetoprotein (AFP), and of liver chemistry (Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), bilirubin, albumin, and estimation of prothrombin time).

For estimation of serum levels of copper, zinc, and selenium, 10 mL of fasting venous blood was drained from ante-cubital vein on plain tubes. After waiting for 30 minutes to allow blood clotting, centrifugation at 3000 rpm for 10 minutes was performed. Serum was separated and stored at -20°C, until batch analysis. The serum levels of the trace elements were measured by atomic absorption spectrophotometry, using Varian Techtron (Varian Inc. Melbourne, Australia).

**Statistical analysis**

Data were analyzed using the Statistical Package for Social Sciences (IBM SPSS Statistics, version 22.0, release 22.0.0.0; IBM Corp) for Microsoft Windows® (64-bit version). Results were expressed as mean ± standard deviation or frequency (percentage) as appropriate. Student's t-test or Mann-Whitney U test, and Yates' corrected chi-squared test or Fischer's exact test as appropriate were used to compare the variables between the study groups. A p value less than 0.05 was considered statistically significant.

**Table 2:** Serum levels of copper, zinc, and selenium among the study population (n=183).

	Control subjects (n=92)	HCC (n=91)	p
Serum copper (mcg/dL)	126 ± 18	178 ± 63	0.018
Serum zinc (mcg/dL)	139 ± 36	76 ± 20	0.022 *
Serum selenium (mcg/dL)	93 ± 5.7	50.6 ± 6.97	0.037 *

HCC: Hepatocellular Carcinoma. Data are expressed as mean ± standard deviation. \*Statistically significant.

**Ethical considerations**

The study was conducted after approval of the Clinical Research Ethical Committee of Assiut Faculty of Medicine and was carried out according to the code of ethics of the World Medical Association (Declaration of Helsinki). All the participants signed a consent certificate after discussing in detail with the investigators the certificate subjects and the study aim. Participants were clearly informed that refusing to participate in the study will not affect having full benefit of the available medical service. Data confidentiality was respected.

**Results**

Demographic and laboratory characteristics of the study population are shown in (Table 1).

Both groups were matching; the mean age of the patients with HCC was 54.3 ± 11.6, while it was 49.7 ± 8.1 for the normal subjects. Male gender represented 69.2% of the patients with HCC and 56.5% of the normal subjects.

All the patients with HCC had the evidence of liver cirrhosis. Liver cirrhosis was related to chronic hepatitis C virus infection among 63 (69.2%) patients, while it was caused by chronic hepatitis B virus infection among 28 (30.8%). Patients with HCC had significantly higher serum levels of ALT, AST, ALP, bilirubin, and AFP, and lower level of albumin compared to the normal subjects.

Table 2 shows the serum levels of copper, zinc, and selenium among the study population. Serum levels of zinc (76 mcg/dL ± 20 mcg/dL vs 139 mcg/dL ± 36 mcg/dL, p=0.022) and selenium (50.6 mcg/dL ± 6.97 mcg/dL vs 93 mcg/dL ± 5.7 mcg/dL, p=0.037) were significantly lower among patients with HCC compared to normal subjects, respectively. Serum levels of copper were higher among patients with HCC compared to the normal subjects; however, this was not statistically significant (126 mcg/dL ± 18 mcg/dL in control vs 178 mcg/dL ± 63 mcg/dL in HCC-group).

Among patients with HCC, the Barcelona Clinic Liver Cancer (BCLC) stage was-A among 8 (8.8%) patients, stage-B among 14 (15.4%), stage-C among 19 (20.9%), and stage-D among 70 (54.9%). When comparing patients with late HCC to those with early HCC, serum levels of zinc were significantly lower among patients with late HCC (51.84 mcg/dL ± 19.2 mcg/dL vs 103.58 mcg/dL ± 24.1 mcg/dL,

**Table 3:** Serum levels of copper, zinc, and selenium among the patients with HCC (n=91).

	Early HCC (n=22)	Late HCC (n=69)	p
Serum copper	157.25 ± 55.7	227.47 ± 49.7	0.076
Serum zinc	103.58 ± 24.1	51.84 ± 19.2	0.016 *
Serum selenium	38.94 ± 4.18	62.11 ± 6.5	0.058

n: number; HCC: Hepatocellular Carcinoma. Early HCC: stage-A and stage-B; late HCC: stage-C and stage-D. Data are expressed as mean ± standard deviation. \*Statistically significant.

p=0.016) as shown in Table 3. Although patients with late HCC had lower serum levels of selenium and higher levels of copper compared to those early HCC, this did not reach the statistical significance point.

## Discussion

There is arising burden of HCC in many countries worldwide. In Egypt, liver cancer represents more than 10% of all malignancies of the digestive tract and HCC constitutes more than 70% of all liver tumors [14]. Many recent studies tried to explore the potential factors that may aggravate or suppress tumor progression. Of these factors, serum levels of trace elements are tackled in many series.

We reported that higher serum copper levels were associated with HCC compared to lower serum levels of zinc and selenium. Comparing late HCC to early HCC, only lower serum levels of zinc were associated with the late stages of HCC. This observation is found also in the study of Pramoolsinsap et al., were they also reported a lower serum levels of zinc to be significantly lower among the normal subjects compared to the patients with HCC [15]. Zinc contributes to several biochemical and physiological functions, including metabolism, enzyme function, protein synthesis, and host defense reactions [16]. Zinc deficiency can result from deficient dietary intake, defective absorption, and hepatocellular necrosis [17, 18]. Zinc levels were found to be significantly lower in neoplastic liver tissue compared to its increased levels in the normal surrounding hepatic tissue [19]. In addition, Zinc supplementation was reported to inhibit carcinogenesis [20]. Alteration in some trace elements may lead to their interaction, such as zinc and copper; the excesses of one trace element may lead to the deficiency of another [21].

The elevated serum levels of copper among our study patients with HCC may be either directly related to HCC development, or secondary to zinc deficiency. Elevation of serum levels of copper are expected with hepatocellular damage; liver is the principal organ responsible for copper storage and excretion [22].

Higher serum levels of selenium were associated with a significantly lower risk of HCC [23]. Even a minor dysfunction of hepatocytes may reduce the serum levels of selenium, through lowering the levels of Selenoprotein-P, suggesting a potential mechanism of hepatocarcinogenesis where the dysregulation of Selenoprotein-P expression and secretion due to impaired selenium organification (defective conversion of dietary selenium into selenoproteins by sub-functional or dedifferentiated hepatocytes) contributes to the damaging effect of oxidative stress to hepatocytes [24].

Single point estimation of the serum levels of trace elements is considered as a limitation of our study. In addition, lack of follow up is another limitation. Early evaluation of the serum level of such trace elements especially in malnourished or in those with hyper-catabolic diseases and concomitant viral hepatitis may serve in replenish their stores and hence protection against HCC. More recently, trace element may add a potential role for cancer treatment through enhancing or suppression of chemotherapeutic agents [25].

In conclusion, lower serum levels of zinc and selenium can be associated with a higher risk of HCC development. In addition, lower serum levels of zinc may be associated with a higher stage of HCC. Further, prospective studies tackle the biologic interplay of these trace elements are warranted to find out if this association is causal or can be considered as a biomarker of HCC development.

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