Research Article ISSN 2639-9458

Microbiology & Infectious Diseases

Total Oxidant Status (TOS), Total Antioxidant Status (TAS), Ferritin and Vitamin D Levels in Patients with Chronic Hepatitis C

Gülten TOPRAK[1], İlhan SABANCILAR[2], Nida ÖZCAN[3*] and Hüseyin ALKAN[4]

[1]*Dicle University, Faculty of Medicine, Department of Medical Biochemistry, Diyarbakır, Turkey*

[2]*Dicle University, Faculty of Veterinary, Department of Biochemistry, Diyarbakır, Turkey.*

[3]*Dicle University, Faculty of Medicine, Department of Medical Microbiology, Diyarbakır, Turkey.*

[4]*Dicle University, Faculty of Pharmacy, Department of Biochemistry, Diyarbakır, Turkey.*

***Correspondence:**
Nida ÖZCAN, Dicle University, Faculty of Medicine, Department of Medical Microbiology, Diyarbakır, Turkey.

Received: 02 March 2020; **Accepted:** 04 April 2020

Citation: Gülten TOPRAK, İlhan SABANCILAR, Nida ÖZCAN, et al. Total Oxidant Status (TOS), Total Antioxidant Status (TAS), Ferritin and Vitamin D Levels in Patients With Chronic Hepatitis C. Microbiol Infect Dis. 2020; 4(2): 1-4.

ABSTRACT

Objectives: *Oxidative stress is thought to play a role in chronic liver diseases, but its effect on chronic hepatitis C (CHC) remains unclear. The aim of this study was to investigate oxidative stress parameters such as total oxidant and antioxidant capacity, ferritin and vitamin D levels among patients with CHC.*

Materials and Methods: *A total of 49 patients with CHC infection were included in the study. The control group (50 subjects) consisted of healthy blood donors. Total oxidant status (TOS), total antioxidant status (TAS), ferritin and vitamin D levels were measured in both groups. TAS and TOS levels were determined spectrophotometrically, ferritin and vitamin D levels and Hepatitis C virus antibodies (Anti-HCV) were determined by electrochemiluminescence method.*

Results: *The mean TOS value of CHC patients was found higher than control group(74.44 ± 55.54 versus 42.73 ± 13.48). Mean ferritin and Vitamin D levels in CHC patients were found as 218.09 ± 27.47 and 23.72 ± 14.60 respectively. TOS and ferritin levels were significantly higher ($p<0.05$) and vitamin D levels were significantly lower ($p <0.05$) in CHC patients. There wasn't any statistically significant difference in TAS levels between CHC patients and the control group($p>0.05$).*

Conclusion: *High oxidative stress markers, TOS and ferritin, suggest that oxidative stress is increased in CHC. There is a need for extensive research involving more patients.*

Keywords

Hepatitis C, Oxidative stress, Ferritin, Vitamin D.

Introduction

Hepatitis C virus (HCV) is a RNA virus affecting about 3–4 million individuals worldwide [1,2]. The people with chronic HCV infection have a high risk to develop cirrhosis and hepatocellular carcinoma (HCC) [2,3]. Some mechanisms are suggested for hepatocarcinogenesis in HCV infection. In mouse model studies, the core protein of the virus has been shown to have the main role in inducing hepatocarcinogenesis [4]. However, the mechanisms of this induction have not yet been clearly defined. Induction of oxidative stress and modulation of some cellular products are mechanisms thought to induce hepatocarcinogenesis [5,6]. Moriya et al., revealed that alterations in the oxidant/antioxidant state of the liver by HCV core protein may contribute to HCC in HCV infection, without developing inflammation. They also revealed that alcohol caused a significant increase in phosphatidylcholine

(PCOOH) levels in transgenic mice, which suggested a synergism between alcohol and HCV in hepatocarcinogenesis [5].

Reactive oxygen species (ROS) are extensively reactive oxygen-containing free radical molecules. These free radicals are produced in small quantities by the metabolic reactions of the body and have the ability to damage cellular molecules including proteins, lipids and genetic materials. The imbalance between production and removal of ROS is known as oxidative stress [7]. Cytochrome P450 enzyme systems in neutrophils, Kupffer cells and the hepatocytes are the major sources of ROS. Hepatocytes and their cellular molecules are among the most affected structures by reactive nitrogen species (RNS) and ROS [8]. Up to date, several studies have been carried out about oxidative system molecules and hepatocytes worldwide [8-12]. Erel evaluated new automated methods to measure total antioxidant status (TAS) in 2004 and total oxidant status (TOS) in 2005 [13,14].

Ferritin is an iron storage protein which acts as an indicator for the level of iron stores. Iron is essential for the survival and proliferation of the cell. Since the presence of excess iron in the cell leads to the formation of toxic ROS, extensively regulated mechanisms are necessary to control intracellular iron levels. Ferritin, with its two subunits –H and L- plays a major role in these mechanisms. The H subunit has ferroxidase activity which not only catalyses the oxidation of ferrous iron but also provides resistance to intracellular oxidative damage [15,16].

Vitamin D [25(OH)D], is not only the main regulator of the calcium and phosphate homeostasis but has several effects on non-skeletal tissues. Recently, numerous studies have been carried out on the antioxidative effect of vitamin D [17–20].

In this study, it was aimed to investigate total oxidant and antioxidant levels, ferritin and vitamin D levels among chronic hepatitis C (CHC) patients. The same oxidative system parameters in healthy blood donors will be measured and compared with CHC patients. Thus, information about oxidative stress in chronic hepatitis C patients will be obtained.

Materials and Methods

Ethics Committee approval of the study was obtained from Dicle University Faculty of Medicine in September 2017 (No: 387). The study was carried out as a randomized case control study between January - December 2018. The study included patients aged between 16-62 years admitting to Dicle Univesity Hospital clinics with the diagnosis of CHC infection. The diagnosis of CHC was based on Anti-HCV and HCV-RNA positivity for at least 6 months. All patients lived in the same region and had no underlying disease. Pregnant women, patients with autoimmune, metabolic or systemic diseases and non-HCV hepatitis were excluded from the study. Control group consisted of healthy blood donors. All patients and healthy volunteers provided written informed consent to participate in the trial according to the approved protocol. The patients have not taken any medication the day before blood intake for testing. Eight milliliters of venous blood samples were collected aseptically from each subject. The sera were obtained by centrifugation of the blood samples at 4000 rpm / min for about 10 minutes and were stored at -80°C till the testing time. Total antioxidant status (TAS), total oxidant status (TOS), ferritin and vitamin D levels of each sample were measured.

Anti-HCV antibody, vitamin D and ferritin levels were measured by Cobas 601 (Roche Diagnostics, USA) autoanalyser with electrochemiluminescence method. TAS and TOS levels were measured by spectrophotometric method with Rel Assay Diagnostics kits (Mega Medicine, Gaziantep, Turkey) developed by Erel [13,14]. In the TAS assay, the absorbance of colored dianisidyl radicals which were generated during potent free radical reactions is measured. The color formation is suppressed in proportion to the concentration of antioxidants in the sample. This reaction is evaluated spectrophotometrically on the automatic analyzer [13]. The TOS assay measures the amount of ferric ions which were generated during the oxidation of ferrous ions by the means of oxidant molecules. The oxidant molecules present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. Glycerol in the medium accelerates this reaction up to about three times. Ferric ions form a colored complex with “xylenol orange” in acidic environment. The intensity of the color associated with the amount of oxidants present in the sample is measured spectrophotometrically [14].

Architect c16000 (Abbott Diagnostics, USA) autoanalyzer was used for assesment. The TOS results were expressed as μmol H_2O_2 Eq/L (micromolar hydrogen peroxide equivalent per litre) due to calibration process with hydrogen peroxide (H_2O_2). The TAS results were stated in terms of μmol Trolox Eq/L.

SSPS 15.0 version software was used for statistical analysis of the data. Mean ± standard deviation values were used in descriptive statistics. Kolmogorov-Smirnov test was used for the normal distribution of data in the groups. Student t test was used for normal distribution and Mann Whitney U test was used for abnormal distribution in the presence of significant difference between the groups. $P<0.05$ was accepted for statistical significance level. Pearson correlation test was used to determine the correlation in parameters where p value was less than 0.05.

Results

Of the CHC patients included in the study, 27 were female and 22 were male. The control group consisted of 21 females and 29 males. The mean TOS value of CHC patients was 74.44 ± 55.54 while it was measured as 42.73 ± 13.48 in the control group. TOS levels were significantly higher in CHC patients compared to control group ($p<0.05$). The mean TAS value was found higher (1.33 ± 0.25 versus 1.29 ± 0.21) in CHC patients compared to control group but the difference wasn’t statistically significant ($p>0.05$).

The mean ferritin level of CHC patients was found as 218.09 ± 27.47 while it was 87.54 ± 48.61 in the control group. Ferritin levels were found as significantly higher in CHC patients (p<

0.05). The mean vitamin D level was measured as lower in CHC patients than control group (23.72 ± 14.60 versus 25.12 ± 7.53). The difference was statistically significant ($p<0.05$). TOS and ferritin levels were positively correlated (r=0.5) while a weak negative correlation (r=0.3) was found between TOS and vitamin D levels in patients with CHC. Age, gender, oxidative stress and antioxidant system parameters of study groups were summarised in the Table 1.

Groups / Parameters	Chronic Hepatitis C	Control	p value
Age (Mean ± SD)	61.08 ± 16.26	47.78 ± 5.69	
Gender	Female/Male = 27/22	Female/Male = 21/29	
TOS (Mean ± SD)	74.44 ± 55.54	42.73 ± 13.48	0.017*
TAS (Mean ± SD)	1.33 ± 0.25	1.29 ± 0.21	0.072
Ferritin (Mean ± SD)	218.09 ± 27.47	87.54 ± 48.61	0.009*
Vitamin D (Mean ± SD)	23.72 ± 14.60	25.12 ± 7.53	0.019*

Table 1: Age, gender, oxidative stress and antioxidant system parameters of study groups.
SD: Standart deviation, TAS: Total Anti-oxidant response, TOS: Total oxidative status.
*: $p<0.05$ was determined as significant.

Discussion

The imbalance in oxidant and antioxidant levels causes many metabolic and functional disorders. Excess free radicals such as superoxide, peroxide, hydroxyl, known as reactive oxygen molecules, threaten the integrity of phospholipids, proteins as well as genetic materials [21,22]. Released protein oxidation products and lipid peroxidation products lead to cell destruction [11,23]. High amounts of oxidative radicals increase the oxidative stress of the cell. Increased oxidative stress in the liver leads to cell death and elevated liver enzymes, which may lead to liver cirrhosis if left untreated [9,12,24,25].

Several testing methods were used to determine the oxidative status; we aimed to evaluate the oxidative status by measuring TOS, TAS, ferritin, and vitamin D levels in the present study. In a study about oxidative status in hepatitis B infection, TOS index and TAS levels were evaluated in chronic hepatitis patients (33 person), inactive HBsAg carriers (31 person), cirrhotic patients (12 person) and healthy control group (16 person). TOS index was found higher while TAS level was lower in chronic hepatitis B (CHB) and cirrhotic patients than in inactive carriers and control group subjects [26]. Testing of TAS and TOS levels was similar to that of our study, but ferritin and vitamin D levels were also tested in current study. Since there was no carrier status in hepatitis C infection, no carriers were evaluated; also cirrhotic patients were not included in our study. Similar to the previous study, our study also found a higher TOS index in patients with hepatitis C infection, compared with the control group. No significant difference was observed in TAS levels of the patient and control groups in the present study. This can be explained by the elevation of TAS levels due to antiviral treatment.

Compared to hepatitis C infection, there have been more studies of hepatitis B infection and oxidant balance. In another study about HB, Duygu et al. found higher lipid peroxidation and TOS index levels in CHB patients compared to inactive carriers [27]. Chrobot et al., found statistically significant decrease of catalase and superoxide dismutase activities and increase of glutathione peroxidase activity in children with chronic viral hepatitis [25]. Dikici et al. determined some oxidative stress and antioxidant parameters in patients with acute and CHB patients. A poly-unsaturated fatty acid peroxidation product -malondialdehyde (MDA)-, and conjugated dienes (CD) were measured as oxidative stress parameters while reduced glutathione (GSH) and beta-caroten as antioxidants. The parameters were evaluated both before and 6 months after interferon-alpha (IFN-α) treatment in CHB patients. Oxidative stress parameters -MDA and CD- were significantly higher while antioxidants -GSH and beta-caroten levels- were significantly lower than the control group. They also mentioned that the parameters returned about to normal levels after 6 months of IFN-α treatment [28]. Our study also revealed that oxidative stress parameters –TOS and ferritin- were higher in CHC patients. In a study about oxidative stress in both CHB and chronic hepatitis C (CHC), Çıragil et al. mentioned significantly higher MDA levels in CHC than CHB. Catalase (CAT) and superoxide dismutase (SOD) activities were significantly lower in patients with CHC compared to CHB patients. MDA levels were lower, CAT and SOD levels were higher in both CHB and CHC patients compared to control group [12]. This suggests that oxidative stress levels may vary in different stages of infections as well as in different liver infections (hepatitis B and C). In the present study, TOS and ferritin were significantly higher in CHC patients than in the control group ($p <0.05$), but we could not compare the different stages of the disease.

Amanzada et al. evaluated pre and post-treatment serum levels of Vitamin D and ferritin among CHC patients. Higher ferritin and lower serum vitamin D levels before treatment were found to be significantly related to hepatic inflammation and fibrosis [29]. Our study is similar to that study, in terms of determination of ferritin and vitamin D levels in hepatitis C patients but in our study, patients were not discriminated according to whether they received treatment or not. The results of current study, higher ferritin levels and lower vitamin D levels in CHC patients, were consistent with Amanzada et al.'s study. A meta-analysis about CHC and vitamin D revealed that low vitamin D levels in CHC patients was associated with a higher possibility of fibrosis progression and a lower possibility of achieving sustained virological reponse [30]. Vitamin D levels of our study were significantly lower in patients with CHC, consistent with previous studies.

Conclusion

In conclusion, there are various oxidative stress parameters and we determined TOS, TAS, ferritin and vitamin D among hepatitis C patients in current study. TOS and ferritin levels were higher, while vitamin D levels were lower in CHC patients compared to healthy controls. The limitations of the study were the small number of cases and the lack of separation of patients according

to the treatment status and stage of the disease. Determination of oxidative stress parameters may help in prognosis monitoring or treatment support of the disease. Further clinical research is needed on the contribution of vitamin D to treatment in patients with CHC.

Acknowledgment

The authors declared that they had no conflict of interest.

This study was supported by Dicle University Scientific Research Projects Coordination Unit (DUBAP), (No: TIP 18.017).

References

1. Purcell RH. The Discovery of the Hepatitis Viruses. Gastroenterology. 1993; 104: 955-963.
2. Lauer GM, Walker BD. Hepatitis C Virus Infection. N Engl J Med. 2001; 345: 41-52.
3. Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol. 2007; 13: 2436-2441.
4. Moriya K, Fujie H, Shintani Y, et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med. 1998; 4: 1065-1067.
5. Moriya K, Nakagawa K, Santa T, et al. Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res. 2001; 61: 4365-4370.
6. Mamiya N, Worman HJ. Hepatitis C virus core protein binds to a DEAD box RNA helicase. J Biol Chem. 1999; 274: 15751-15756.
7. Wu D, Cederbaum AI. Alcohol, Oxidative Stress, and Free Radical Damage. Alcohol Res Heal. 2003; 27: 277-284.
8. Arauz J, Ramos-Tovar E, Muriel P. Redox state and methods to evaluate oxidative stress in liver damage: From bench to bedside. Ann Hepatol. 2016; 15: 160-173.
9. Yamamoto Y, Yamashita S, Fujisawa A, et al. Oxidative Stress in Patients with Hepatitis, Cirrhosis, and Hepatoma Evaluated by Plasma Antioxidants. Biochem Biophys Res Commun. 1998; 247: 166-170.
10. Esrefoglu M. Oxidative stress and benefits of antioxidant agents in acute and chronic hepatitis. Hepat Mon. 2012; 12: 160-167.
11. Sorrentino P, Terracciano L, D'Angelo S, et al. Oxidative stress and steatosis are cofactors of liver injury in primary biliary cirrhosis. J Gastroenterol. 2010; 45: 1053-1062.
12. Ciragil P, Belge Kurutas E, Kokoglu OF, et al. Oxidative Stress In Patients With Chronic Hepatitis B And C. Med J Trak Univ. 2010; 2011: 300-303.
13. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem. 2004; 37: 112-119.
14. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005; 38: 1103-1111.
15. Orino K, Lehman L, Tsuji Y, et al. Ferritin and the response to oxidative stress. Biochem J. 2001; 357: 241-247.
16. Arosio P, Levi S. Ferritin, iron homeostasis, and oxidative damage. Free Radic Biol Med. 2002; 33: 457-463.
17. Bao B-Y, Ting H-J, Hsu J-Wet al. Protective role of 1α, 25-dihydroxyvitamin D3 against oxidative stress in nonmalignant human prostate epithelial cells. Int J Cancer. 2008; 122: 2699-2706.
18. Pang Q, Qu K, Zhang J-Y, et al. Evidence supporting a beneficial role of vitamin D in chronic hepatitis C. J Hepatol. 2015; 63: 530-531.
19. Kitson MT, Sarrazin C, Toniutto P, et al. Vitamin D level and sustained virologic response to interferon-based antiviral therapy in chronic hepatitis C: A systematic review and meta-analysis. J Hepatol. 2014; 61: 1247-1252.
20. Sales de Almeida JP, Liberatti LS, Nascimento Barros FE, et al. Profile of oxidative stress markers is dependent on vitamin D levels in patients with chronic hepatitis C. Nutrition. 2016; 32: 362-367.
21. Frei B. Reactive oxygen species and antioxidant vitamins: mechanisms of action. Am J Med. 1994; 97: 22S-28S.
22. Farinati F, Cardin R, Degan P, et al. Oxidative DNA damage in circulating leukocytes occurs as an early event in chronic HCV infection. Free Radic Biol Med. 1999; 27: 1284-1291.
23. Bast A, Haenen GR, Doelman CJ. Oxidants and antioxidants: state of the art. Am J Med. 1991; 91: 2S-13S.
24. Poli G. Pathogenesis of liver fibrosis: role of oxidative stress. Mol Aspects Med. 2000; 21: 49-98.
25. Chrobot AM, Szaflarska-Szczepanik A, Drewa G. Antioxidant defense in children with chronic viral hepatitis B and C. Med Sci Monit. 2000; 6: 713-718.
26. Bolukbas C, Bolukbas FF, Horoz M, et al. Increased oxidative stress associated with the severity of the liver disease in various forms of hepatitis B virus infection. BMC Infect Dis. 2005; 5: 95.
27. Duygu F, Karsen H, Aksoy N, et al. Relationship of oxidative stress in hepatitis B infection activity with HBV DNA and fibrosis. Ann Lab Med. 2012; 32: 113-118.
28. Dikici I, Mehmetoglu I, Dikici N, et al. Investigation of oxidative stress and some antioxidants in patients with acute and chronic viral hepatitis B and the effect of interferon-α treatment. Clin Biochem. 2005; 38: 1141-1144.
29. Amanzada A, Goralczyk AD, Moriconi F, et al. Vitamin D status and serum ferritin concentration in chronic hepatitis C virus type 1 infection. J Med Virol. 2013; 85: 1534-1541.
30. Villar LM, Del Campo JA, Ranchal I, et al. Association between vitamin D and hepatitis C virus infection: a meta-analysis. World J Gastroenterol. 2013; 19: 5917-5924.

© 2020 Gülten TOPRAK, et al. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License