Thiol-disulfide balance and total oxidant-antioxidant status in patients with chronic hepatitis C

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Abstract

Objective: Increasing evidences suggest that oxidative stress is closely related to the pathogenesis of hepatitis C virus (HCV) infection. The purpose of this study was to examine the dynamic thiol/disulfide homeostasis (DTDH) and total oxidant/antioxidant status in patients with HCV infection. Methods: Levels of serum total thiol (TT), native thiol (NT), disulfide (DS), total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI; TOS/TAS ratio) as oxidative stress markers were determined in 162 individuals, including 74 patients with HCV infection and 88 non-HCV controls. Results: The NT, TT levels and NT/TT ratio were significantly lower and DS level, DS/NT and DS/TT ratios were significantly higher in HCV group compared to the control group. The TOS and OSI values were significantly higher and the TAS level was significantly lower in the HCV group than in the control group. No significant correlations were found between oxidative stress markers and albumin, alanine aminotransferase, aspartate aminotransferase and bilirubin levels in patients with HCV infection. A negative correlation was found only between OSI and albumin. Conclusion: These results indicate that patients with HCV infection are vulnerable to oxidative stress and have disturbed status of oxidant and antioxidant.

What's known

Oxidative stress is associated with pathogenesis of hepatitis C virus (HCV) infection.

Thiols are oxidized by oxidant radicals and form disulfide bonds.

Dynamic thiol-disulfide homeostasis is an important indicator used to evaluate the presence and intensity of oxidative stress.

Abnormal dynamic thiol-disulfide homeostasis plays a role in the pathogenesis of various diseases.

What's new

This study is the first report of thiol-disulfide homeostasis in HCV-infected patients.

Thiol/disulfide homeostasis is disturbed and shifts to the disulfide side in patients with HCV infection.

TOS and OSI were higher and TAS was lower in HCV-infected patients compared to healthy controls.

This study shows that patients with HCV infection are vulnerable to oxidative stress and have disturbed status of oxidant- antioxidant.

Introduction

Chronic hepatitis C (CHC) infection is a liver disease caused by the hepatitis C virus (HCV). It is a major public health problem, affecting approximately 71 million people worldwide, and is one of the leading causes of cirrhosis, hepatocellular cancer, and liver-related deaths. It is estimated that approximately 400,000 people died in 2015 from complications related to CHC.[1] Although the mechanism of how HCV induces liver damage is not fully understood to date, it has been suggested that oxidative stress plays an important role in the pathogenesis of CHC.[2-4] Oxidative stress, caused by the deterioration of homeostasis between reactive oxygen species (ROS) and antioxidants, triggers liver damage by inducing DNA damage, lipid peroxidation, and protein oxidation in the organism. The increased oxidative stress in HCV infection induces hepatic stellate cell activation, DNA damage, and gene mutation, which progresses toward liver fibrosis/cirrhosis and hepatocellular carcinoma.[5]

Thiols containing the sulfhydryl (SH) group are part of the antioxidant defense system as they are metal chelators, free radical scavengers, and thiol/disulfide redox buffer components. The thiol groups of sulfur-containing compounds, the main targets of ROS, react with oxidant molecules to form disulfide bonds (S-S). This process is reversible; disulfide bonds can be reduced to obtain the thiol groups.[6] The redox cycle between thiol groups and disulfide bonds maintains a dynamic thiol–disulfide homeostasis (DTDH), which plays an important role in many physiological processes such as cellular growth, cell signaling, protein conformation, detoxification of xenobiotics, apoptosis, and the antioxidant defense system.[7] While the thiol side of the thiol–disulfide system has been measured since 1979, it was only in 2014 that Erel and Neselioğlu developed a reliable way to measure the level of both components of this system quickly and cheaply.[8]Previous studies have reported that impaired DTDH plays a role in the pathogenesis of various diseases such as chronic hepatitis B,[9] Crimean-Congo hemorrhagic fever,[10] diabetes[11] and familial Mediterranean fever.[12] However, DTDH, an alternative indicator of oxidative stress, has not yet been investigated using the new method in HCV-infected patients. Therefore, the objective of this study was to examine the DTDH as well as total antioxidant status (TAS) and total oxidant status (TOS) in patients with HCV infection.

Methods

Subjects

This case-control study was conducted between March and July 2019 at the Infectious Diseases and Clinical Microbiology Department of Harran University School of Medicine, Sanliurfa, Turkey. The study included 74 patients with HCV and 88 subjects without HCV infection as the control group. CHC was diagnosed based on the presence of anti-HCV antibodies and HCV-RNA positivity. Seven cases had been previously treated and relapsed, while 67 patients were treatment-naïve.

A detailed medical history of all participants was obtained, and physical examinations and laboratory tests were performed. Patients with malignancy, hematological disease, endocrine disorder (excluding diabetes mellitus), HIV infection, hepatitis A or B virus infection, liver cirrhosis, hepatocellular carcinoma and other chronic liver diseases, pregnancy, antioxidant medication use, or the use of alcohol and/or vitamin supplements were excluded from the study. This study was approved by the Harran University School of Medicine Ethics Committee Commission (protocol number HRU/19.03.31). Written informed consent was obtained from all participants before blood samples were collected.

Laboratory Tests

Fasting blood specimens were taken into serum gel tubes via venipuncture from each participant, and afterwards centrifuged at 3000 rpm for 10 min. Separated serums were aliquoted and kept at -80degC until DTDH, TAS, and TOS measurements were performed.

Complete blood counts were determined using an Alinity hq hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA). The levels of clinical chemistry parameters (serum albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin, total protein, urea, and creatinine) were determined using an Architect c16000 analyzer (Abbott Diagnostics, Abbott Park, IL, USA) with standard methods. Prothrombin time (PT) and international nor-

malized ratio (INR) values were determined using a Sysmex CS-2100i coagulation analyzer (Sysmex Corp., Kobe, Japan). Viral markers were measured using the chemiluminescent microparticle immunoassay method in an Architect i2000 SR analyzer (Abbott Laboratories, Lake Bluff, IL, USA). Serum HCVRNA levels and HCV genotypes were determined using the Abbott RealTime HCV quantification, Abbott RealTimeHCV Genotype II kits, and Abbott m2000 RealTime polymerase chain reaction system (Abbott Laboratories, Lake Bluff, IL, USA).

Oxidative stress markers

Native thiol (NT), total thiol (TT) and disulfide (DS) levels were analyzed according to the method of Erel and Neselioglu.[8] DS (S-S) levels were determined by calculating half of the difference between TT (-SH+S-S) and NT (-SH); subsequently, DS/NT, DS/TT, and NT/TT ratios were calculated.[8]

Serum TAS and TOS levels were measured using the automated colorimetric method developed by Erel O.[13,14] After measuring the TOS and TAS levels, as a further good marker of oxidative stress, the oxidative stress index (OSI) was calculated as follows: OSI= [(TOS /TAS)x100].[15]

Statistical analysis

Statistical analyses were performed using SPSS version 21.0 (Chicago IL, USA) . A $p < 0.05$ value was accepted as significant. For normality testing, the Kolmogorov–Smirnov test was used. The comparisons of variables between groups were performed using Student's t-test, Mann-Whitney U test and χ^2 test. The correlation between oxidative stress-related markers and liver function tests was determined using the Spearman test or the Pearson test.

Results

Our study included 74 patients with HCV infection and 88 individuals without HCV infection. Demographic characteristics and laboratory findings of the patient and control groups are summarized in Table1. The mean HCV-RNA of patients with HCV infection was 1513297.6 ± 2898584 IU/mL, and the distribution of the genotypes (gp) was as follows: gp 1a, 10%; gp 1b, 47%; gp 1, 12%; gp 2, 20%; gp 3, 8%; and gp 5, 3%.

There was no significant difference in age, gender distribution, body mass index, smoking, or comorbidities (diabetes mellitus, hypertension, cardiovascular disease, chronic renal failure, hyperlipidemia, and asthma/chronic obstructive pulmonary disease) between the HCV patients and controls ($p > 0.05$). ALT, AST, GGT, total protein, total bilirubin and direct bilirubin levels were significantly higher in the HCV group compared with the control group, whereas the albumin level was significantly lower ($p < 0.05$ for all). Leukocyte and platelet counts, as well as urea, creatinine, PT, and INR values were similar in the two groups ($p > 0.05$) (Table1).

As shown in Table 2, the NT, TT, and NT/TT levels were significantly lower in the HCV group than in the control group, whereas the DS, DS/NT, and DS/TT levels were significantly higher ($p < 0.05$ for all). The TOS and OSI levels were significantly higher and the TAS levels were significantly lower in the HCV group than in the control group ($p < 0.001$ for all).

In the correlation analysis between oxidative stress markers (TAS, TOS, OSI and DTDH) and liver function tests (albumin, ALT, AST and bilirubin) in the HCV group, there was only a negative correlation between OSI and albumin ($r = -0.301$, $p = 0.009$).

DiscussionGrowing evidences suggest that oxidative stress caused by disruption of the balance between ROS and antioxidants is associated with the pathogenesis and progression of CHC.[2-4] Induction of NADPH oxidases by HCV proteins, HCV protein-mediated mitochondrial dysfunction, production of various cytokines, decreased GSH output, iron overload, induced antioxidant gene expression and increased expression of HCV core protein and cytochrome P450 2E1 have been reported as the possible sources of ROS in HCV infection.[16]Antioxidant defense systems remove increased ROS to maintain the redox balance in the liver.[5] Thiols are one of the antioxidants that protect cells and tissues against oxidative damage. Under oxidative stress, thiols can be oxidized by oxidant molecules to form reversible DS bonds and thereby resulting in

altered DTDH. Therefore, DTDH is an important indicator used to evaluate the presence and intensity of oxidative stress.[8]

Many previous studies found that total thiol levels measured using the Ellmann's method were significantly lower in patients with HCV infection than in those without.[17-19] However, there were no data on DTDH in these studies because the level of DS, the thiol oxidation product, could not be determined with this method. Both components of the DTDH can be measured simultaneously using the automated colorimetric method developed by Erel and Neselioglu in 2014, thereby this balance can be fully evaluated.[8] Prior to the availability of this technique, the thiol and disulfide levels of low molecular weight thiols (LMWTs), such as glutathione (GSH), glutathione disulfide (GSSG), cysteine (Cys) and cystine (CySS), were generally measured to evaluate the status of DTDH.[8]However, LMWTs constitute only a small part of the plasma thiol pool; a large part primarily comprises albumin and protein thiols.[20] Therefore, we assume that the low molecular weight thiol/disulfide homeostasis such as GSG/GSSG and cys/CySS may not precisely reflect body DTDH.

Lin et al.[21] found that the levels of GSH, a predominant LMWT, were low and GSSG were high in HCV group before treatment compared with the control group; however, it was observed that the GSSG level decreased after treatment. Jain et al.[4]showed that the plasma GSSG/(GSH + GSSG) ratio in patients with HCV infection was significantly higher than in the controls. Similarly, another study demonstrated low GSH and high GSSG levels in HCV patients, suggesting a high GSH turnover.[22] Consistent with these studies, we found that NT level, TT level and NT/TT ratio were significantly lower and DS level, DS/NT ratio and DS/TT ratio were significantly higher in HCV group compared to the control group. These results indicate that DTDH is disturbed and shifts to the DS side in patients with HCV infection. This shift reveals that patients with HCV infection are exposed to high oxidative stress. This increased oxidative stress in CHC may be due to chronic inflammation and continuous production of ROS in HCV-infected cells.[16] Similarly, researchers have observed that the DTDH shifted to the DS side in other infectious diseases such as chronic hepatitis B,[9] and Crimean-Congo hemorrhagic fever,[10]brucellosis.[23]

In our study, we also found no correlation between DTDH parameters and albumin, ALT, AST, and bilirubin levels, which is in concordance with some previous studies.[3,17,18] In contrast, some researchers reported that ALT and AST were negatively correlated with GSH and positively correlated with GSSG in HCV-infected patients.[24-26] These conflicting results may be due to differences in the characteristics of the patients with HCV infection included in the studies, such as disease stage and the presence or absence of HCV-related liver disease.

Several studies have demonstrated that antioxidant therapy may have a beneficial effect on normalization of ALT, decrease in viral load and improvement of liver histology in patients with HCV infection.[27,28] In that aspect, we believe that compensation for the thiol deficiency with thiol resources such as N-acetylcysteine and alpha-lipoic acid may improve the efficacy of treatment in these patients.

To assess oxidative stress, TOS and TAS measurements may provide more valuable information than individual measurements of each oxidant and antioxidant molecule.[13,14] Therefore, in this study, TAS, TOS, and OSI values of the HCV and control groups were also compared. We observed that the TOS and OSI values were higher and the TAS level was lower in the HCV group compared with the control group, as in a previous study.[19] These results indicate increased oxidative stress in HCV infected patients.

There are some limitations in this study. It was conducted with a small number of participants in a single center. Furthermore, the relationship between the progression of CHC disease and the DTDH status was not examined.

ConclusionTo the best of our knowledge, the present study is the first to examine the DTDH status in patients with HCV infection. DTDH shifted toward DS formation in patients with HCV infection. The TOS and OSI levels were significantly higher and TAS level was significantly lower in HCV group compared to the control group. These results indicate that patients with HCV infection are vulnerable to oxidative stress and have disturbed status of oxidant- antioxidant. This study supports the view that oxidative stress plays

a role in the pathogenesis of CHC.

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