



Research Article

Evaluation of serum proinflammatory cytokines, oxidative stress, and other biochemical markers in chronic viral hepatitis B and C infections

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Abstract

Objectives: Infection with the hepatitis B virus (HBV) or the hepatitis C virus (HCV) results in inflammatory responses, which can subsequently lead to severe progressive hepatic disease. Oxidative stress resulting from changes in the activity of antioxidant enzymes and cytokines and causing an excessive accumulation of reactive oxygen species has been identified as a key factor in the pathogenesis and progression of chronic liver inflammation and disease. Minerals such as zinc (Zn) and copper (Cu), which are part of the biochemical structure of some antioxidant enzymes, also play a part in the complicated pathogenesis of HBV and HCV infections. The aim of this study was to investigate the effects of chronic viral HBV and HCV infection on the status of some serum cytokines, antioxidant enzymes, lipid peroxidation, and serum Zn and Cu levels, and to determine if there was any relationship between them.

Methods: A total of 78 patients with positive clinical and serological markers of HBV or HCV infection were included: 40 chronic HBV-positive patients, 30 inactive HBV carriers, and 8 chronic HCV-positive patients. Thirty healthy subjects were also included in the study as a control group. The level of serum cytokines tumor necrosis factor- α (TNF- α), interleukin-1 β , (IL-1 β), interleukin-2R (IL-2R), interleukin-6 (IL-6), and interleukin-8 (IL-8) was analyzed with a chemiluminescent enzyme immunoassay test, the activity of antioxidant enzymes superoxide dismutase (SOD), phospholipid hydroperoxide glutathione peroxidase (GSH-Px), and catalase (CAT) was measured in erythrocytes, and the malondialdehyde (MDA) level was measured in plasma using a fluorescence spectrophotometric method. The serum Zn and Cu concentration was measured with a flame atomic absorption spectrophotometer.

Results: Serum cytokine (TNF- α , IL-2R, IL-6, and IL-8) levels were significantly higher in both the HBV- and HCV-positive patient groups when compared with the control group ($p < 0.05$). No statistically significant difference was found in IL-1 β values between chronic HBV patients and inactive HBV carriers ($p > 0.05$). Erythrocyte SOD, GSH-Px, and CAT activity, and the serum Zn level were low in both HBV and HCV patients ($p < 0.05$), whereas the plasma MDA and serum Cu levels were found to be significantly elevated ($p < 0.05$).

Conclusion: An impaired oxidative stress reaction and increased proinflammatory cytokines were observed in patients infected with chronic HBV or HCV. Chronic viral hepatitis infection was also observed to affect the homeostasis of Zn and Cu, leading to an increase in the serum Cu level and a decrease in Zn as a result of metabolic interactions. The lipid peroxidation marker MDA may be a useful tool for observing and following up pathogenic mechanisms and the course of chronic HBV and HCV.

Keywords: Chronic hepatitis, Cu, HBV, HCV, MDA, oxidative stress, pro-inflammatory cytokines, Zn

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are a global health problem that is endemic in Turkey. These viruses attack the liver and can cause both acute and

chronic hepatitis infection [1, 2]. Chronic hepatitis infection presents a major risk for the development of hepatocellular carcinoma [2, 3, 4]. These viruses directly induce oxidative stress

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and liver injury, often resulting in chronic infection, which can be followed by cirrhosis and hepatocellular carcinoma [5, 6]. The induction and subsequent production of reactive oxygen species cause DNA damage and affect the repair mechanism in HCV infections [7]. It has been determined that superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) antioxidants have key roles as cytoprotective enzymes with antioxidant and anti-inflammatory activity that prevents liver damage [8]. Lipid peroxidation occurs as result of liver cytotoxicity and oxidative stress in viral hepatitis. MDA is a maker of lipid peroxidation and indirectly of oxidative stress [9].

Cytokines are important in the course viral hepatitis infections. Cytokines can be synthesized by various immune cells and have the ability to remove the virus by creating an immune response [10, 11]. Some proinflammatory cytokines may serve as biomarkers in pathogenesis of chronic HBV and HCV infections [12]. Acute HCV infection is followed by a type 1 helper T cell (Th1) response that produces cytokines (IL-2, interferon gamma, IL-6, and TNF- α) and promotes inflammation as well as cell-mediated immunity in an attempt to control infection [13]. A Th2 response follows, which regulates humoral immunity and the proinflammatory Th1 response. An imbalance between Th1 and Th2 responses has been seen to result in progression and persistence of HCV infection [14]. Th1 cytokines have been associated with progressive liver injury [14, 15]. Some studies of immune response to HCV infection have been conducted in vitro and in animal models [16].

Important trace minerals, such as zinc (Zn) and copper (Cu), act as antioxidants and play a vital role in the immune system. A Zn deficiency leads to reduced activation of natural killer cells and T8 cells [17, 18]. Mineral disorders tend to lead to protection of the cell's own metabolism and may cause oxidative stress and inflammatory reactions, thereby increasing HCV inflammatory processes, increasing liver fibrosis, and decreasing the effectiveness of anti-viral drugs in patients infected with chronic HCV [19, 20]. Liver diseases may be improved by the preservation of Zn and Cu metabolism. Zn treatment has been found to prevent the increase of liver enzymes, reduce liver fibrosis and the synthesis of fatty acids, and to improve the treatment and prognosis in HCV [20]. The maintenance of plasma concentrations of essential minerals, particularly Zn and Cu, is an important therapeutic target that can prevent deterioration in HCV [20].

The aim of this study was to measure IL-1 β , IL-2R, IL-6, IL-8, and TNF- α levels in patients with a chronic HBV or HCV infection. In addition, changes in antioxidants, lipid peroxidation, and trace elements (Zn and Cu) concentrations were investigated.

Materials and Methods

Patients

This study was approved by the ethics committee of the Haran University Faculty of Medicine (date: 16.12.2019, no: 07). Informed consent was obtained from all of the patients in-

cluded in this study and the research was conducted according to the ethical principles of the Declaration of Helsinki.

A total of 78 patients were included in this study: 40 chronic HBV-positive patients, 30 inactive carriers, and 8 HCV-positive patients. All of the patients included had positive hepatitis B surface antigen or anti-HCV results for at least 6 months. Patients with HIV infection and those with chronic inflammatory diseases, heart disease, or diabetes were excluded. There was no history of disease in a healthy control group of 30 healthy individuals used for comparison.

Laboratory method

A venous blood sample was collected from all of the patients and the healthy group after 12 hours of fasting. After centrifugation, the serum was separated and stored at -80°C until the time of analysis.

Cytokine measurement

The analyses were performed using a chemiluminescent enzyme immunometric test (Immulite; Diagnostic Products Corp., Los Angeles, CA, USA). For each cytokine (TNF- α , IL-1 β , IL-2R, IL-6, IL-8) calibration, the calibration curve was plotted according to the standards of the National Institute for Biological Standards and Control (NIBSC) of the UK and the test manufacturer's information. The control reference preparation was also standardized by the NIBSC. Intra- and inter-assay coefficients of variance were also determined.

Analysis of antioxidant enzymes

After fasting overnight, blood samples were drawn into hemogram tubes containing ethylenediaminetetraacetic acid and centrifuged for 10 minutes at 4000 g and 4°C. After plasma separation, the buffy coat was removed and the erythrocytes were washed 3 times with 2 volumes of isotonic solution. Some erythrocytes were then fractionated with cold distilled water (1/4), refrigerated at 4°C for about 20 minutes, and then centrifuged at 4°C for 10 minutes at 2000g to separate the supernatant. The erythrocytes were stored at -70°C until the time of analysis.

HBV and HCV were determined serologically with the respective enzyme-linked immunosorbent assay kit (Microelisa; Biokit SA, Barcelona, Spain). SOD activity was measured as described by Sun et al. [21]. This method uses the inhibition of nitroblue tetrazolium with xanthine-xanthine oxidase as a superoxide generator. The reduction of nitroblue tetrazolium by the superoxide anion to blue formazone was measured spectrophotometrically at the 560 nm wavelength. Unit SOD activity was defined as the amount of protein causing a 50% inhibition of nitroblue tetrazolium reduction with the superoxide. The activity of GSH-PX in erythrocytes was analyzed using a spectrophotometric method. The reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation was seen at the 340 nm wavelength. The amount of

enzyme that oxidized 1 micromole NADPH per minute was shown as 1 unit of GSH-PX [22]. The activity of erythrocyte CAT was determined spectrophotometrically. Solubility of the hydrogen peroxide (H₂O₂) substrate was observed at the 240 nm wavelength as the enzyme reaction decreased. One unit of CAT activity was defined as 1 micromole of H₂O₂ utilized per minute [23]. The MDA concentration was assessed using a fluorescence spectrophotometric method [24].

Evaluation of Zn and Cu

A flame atomic absorption spectrophotometer was used to measure the essential elements of Zn and Cu.

Statistical analysis

The mean and SD were calculated for each biochemical parameter studied. Analysis of variance was performed to determine statistical significance for chronic HBV patients, inactive HBV carriers, and the healthy controls, while Student’s t-test was used to evaluate statistical significance between chronic HCV patients and healthy controls. The level of significance was defined as p<0.05. SPSS for Windows software, Version 11.0 (SPSS Inc., Chicago, IL, USA) was used to perform the analyses.

Results

The cytokine levels in HBV patients were measured and it was observed that the serum level of TNF-α, IL-1β, IL-2R, IL-6, and

IL-8 was significantly increased in chronic HBV patients and inactive HBV carriers compared with the controls (p<0.05; Table 1). There was no significant difference in the level of IL-1β between chronic HBV patients and inactive HBV carriers (p>0.05; Table 1).

The TNF-α, IL-1β, IL-2R, IL-6, and IL-8 values were found to be significantly increased in the chronic HCV patients (p<0.001; Table 1). Erythrocyte antioxidant enzyme (SOD, GSH-Px, CAT) activity in the chronic HBV patients and inactive HBV carriers was significantly decreased when compared with the controls. MDA was significantly increased when the chronic HBV patients and inactive HBV carriers were compared with the controls (p<0.05; Table 2). Analysis of trace elements revealed that the serum Cu level in the chronic HBV patients, chronic HCV patients, and inactive HBV carriers was significantly increased when compared with the controls, but Zn values were significantly decreased when compared with the healthy group (p<0.05; Table 3).

Discussion

Both HBV and HCV can cause chronic liver disease followed by hepatocellular carcinoma, with the majority of deaths from liver cancer attributed to HBV [4]. There has been exploration of the involvement of oxidative stress in inducing liver damage and hepatocarcinogenesis in HBV and HCV [4, 6]. Cytokines have been reported to have a major role in regulating

Table 1. The mean±SD serum level of TNF-α, IL-1β, IL-2R, IL-6, and IL-8 in chronic HBV patients, inactive HBV carriers, and chronic HCV patients, as well as healthy controls

Parameters	Chronic HBV patients (n=40)	Inactive HBV carriers (n=30)	HCV patients (n=8)	Control group (n=30)	p
TNF-α (pg/mL)	12.93±1.64	8.43±1.08	12.94±2.13	4.11±0.78	<0.05*
IL-1β (pg/mL)	4.10±6.08	4.00±0.67	4.01±6.59	3.74±0.53	0.136** <0.05***
IL-2R (U/mL)	668.36±62.89	576.56±55.9	995.31±64.95	535.69±21.16	<0.05*
IL-6 (pg/mL)	17.64±2.51	13.58±2.04	18.88±1.92	4.50±1.17	<0.05*
IL-8 (pg/mL)	11.26±1.34	8.28±0.71	12.05±1.22	4.83±0.62	<0.05*

*Statistically significant for both HBV and HCV patients. **Statistically not significant only for HBV patients. ***Statistically significant only for HCV patients. IL: Interleukin; HBV: Hepatitis B virus; TNF-α: Tumor necrosis factor alpha.

Table 2. The mean±SD serum level of SOD, GSH-Px, CAT, and MDA in chronic HBV patients, inactive HBV carriers, chronic HCV patients, and healthy controls

Parameters	Chronic HBV patients (n=40)	Inactive HBV carriers (n=30)	HCV patients (n=8)	Controls (n=30)	p
SOD U/g Hb	1085.17±86.45	1207.13±36.71	958.33±76.31	1521.15±349.06	<0.05*
GSH-Px U/g Hb	1.478±2.24	2.386±2.08	1.261±1.33	7.915±43.50	<0.05*
CATU/g Hb	13.79±1.50	21.74±1.96	12.85±0.86	32.07±7.17	<0.05*
MDA μmol/dL	4.46±0.78	2.31±0.46	5.04±0.65	1.88±0.94	<0.05*

*Statistically significant for both HBV and HCV patients. CAT: Catalase, GSH-Px: Glutathione peroxidase; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MDA: Malondialdehyde; SOD: Superoxide dismutase.

Table 3. The mean±SD serum level of Cu and Zn in chronic HBV patients, inactive HBV carriers, chronic HCV patients, and healthy controls

Parameters	Chronic HBV patients (n=40)	Inactive HBV carriers (n=30)	HCV patients (n=8)	Controls (n=30)	p
Cu µ/L	159.02±11.33	132.07±6.71	173.52±27.63	119.18±9.23	<0.05*
Zn µ/L	62.14±13.58	72.31±11.12*	23.41±9.73	94.64±6.22	<0.05*

*Statistically significant for both HBV and HCV patients.

inflammation, tissue repair, and even control and cure of viral hepatitis [14, 25, 26].

In this study, proinflammatory cytokines (IL-2R, IL-6, IL-8, TNF- α) were elevated in chronic HBV-positive patients and inactive HBV carriers. These findings were associated with disease activity and severity. Our results indicated that while the IL-1 β level was increased in chronic HBV patients, it was without a statistically significant difference when HBV-positive patients and inactive HBV carriers were compared, while as has been previously reported [27], significant HCV activation of IL-1 β was seen ($p>0.05$; Table 1). Increased serum proinflammatory cytokines has been reported in several series examining chronic HCV infection [28-30]. These cytokines have also been detected in hepatocytes, liver sinusoidal cells, and leukocytes in portal areas of chronic HCV patients [31].

HBV and HCV cause oxidative stress and lipid peroxidation. One of the multifunctional properties of antioxidant enzymes is to relieve oxidative stress [32]. Our data also showed that SOD, GSH-Px, and CAT were significantly decreased and that MDA was significantly increased in both chronic HBV and HCV infection as well as in inactive HBV carriers when compared with healthy subjects ($p<0.05$; Table 2). Our findings were consistent with other studies of HBV patients and support the presence of oxidative stress [5, 7, 33]. Our results confirm the contribution of oxidative stress as part of the pathophysiology of both HBV and HCV infections.

Cu and Zn are trace elements necessary for body metabolism. They are vital enzyme components in cell metabolism and antioxidant defense systems [20, 21, 34]. Cu and Zn are members of a superoxide dismutase enzyme group (CuZnSOD), which catalyzes the superoxide anion dismutation into H₂O₂ and oxygen. Therefore, they have a significant effect on the activity of these antioxidants [35]. Even a slight change in the level of these elements may result in the change of antioxidant activity. Thus, alteration in the homeostasis of these trace elements affects the development of liver disease, especially in patients with chronic HBV and HCV infections [21, 35, 36]. Trace elements are necessary in the treatment of viral hepatitis [34, 37]. In our study, the serum Zn level was found to be decreased while the Cu level was increased. These results support previous studies [19, 21]. Antioxidants increase the immune response by producing proinflammatory cytokines, and positively or negatively regulate Cu and Zn levels. Some proinflammatory cytokines and trace elements, Zn in particular, can

alleviate disease progression in patients with chronic HBV or HCV, since cytokines promote a T cell response and Zn accelerates the antiviral effects of some cysteine proteins, such as metallothioneins [26, 37].

Conclusion

Chronic HBV and HCV infections upregulate some cytokines as result of oxidative stress, liver injury, and lipid peroxidation. A significant increase in MDA and Cu levels and a significant decline in the free radical scavenging capacity and Zn level was observed in this study. Although the exact mechanism underlying the relationship between plasma minerals and antioxidant enzymes in patients with chronic HBV and HCV is unknown, it may be due to deterioration in the homeostasis of these minerals. Therefore, we strongly recommend the use of Zn and some cytokines to facilitate therapeutic mechanisms in the treatment of chronic HBV and HCV infections.

Conflict of interest: None declared.

Ethics Committee Approval: This study was approved by the Ethics Committee of the Harran University Faculty of Medicine (Date: 16.12.2019, No: 07).

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References

- Özkan H. Epidemiology of Chronic Hepatitis B in Turkey. *Euroasian J Hepatogastroenterol* 2018;8(1):73–4. [\[CrossRef\]](#)
- Lavanchy D. Chronic viral hepatitis as a public health issue in the world. *Best Pract Res Clin Gastroenterol* 2008;22(6):991–1008.
- Petruzzello A. Epidemiology of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) Related Hepatocellular Carcinoma. *Open Virol J* 2018;12:26–32. [\[CrossRef\]](#)
- Tanaka J, Akita T, Ko K, Miura Y, Satake M; Epidemiological Research Group on Viral Hepatitis and its Long-term Course, et al. Countermeasures against viral hepatitis B and C in Japan: An

- epidemiological point of view. *Hepatol Res* 2019;49(9):990–1002. [\[CrossRef\]](#)
5. Acar A, Görenek L, Aydin A, Eyigün CP, Eken A, Sayal A, et al. Investigation of oxidative stress and antioxidant defense in patients with hepatitis B virus infection and the effect of interferon-alpha plus lamivudine combination therapy on oxidative stress. *Mikrobiyol Bul* 2009;43(3):411–23.
 6. Ivanov AV, Valuev-Elliston VT, Tyurina DA, Ivanova ON, Kochetkov SN, Bartosch B, et al. Oxidative stress, a trigger of hepatitis C and B virus-induced liver carcinogenesis. *Oncotarget* 2017;8(3):3895–932. [\[CrossRef\]](#)
 7. Anticoli S, Amatore D, Matarrese P, De Angelis M, Palamara AT, Nencioni L, et al. Counteraction of HCV-Induced Oxidative Stress Concur to Establish Chronic Infection in Liver Cell Cultures. *Oxid Med Cell Longev* 2019;2019:6452390. [\[CrossRef\]](#)
 8. Pár A, Róth E, Rumi G Jr, Kovács Z, Nemes J, Mózsik G. Oxidative stress and antioxidant defense in alcoholic liver disease and chronic hepatitis C. [Article in Hungarian] *Orv Hetil* 2000;141(30):1655–9.
 9. Kaya S, Sütüçü R, Sesli Cetin E, Cicioğlu Aridoğan B, Aktütrk O, Delibaş N. The relationship between viral load and malondialdehyde and antioxidant enzymes in patients with hepatitis C virus infection. [Article in Turkish] *Mikrobiyol Bul* 2006;40(1-2):55–61.
 10. Koziel MJ. Cytokines in viral hepatitis. *Semin Liver Dis* 1999;19(2):157–69. [\[CrossRef\]](#)
 11. Missale G, Ferrari C, Fiaccadori F. Cytokine mediators in acute inflammation and chronic course of viral hepatitis. *Ann Ital Med Int* 1995;10(1):14–8.
 12. Li H, Huang MH, Jiang JD, Peng ZG. Hepatitis C: From inflammatory pathogenesis to anti-inflammatory/hepatoprotective therapy. *World J Gastroenterol* 2018;24(47):5297–311. [\[CrossRef\]](#)
 13. de Avila L, Weinstein AA, Estep JM, Curry MP, Golabi P, Escheik C, et al. Cytokine balance is restored as patient-reported outcomes improve in patients recovering from chronic hepatitis C. *Liver Int* 2019;39(9):1631–40. [\[CrossRef\]](#)
 14. Beltra JC, Decaluwe H. Cytokines and persistent viral infections. *Cytokine* 2016;82:4–15. [\[CrossRef\]](#)
 15. Baskic D, Vukovic VR, Popovic S, Djurdjevic P, Zaric M, Nikolic I, et al. Cytokine profile in chronic hepatitis C: An observation. *Cytokine* 2017;96:185–8. [\[CrossRef\]](#)
 16. Koike K, Moriya K, Matsuura Y. Animal models for hepatitis C and related liver disease. *Hepatol Res* 2010;40(1):69–82. [\[CrossRef\]](#)
 17. Marion O, Abravanel F, Izopet J, Kamar N. Failure to respond to ribavirin despite elevated intra-erythrocyte zinc level in transplant-patients with chronic hepatitis E virus infection. *Transpl Infect Dis* 2019;21(2):e13050. [\[CrossRef\]](#)
 18. Ko YL, Morihara D, Shibata K, Yamauchi R, Fukuda H, Kunimoto H, et al. Factors Attenuating Zinc Deficiency Improvement in Direct-Acting Antiviral Agent-Treated Chronic Hepatitis C Virus Infection. *Nutrients* 2018;10(11):1620. [\[CrossRef\]](#)
 19. Murakami Y, Koyabu T, Kawashima A, Kakibuchi N, Kawakami T, Takaguchi K, et al. Zinc supplementation prevents the increase of transaminase in chronic hepatitis C patients during combination therapy with pegylated interferon alpha-2b and ribavirin. *J Nutr Sci Vitaminol (Tokyo)* 2007;53(3):213–8. [\[CrossRef\]](#)
 20. Papanikolopoulos K, Alexopoulou A, Dona A, Hadziyanni E, Vasilieva L, Dourakis S. Abnormalities in Cu and Zn levels in acute hepatitis of different etiologies. *Hippokratia* 2014;18(2):144–9.
 21. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988;34(3):497–500.
 22. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70(1):158–69.
 23. Aebi H. Catalase. In: Bergmeyer H, editor. *U Methods of enzymatic analysis*. New York: Academic Press; 1974. p. 673–80.
 24. Wasowicz W, Nève J, Peretz A. Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. *Clin Chem* 1993;39(12):2522–6.
 25. Mounieb F, Ramadan L, Akool ES, Balah A. Propolis alleviates concanavalin A-induced hepatitis by modulating cytokine secretion and inhibition of reactive oxygen species. *Naunyn Schmiedebergs Arch Pharmacol* 2017;390(11):1105–15.
 26. Xia Y, Protzer U. Control of Hepatitis B Virus by Cytokines. *Viruses* 2017;9(1):18. [\[CrossRef\]](#)
 27. Beech H. Expression of concern: Hepatitis C virus activates interleukin-1 β via caspase-1-inflammasome complex. *J Gen Virol* 2019;100(9):1342. [\[CrossRef\]](#)
 28. Batsaikhan B, Lu MY, Yeh ML, Huang CI, Huang CF, Lin ZY, et al. Elevated interleukin-4 levels predicted advanced fibrosis in chronic hepatitis C. *J Chin Med Assoc* 2019;82(4):277–81.
 29. Afify M, Hamza AH, Alomari RA. Correlation Between Serum Cytokines, Interferons, and Liver Functions in Hepatitis C Virus Patients. *J Interferon Cytokine Res* 2017;37(1):32–8. [\[CrossRef\]](#)
 30. Estevez J, Chen VL, Podlaha O, Li B, Le A, Vutien P, et al. Differential Serum Cytokine Profiles in Patients with Chronic Hepatitis B, C, and Hepatocellular Carcinoma. *Sci Rep* 2017;7(1):11867.
 31. Abouelasrar Salama S, Lavie M, De Buck M, Van Damme J, Struyf S. Cytokines and serum amyloid A in the pathogenesis of hepatitis C virus infection. *Cytokine Growth Factor Rev* 2019;50:29–42. [\[CrossRef\]](#)
 32. Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. *World J Gastroenterol* 2014;20(25):8082–91.
 33. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, et al. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *Int J Mol Sci* 2015;16(11):26087–124. [\[CrossRef\]](#)
 34. Sahin M, Karayakar F, Koksall AR, Yetim A, İyisoy MS, Şen İ, et al. Changes in Liver Tissue Trace Element Concentrations During Hepatitis B Viral Infection Treatment. *Biol Trace Elem Res* 2019;188(2):245–50. [\[CrossRef\]](#)
 35. Wołoncziej M, Milewska E, Roszkowska-Jakimiec W. Trace elements as an activator of antioxidant enzymes. *Postepy Hig Med Dosw (Online)* 2016;70(0):1483–98. [\[CrossRef\]](#)
 36. Huang Y, Zhang Y, Lin Z, Han M, Cheng H. Altered serum copper homeostasis suggests higher oxidative stress and lower antioxidant capability in patients with chronic hepatitis B. *Medicine (Baltimore)* 2018;97(24):e11137. [\[CrossRef\]](#)
 37. Read SA, Parnell G, Booth D, Douglas MW, George J, Ahlenstiel G. The antiviral role of zinc and metallothioneins in hepatitis C infection. *J Viral Hepat* 2018;25(5):491–501. [\[CrossRef\]](#)