ORIGINAL ARTICLE

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COMPARATIVE ANALYSIS OF COPPER AND ZINC LEVELS IN HEPATITIS C, ONCOLOGY AND HEALTHY GROUP

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Abstract. *Copper (Cu) and Zinc (Zn) are essential trace elements whose homeostasis is often dysregulated in chronic diseases. Imbalances, particularly an elevated serum copper-to-zinc (Cu/Zn) ratio, are linked to chronic hepatitis C (HCV) and various cancers, which suggests this ratio has value as a biomarker. The aim of the study was to analyze and compare serum Cu and Zn levels in patients with chronic hepatitis C, patients with oncological diseases, and patients in a healthy control group. This study enrolled 333 patients (66 HCV, 46 Oncology, 221 Healthy controls) from 2020 to 2025. Serum Cu and Zn were measured using flame atomic absorption spectrophotometry. Analysis of variance (ANOVA) was used to compare group means ($p<0.05$ considered significant). Significant differences were found among groups ($p<0.001$). Mean serum Cu levels were significantly elevated in the Oncology and HCV groups compared to the Healthy control group. Conversely, mean serum Zn levels were significantly lower in both disease groups versus the control group. Consequently, the mean Cu/Zn ratio was highest in the Oncology group, followed by the HCV group, and lowest in the Healthy group ($p<0.001$). The observed Cu/Zn dysregulation in HCV and oncology patients points to its role in pathogenesis. Elevated Cu may promote tumor angiogenesis, while Zn deficiency could impair immune function and increase liver damage. These findings suggest that Cu and Zn metabolism could be viable therapeutic targets, for example, through copper-chelating agents in cancer. Serum Cu and Zn levels and the Cu/Zn ratio are significantly altered in patients with chronic hepatitis C and oncological diseases. The Cu/Zn ratio can be a strong, sensitive marker of this pathological dysregulation. Monitoring these trace elements may be crucial for patient care and for applying individualized treatment strategies.*

Key words: *copper, zinc, oncology, HCV, hepatitis C*

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INTRODUCTION

Copper (Cu) and zinc (Zn) are essential trace elements for the human body [1]. They are involved in many physiological processes, such as cellular growth and differentiation, apoptosis, immunological response, oxidative stress regulation, and enzymatic activity [2, 3]. Cu and Zn have a dual nature. That is why their regulation is very strict, and their physiology in the body is mediated by a number of molecules that play the role of specific transporters. Transporters, importers, and metallothioneins (MTs) are involved in the mechanisms that regulate the homeostasis of Cu and Zn within the body, guaranteeing a balance in their status. Serious micronutrient imbalances, particularly Zn deficiencies and concurrent excesses of copper and iron, are commonly linked to chronic Hepatitis C Virus (HCV) infection. The immune, inflammatory, and antioxidant reactions to HCV are significantly impacted by these imbalances [4, 5]. A common characteristic seen in many cancer types is the dysregulation of vital minerals, such as Cu and Zn, which has a substantial impact on the development of cancer by changing metabolic pathways [6, 7, 8].

An elevated serum copper-to-zinc (Cu/Zn) ratio has emerged as a potentially valuable tool in cancer diagnosis, prognosis, tumor staging, and predicting overall patient survival [9].

The aim of this study was to quantitatively assess and compare the serum balance of Cu and Zn in three distinct cohorts: a healthy control group, patients with HCV, and patients with specific oncological diseases. Primary objectives were to determine and compare the mean serum concentrations of both Cu and Zn across the three groups. To investigate the potential correlation between Cu and Zn within each group and to calculate and analyze Cu/Zn, evaluating its significance as a potential differentiating biomarker between the Healthy, HCV, and Oncology groups.

MATERIALS AND METHODS

From 2020 to 2025, a total of 333 patients were enrolled into the study, and Cu and Zn levels were measured in serum. A flame atomic absorption spectrophotometry was used (AAnalyst analyzer, PerkinElmer).

The patients were divided into three distinct groups based on their medical conditions. These patient groups were selected because both HCV infection and oncological diseases are strongly associated in existing literature with significant dysregulation of Cu and Zn homeostasis. The healthy cohort was included to provide an essential baseline control, allowing for a direct comparison to determine the extent and direction of these mineral imbalances in each disease state.

1. Chronic HCV group: This group comprised 66 patients diagnosed with chronic hepatitis C. It consisted of 22 males and 44 females, with a mean age of 55.3 +11.6 years.

2. Oncology group (Onco group): This group included 46 oncology patients. There were 22 males and 24 females, with a mean age of 66.9 ± 7.4 years. This cohort was further subdivided by primary cancer diagnosis. 15 patients presented with non-metastatic colon cancer, 21 with metastatic colon cancer, and 10 with metastatic pancreatic cancer.

3. Healthy group: This group consisted of 221 patients. It included 120 males and 101 females, with a mean age of 58.53 ± 16.97 years. This group served as a control group.

All statistical procedures were performed using SPSS. Continuous variables were analyzed using ANOVA to compare means across groups. Relationships between variables were assessed using correlation analyses. A p-value <0.05 was considered statistically significant.

RESULTS

1. Copper [µmol/L]

Mean serum Cu levels differed among the studied groups. The Healthy group exhibited a mean serum copper level of 16.4 ± 4.65 µmol/L. In contrast, the Onco group showed significantly higher levels of 18.9 ± 5.8 µmol/L (+16% compared to the Healthy group; $p < 0.05$). The HCV group also presented with a higher mean copper value of 17.4 ± 2.76 µmol/L (+6.6% compared to the Healthy group; $p < 0.05$). We compared the three groups using a one-way ANOVA test. With an F-statistic = 7.77 and p = 0.00, there is a statistically significant difference in the mean values among the three groups (Figure 1).

This suggests that patients with neoplasms and hepatitis C have noticeably higher serum copper concentrations than the healthy population.

In the present study, the following reference ranges were used in the assessment of patients‘ copper status: 18.7-36.1 µmol/L [10]. The distribution relative to the reference interval of all subjects included in the study is presented in Table 1.

Based on 221 patients in the Healthy group, we calculated the mean Cu level among patients within the

reference range, which was 22.2 ± 3.65 µmol/L. In the Onco group, the mean Cu was 23.4 ±3.64 µmol/L, and in the HCV Group - 20.7 ±1.61. We performed once again the one-way ANOVA test, which indicated a statistical difference between the three groups (F = 3.13, p = 0.04). These differences are shown in Figure 2.

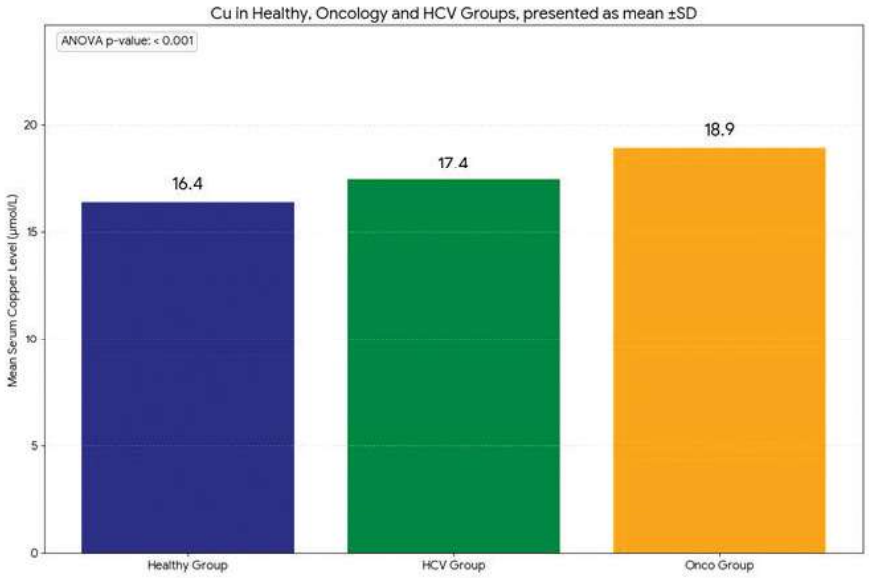

Fig. 1. Mean levels of copper between the oncology, HCV, and healthy groups

Table 1. Distribution of patients by groups according to the reference interval of Cu in serum

Copper [µmol/L]	Total n	n (%) in ref. interval	n (%) under ref. interval	n (%) above ref. interval
Healthy	221	47 (22%)	174 (78%)	0 (0%)
HCV	66	19 (29%)	47 (71%)	0 (0%)
Oncology	46	19 (41%)	26 (57%)	1 (2%)

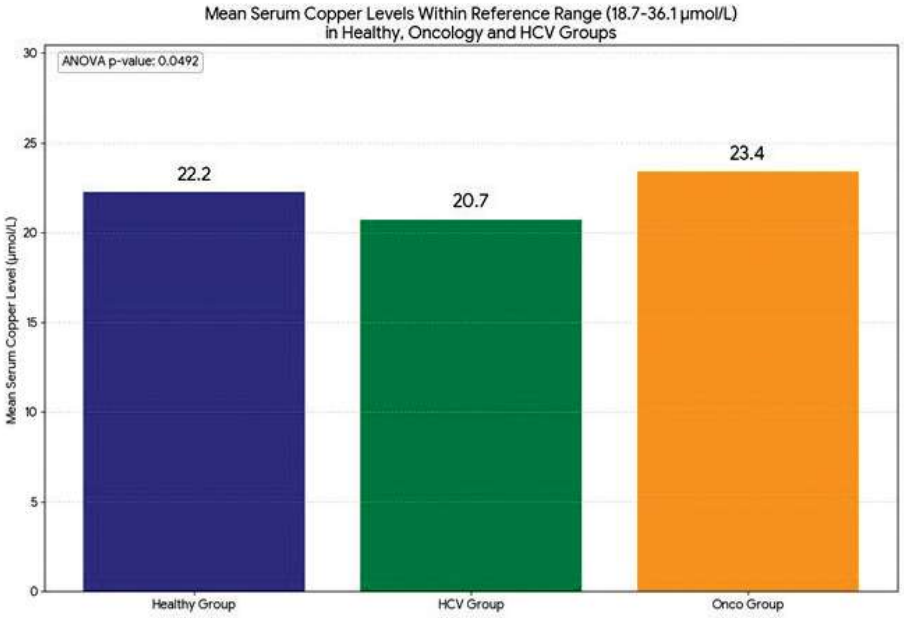

Fig. 2. Mean levels of Cu within the reference range between the three groups

2. Zinc [µmol/L]

The mean value for zinc in the Healthy group is 15.4 ± 5.31 µmol/L, for the HVC group it is 12.7+2.12 µmol/L, and for the Oncology group it is 11.8+2.25 µmol/L. With an F-statistic = 17.76 and p = 0.00, there is a statistically significant difference in the mean values among the three groups (Figure 3).

In the present study, the following reference ranges were used in the assessment of patients' zinc status: 16.5–27.4 µmol/L [6]. The distribution is presented in Table 2.

The mean zinc level among patients in the Healthy group within the reference range was 19.6 ± 2.31 µmol/L. For the Onco group, it was 17.4 ± 011 µmol/L, and for the HCV group, 17.2 ± 0.86 µmol/L. With F = 11.41, p = 0.00, we found a significant difference in the mean zinc levels among the three groups. (Figure 4).

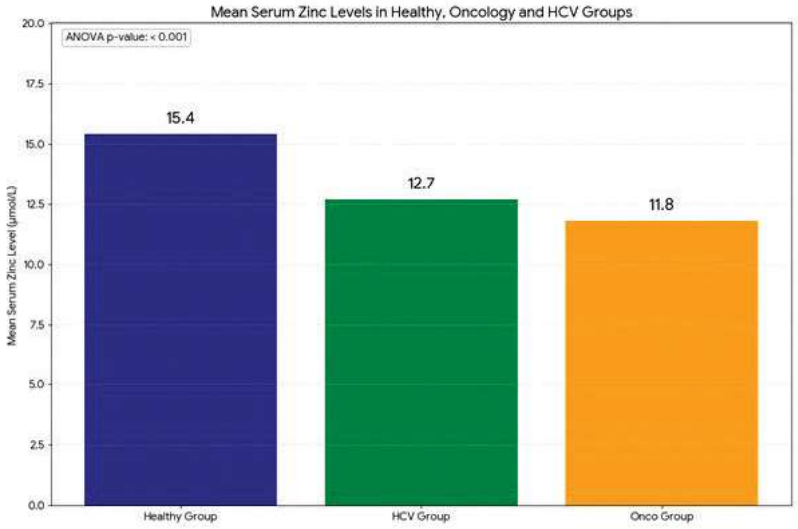

Fig. 3. Mean levels of zinc between the oncology, HCV, and healthy groups

Table 2. Distribution of patients by groups according to the reference interval of zinc in serum

Zinc	Total n	n (%) in ref. interval	n (%) under ref. interval	n (%) above ref. interval
Healthy Group	221	57 (27%)	152 (68%)	12 (5%)
HCV Group	66	2 (3%)	64 (97%)	0 (0%)
Oncology Group	46	2 (4%)	44 (96%)	0 (0%)

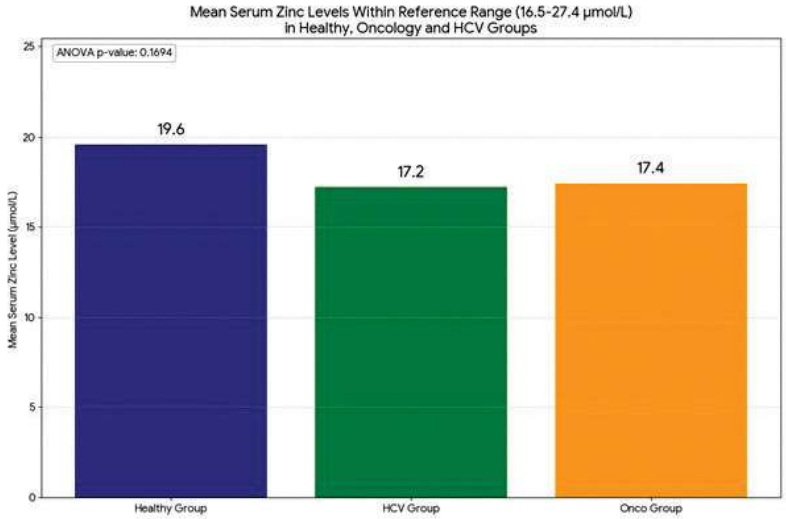

Fig. 4. Mean levels of zinc within the reference range between the oncology, HCV, and healthy groups

3. Copper and zinc together

With a p-value of 0.41, the Pearson correlation coefficient between copper and zinc levels in the Oncology group was r = –0.13. This suggests a negligible negative correlation that was not statistically significant (p>0.05). We performed the same for the Healthy group (r = -0.111, p>0.05) and for the HCV group (r = -0.012, p>0.05).

4. Cu/Zn ratio

Each group's mean serum Cu/Zn ratios were determined to be 1.22 ±0.63, 1.41 ± 0.34, and 1.67 ± 0.66, respectively, for the Healthy, HCV, and Oncology groups.

These mean ratios were compared among the three groups using the ANOVA. There were significant statistical differences in the mean Cu/Zn ratios for the Healthy, HCV, and Oncology groups according to the analysis (F = 12.06, p<0.001).

The percentage of people in each group with Cu/Zn ratios above and below 1.0 was ascertained by additional analysis. Table 3 provides a summary of these findings.

Table 3. Cu/Zn Levels above and below 1

Cu/Zn	Cu/Zn Healthy	Cu/Zn Onco	Cu/Zn HCV
>1	126	42	60
<1	95	4	6

DISCUSSION

Our findings confirm that significant disturbances in the homeostasis of these essential trace elements are characteristic of both chronic HCV and the studied oncological diseases, aligning with the study's primary objective [11]. The oncology group's significantly higher serum Cu levels and the hepatitis C cohort's lower levels suggest a possible role for copper dysregulation in the etiology or development of these diseases [12]. The observed hypercupremia in the Onco group is a well-documented phenomenon. It is often attributed to the systemic inflammatory response, which stimulates the hepatic synthesis of ceruloplasmin, a copper-carrying acute-phase reactant protein. Furthermore, copper is a known cofactor for enzymes involved in angiogenesis and cell proliferation, suggesting that the elevated copper levels may not only be a consequence of the disease, but could also actively contribute to tumor growth and progression.

On the other hand, the decreased Cu levels seen in Hepatitis C patients may be the result of altered hepatic metabolism or increased Cu usage in response to the viral infection's oxidative stress and chronic inflammation [13].

The observed increase in serum Cu levels in cancer patients corresponds with previous studies showing an association between copper and cancer [14]. Studies have demonstrated that elevated Cu levels can promote angiogenesis, a process vital for tumor growth and metastasis [15]. This pro-angiogenic effect comes from Cu's capability to increase the synthesis of vascular endothelial growth factor, a strong signaling molecule promoting the development of new blood vessels [16, 18]. In addition, it has been found that Cu ions activate proangiogenic factors like interleukin 1, tumor necrosis factor alpha, and basic fibroblast growth factor [17]. The observed decrease in serum Cu levels in the case of hepatitis C may be due to the virus's effect on hepatic Cu metabolism. Chronic liver inflammation and fibrosis caused by Hepatitis C virus infection may interfere with the liver's capacity to properly maintain Cu homeostasis. The distribution and excretion of Cu can be changed when the liver, which is essential to Cu metabolism, is impaired.

Metallothioneins, intracellular proteins with an ability to bind metallic ions, are essential storage proteins for both Zn and Cu [19]. Zn transporter dysfunction may play a role in the development or spread of a number of cancers [20]. Reduced Zn levels are common in hepatitis C patients, which could worsen liver damage and interfere with immune function.

These findings have implications for possible therapeutic approaches focusing on the metabolism of Cu and Zn. Considering these findings, using drugs that bind Cu and remove it from the body can be a potential additional cancer treatment [21].

Interestingly, our analysis found no significant correlation between Cu and Zn levels within any of the three groups. This suggests that while both minerals are affected by the disease states, the specific mechanisms driving hypercupremia (e.g., ceruloplasmin synthesis) and hypozincemia (e.g., depletion by oxidative stress) may be largely independent.

The most interesting finding of this study, directly addressing our aim, is the diagnostic potential of the Cu/Zn ratio. While individual Cu and Zn levels were significantly different, the ratio magnified this divergence, showing a clear, progressive increase from the Healthy (1.22) to HCV (1.41) and Onco (1.67) groups (p<0.001). This ratio serves as a more sensitive composite marker of this specific elemental imbalance. The data in Table 3 are particularly stark: while a significant portion of the Healthy group (43%) had a ratio below 1.0, this was true for only 9% of the HCV group and 8.7% of the Onco group. This suggests that a Cu/Zn ratio greater than 1.0 is a strong indicator of a pathological state in this context, supporting its utility as a potential differentiating biomarker. These findings are strongly reinforced by the existing scientific literature, which validates the Cu/Zn ratio as a key indicator in hepatic pathology

and oncogenesis. The clear, progressive increase we observed (Healthy: 1.22, HCV: 1.41, Onco: 1.67) is precisely the trend identified by other researchers.

Our data on the Onco group is strongly supported by Tamai et al., who studied biomarkers in HCC [22]. They concluded that the Cu/Zn ratio could serve as a useful predictive marker for survival in cases of HCC and found that patients with a Cu/Zn ratio ≥0.999 had significantly different survival rates. This directly supports our finding that a ratio greater than 1.0 is a powerful indicator of a pathological state, as seen in 91.3% of our Onco group.

Furthermore, our HCV group (1.41) reflects the state of chronic liver damage. This is consistent with Martinez-Peinado et al., who specifically found that the Cu/Zn ratio correlates with the severity index in cirrhotic patients [23]. This confirms that the ratio is not just elevated, but progressively increases with the severity of liver disease, just as our data shows in the step from HCV to Onco.

This elemental imbalance is a long-observed hallmark of chronic liver disease. Early research by González-Reimers et al. also identified significant alterations in copper and zinc in patients with chronic alcoholic liver disease, reinforcing that disruption of this elemental homeostasis is a fundamental component of hepatic pathology, whether viral or metabolic [24].

CONCLUSIONS

In conclusion, our study demonstrates a significant and progressive imbalance of serum copper and zinc in patients with chronic HCV and oncological diseases. The Cu/Zn ratio can be a strong, sensitive marker of this pathological dysregulation. It can show clear promise as a simple, low-cost biomarker to aid in differentiating these disease states from a healthy population. More studies are needed to validate these findings and correlate them with disease severity and prognosis.

Conflict of Interest Statement: *The authors declare no conflicts of interest related to this work.*

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Ethical statement: *This study has been performed in accordance with the ethical standards as laid down in the Declaration of Helsinki.*

Informed Consent from Participants: *Informed consent was obtained from all participants included in the study.*

REFERENCES

1. Ivanova I, Atanasova B, Kostadinova A, et al. A serum copper and zinc in a representative sample of Bulgarian population. Acta Med Bulg, 2016, 43(2):21-31.
2. Ferreira R, William G. Disorders of metal metabolism. Transl Sci Rare Dis, 2017, 2(3-4):101-139.
3. Michalczyk K, Cymbaluk-Płoska A. The role of zinc and copper in gynecological malignancies. Nutrients, 2020, 12(12):3732.
4. Strashimirov D, Milanov V, Yordanova R, et al. Patients with HIV and tuberculosis coinfection, treated in the Specialized Hospital for Infectious and Parasitic Diseases in Sofia, Bulgaria, for the period 2017–2019. Acta Med Bulg, 2024, 51(Suppl 2):13-18.
5. Gupta S, Read A, Shackel A, et al. The role of micronutrients in the infection and subsequent response to hepatitis C virus. Cells, 2019, 8(6):603.
6. Gulubova M, Chonov D, Ignatova M, et al. New approach in understanding colorectal cancer immunosuppression and immunotherapy-based strategies in the treatment of microsatellite stable colorectal cancer. Acta Med Bulg, 2024, 51(2):65-76.
7. Bhatnagar K, Raju S, Patki N, et al. Targeting mineral metabolism in cancer: insights into signaling pathways and therapeutic strategies. Semin Cancer Biol, 2025, 112:1-19.
8. Złowocka-Perłowska E, Baszuk P, Marciniak W, et al. Blood and serum copper and zinc levels and 10-year survival of patients after kidney cancer diagnosis. Nutrients, 2025, 17(6):944.
9. Popov T, Giragosyan S, Petkova V, et al. Regulatory role of microRNAs in tumor angiogenesis. Acta Med Bulg, 2020, 47:67-72.
10. Mayo Rochester. Interpretive Handbook. Rochester, Mayo Clinic, 2022.
11. Malavolta M, Piacenza F, Basso A, et al. Serum copper to zinc ratio: relationship with aging and health status. Mech Ageing Dev, 2015, 151:93-100.
12. Brewer J, Merajver S. Cancer therapy with tetrathiomolybdate: antiangiogenesis by lowering body copper – a review. Integr Cancer Ther, 2002, 1(4):327-339.
13. Escobedo-Monge M, Barrado E, Parodi-Román J, et al. Copper/zinc ratio in childhood and adolescence: a review. Metabolites, 2023, 13(1):82.
14. Margalioth E, Schenker J, Chevion M. Copper and zinc levels in normal and malignant tissues. Cancer, 1983, 52(5):868-872.
15. Costello L, Franklin R. Cytotoxic/tumor suppressor role of zinc for the treatment of cancer: an enigma and an opportunity. Expert Rev Anticancer Ther, 2011, 12(1):121-128.
16. Heuberger D, Harankhedkar S, et al. High-affinity Cu(I) chelator PSP-2 as potential anti-angiogenic agent. Sci Rep, 2019, 9(1):1-10.
17. Nasulewicz-Goldeman A, Mazur A, Opolski A. Role of copper in tumour angiogenesis-clinical implications. J Trace Elem Med Biol, 2004, 18(1):1-8.
18. Kong R, Sun G. Targeting copper metabolism: a promising strategy for cancer treatment. Front Pharmacol, 2023, 14:1-12.
19. Cai L, Li X, Song Y, Cherian M. Essentiality, toxicology and chelation therapy of zinc and copper. Curr Med Chem, 2005, 12(23):2753-2763.
20. Wang J, Zhao H, Xu Z, Cheng X. Zinc dysregulation in cancers and its potential as a therapeutic target. Cancer Biol Med, 2020, 17(3):612-625.
21. Antoniades V, Sioga A, Dietrich E, et al. Is copper chelation an effective anti-angiogenic strategy for cancer treatment? Med Hypotheses, 2013, 81(6):1159-1163.
22. Tamai Y, Iwasa M, Eguchi A, et al. Serum copper, zinc and metallothionein serve as potential biomarkers for hepatocellular carcinoma. PLoS One, 2020, 15(8):1-12.
23. Martínez-Peinado M, Rueda-Robles A, Nogueras-López F, et al. Serum zinc and copper concentrations and ratios in cirrhotic patients: correlation with severity index. Nutr Hosp, 2018, 35(3):627-632.
24. Rodriguez-Moreno F, González-Reimers E, Santolaria-Fernandez F, et al. Zinc, copper, manganese, and iron in chronic alcoholic liver disease. Alcohol, 1997, 14(1):39-44.