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Serum levels of advanced oxidation protein products, malonyldialdehyde, and total radical trapping antioxidant parameter in patients with chronic hepatitis C

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Background/aims: *Oxidative stress increases in chronic hepatitis C, and antioxidant defense mechanisms are impaired. The aims of this study were to compare chronic hepatitis C patients and healthy subjects according to oxidative stress and antioxidant system markers, and to determine the relationship between oxidative stress and hepatosteatosis.* ***Methods:*** *This is an observational study in a tertiary center. Twenty-nine biopsy-proven chronic hepatitis C patients, with no prior anti-viral treatment and persistently elevated serum transaminase levels for 6 months, were included. The control group included 46 healthy subjects. Advanced oxidation protein products and malonyldialdehyde levels were measured. Total radical-trapping antioxidant parameter was calculated.* ***Results:*** *Baseline characteristics were similar in the patient and control groups. In chronic hepatitis C patients, serum levels of advanced oxidation protein products were significantly higher than in the control group (235.0±142.8 vs 116.7±79.5, p<0.001). Serum levels of malonyldialdehyde were also significantly higher than in the control group (9.3±2.1 vs 6.5±1.1, p<0.001). However, there was no significant difference in total radical-trapping antioxidant parameter. The total radical-trapping antioxidant parameter/advanced oxidation protein products index was significantly lower in chronic hepatitis C patients than in healthy controls (p<0.05). There was no significant correlation between advanced oxidation protein products and malonyldialdehyde and hepatosteatosis.* ***Conclusions:*** *We conclude that oxidative stress occurs in chronic hepatitis C, and antioxidant defense mechanisms are inadequate. Serum levels of advanced oxidation protein products and malonyldialdehyde are higher in chronic hepatitis C patients when compared to healthy individuals, and may be useful markers in chronic hepatitis C.*

Key words: Hepatitis C virus, oxidative stress, advanced oxidation protein products, malonyldialdehyde, total radical-trapping antioxidant parameter

Kronik hepatit C hastalarında serum ileri oksidasyon protein ürünleri, malonildialdehid ve total radikal tutucu antioksidan parametre düzeyleri

Amaç: *Kronik hepatit C'de oksidatif stres artmış, antioksidan savunma mekanizmaları zayıflamıştır. Bu çalışmanın amacı, kronik hepatit C hastalarını ve sağlıklı kontrolleri oksidatif stres ve antioksidan sistem belirteçleri açısından karşılaştırmak ve oksidatif stres ile hepatosteatoz arasındaki ilişkiyi araştırmaktır.* ***Yöntem:*** *Bu, üçüncü basamak bir merkezde yürütülen gözlemsel bir çalışmadır. Biyopsi ile tanıları doğrulanmış olan, daha önce antiviral tedavi almamış, son 6 aydır ısrarcı serum transaminaz yüksekliği gösteren 29 kronik hepatit C hastası çalışmaya alınmıştır. Kontrol grubu 46 sağlıklı bireyden oluşmaktadır. İleri oksidasyon protein ürünleri'nin ve malonildialdehid'in serum düzeyleri ölçülmüştür. Total radikal tutucu antioksidan parametre hesaplanmıştır.* ***Bulgular:*** *Hasta ve kontrol gruplarında bazal karakteristikler benzerdi. Kronik hepatit C hastalarında, ileri oksidasyon protein ürünleri (235.0±142.8 vs 116.7±79.5, p<0.001) ve malonildialdehid'in (9.3±2.1 vs 6.5±1.1, p<0.001) serum düzeyleri, kontrol grubuna göre anlamlı derecede daha yüksekti (p<0.05). Ancak, total radikal tutucu antioksidan parametre düzeyleri açısından iki grup arasında anlamlı fark gözlenmedi. Kronik hepatit C hastalarında, total radikal tutucu antioksidan parametre/ileri oksidasyon protein ürünleri indeksi, sağlıklı kontrollere göre anlamlı derecede daha düşük bulundu (p<0.05). Hepatosteatoz derecesi ile ileri oksidasyon protein ürünleri ya da malonildialdehid düzeyleri arasında anlamlı ilişki saptanmadı.* ***Sonuç:*** *Sonuç olarak, kronik hepatit C hastalarında oksidatif stres artmıştır ve antioksidan savunma mekanizmaları yetersizdir. Kronik hepatit C hastalarında ileri oksidasyon protein ürünleri'nin ve malonildialdehid'in serum düzeyleri armıştır, bu parametreler kronik hepatit C'de yararlı belirteçler olabilir. Kronik hepatit C hastalarında total radikal tutucu antioksidan parametre/ileri oksidasyon protein ürünleri indeksi, oksidatif stres ve antioksidan savunma sistemi arasındaki dengeyi değerlendirmek açısından faydalı bir parametre olabilir.*

Anahtar kelimeler: HCV, oksidatif stres, ileri oksidasyon protein ürünleri, malonildialdehid, total radikal tutucu antioksidan parametre

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INTRODUCTION

Hepatitis C virus (HCV) is one of the main causative agents of chronic viral hepatitis. Chronic hepatitis C (CHC) can progress to cirrhosis and eventually to hepatocellular carcinoma over a period of 20 to 30 years. The mechanisms by which HCV causes cell damage are not well understood. Different mechanisms including immunological liver damage, direct cytotoxicity mediated by different viral products and oxidative stress (OS) have been suggested as playing a role in the pathogenesis of CHC (1,2).

Oxidative stress (OS) is a general term used to describe the steady state level of oxidative damage in a cell, tissue or organ, caused by reactive oxygen species (ROS). Most ROS come from endogenous sources as by-products of normal and essential reactions such as energy generation from mitochondria. Free radicals and reactive non-radical species derived from radicals exist in biological cells and tissues at low but measurable concentrations. Their concentrations are determined by the balance between their rates of production and clearance by various antioxidant compounds and enzymes (3). Toxic oxygen free radicals and reactive non-radical species have been implicated as important pathologic mediators in many clinical disorders (4).

Non-enzymatic glycation of proteins by glucose, leading to the formation of toxic and immunogenic advanced glycation end-products (AGEs), may be a major contributor to the pathological manifestations of diabetes mellitus, aging, and possibly, neurodegenerative diseases such as Alzheimer's. There is a close relationship between carbonyl stress (CS) and OS. Both OS and CS result in formation of advanced oxidation protein products (AOPP), AGEs, and lipoperoxidation end-products (ALEs). AOPP is a new parameter that is used as an OS marker (5,6).

The use of total radical-trapping antioxidant parameter (TRAP) has been proposed recently to assess the antioxidant property of a plasma sample. TRAP may be either directly measured by a fluorescence-based method (TRAPm), or calculated (TRAPc) by a mathematical formula, taking into account the serum levels of five natural antioxidants: protein-bound SH (thiol) groups, uric acid, bilirubin, vitamin E, and vitamin C. The difference between TRAPm and TRAPc may be due to unidentified antioxidants and to the possible synergism among antioxidants (7).

Malonyldialdehyde (MDA) is the end-product of lipid peroxidation, which is a process wherein ROS degrade polyunsaturated lipids. MDA is a marker of lipid peroxidation and it contributes to oxidatively generated DNA damage (8).

Oxidative stress (OS) plays a major role in CHC. Various OS markers were found to be elevated in HCV-related liver disease. Some studies about oxidative DNA damage in CHC have been reported, and one of them was related to hepatic 8-hydroxydeoxyguanosine (8-OHdG), which was quantified in liver biopsy samples from 118 naive patients. This study showed that oxidative DNA damage is associated with increased risk for HCC, and hepatic 8-OHdG levels are useful markers to identify the extreme high-risk subgroup (9).

The aims of this study were:

1) To compare patients with CHC and healthy subjects according to the markers of OS (AOPP, MDA) and markers of the antioxidant defense system (TRAP);
2) To assess the balance between the antioxidant system and OS (TRAP/AOPP);
3) To determine whether or not a relationship exists between OS and hepatic steatosis.

MATERIALS AND METHODS

The study initially included 36 patients with CHC referred to the Gastroenterology Department. HCV genotype 1b was determined in all patients. None of the patients had received any anti-viral treatment before. Informed consents were obtained from each patient, and the study was approved by the university ethical committee.

All patients fulfilled the following inclusion criteria:

1) Persistently elevated serum transaminase levels for 6 months;
2) Positive for both anti-HCV antibody and HCV-RNA;
3) Liver biopsy specimens compatible with CHC.

Exclusion criteria were as follows:

1) Liver cirrhosis
2) Contraindication for liver biopsy
3) Positive serological markers for hepatitis B virus
4) Seropositivity for anti-HIV antibody

5) Chronic alcohol consumption in excess of 20 g per day
6) Signs associated with autoimmune or metabolic liver disease
7) Severe cardiac and renal disease
8) Diabetes mellitus or impaired fasting glucose or impaired oral glucose tolerance
9) Drug addiction
10) Drugs such as interferon, immunosuppressive agents or medications that cause steatosis
11) Use of vitamins or antioxidant drugs within the last 6 months
12) Patients with elevated bilirubin levels (serum total bilirubin >1.4 mg/dl).

We excluded patients with elevated bilirubin levels in order to avoid a bias in TRAP calculation; bilirubin is a component of the TRAP formula. Patients with diabetes or impaired glucose tolerance were excluded to avoid confounding by increased OS and/or hepatosteatosis that is associated with these conditions. Interferon therapy also mandated exclusion because of its possible effects on oxidative status. According to these criteria, we excluded 3 patients because of newly-diagnosed diabetes, 2 patients who developed hyperbilirubinemia and 2 patients due to antioxidant supplement use. Thus, the final patient group consisted of 29 individuals.

The control group consisted of 46 healthy individuals with no medical history of systemic disease whose physical examinations, biochemical, hematological and virological serum markers (HbsAg, antiHbctot, anti-HCV, antiHIV) and abdominal ultrasonographies were normal.

Medical histories were taken and detailed physical examinations were performed in both patients and healthy control group subjects, and individual body mass index (BMI) (kg/m^2) was calculated before including them in the study.

Liver biopsies were performed, and all of the specimens were evaluated by the same pathologist using the Metavir scoring system. The degree of steatosis was determined by calculating the percentage of affected hepatocytes.

Serum HCV-RNA level was determined by reverse transcriptase – polymerase chain reaction (PCR) using a commercial kit (MagAttract Virus Mini M48 Kit, *RealTime*™ HCV Amplification Reagent Kit, Abbott), and anti-HCV antibody was determined by ELISA (chemiluminescence).

Genotype analysis of all subjects was performed by the Line Assay (Inno-LiPA) strip method and all were determined as genotype 1b.

Concentration of thiol groups was measured with Ellman's reagent in 1 M potassium-phosphate buffer, pH = 8.0; absorbency was read at 410 nm and calculations were performed from the standard curve with reduced glutathione (0.1-1.0 mmol/L) for calibration (10).

Advanced oxidation protein products (AOPP) were measured by spectrophotometry according to the Witko-Sarsat method (11); absorbency was read at 340 nm and results were expressed in chloramine T units (µmol/L).

Malonyldialdehyde (MDA) levels were measured using the thiobarbituric acid method (12); calculations were performed from the standard curve with 1,1,3,3-tetramethoxypropane (5-20µmol/L) for calibration.

Serum vitamin C and vitamin E levels were measured using the high performance liquid chromatography (HPLC) system (Shimadzu-Prominence). A C18 column was used as analytical column for vitamin E. The Bischoff prontosil AQ column was used as analytical column for vitamin C. Detection was applied at 300 nm for vitamin E and at 254 nm for vitamin C with an ultraviolet-visible detector.

Total radical-trapping antioxidant capacity (TRAP) was calculated by a mathematical formula associated with serum levels of five natural antioxidants: [1.7 (vitamin C) +2.0 (vitamin E) +2.0 (bilirubin) +1.3 (uric acid) +0.33 (thiol group)] (13).

Data collected in this study was evaluated using the SPSS 12.0 software (SPSS Inc., Chicago, IL, USA). Comparisons between two groups were made using the Student's t test. For the analysis of ranked data when samples failed to show a normal distribution, Kruskal-Wallis test was employed for comparison between three groups; the Mann-Whitney U test was employed for comparison between two groups. The Pearson chi-square test and Fisher's exact test were used for categorical data. The relation between variables was examined with correlation analysis. P values of less than 0.05 were considered as statistically significant.

RESULTS

There were no statistically significant differences in terms of sex, age and BMI between CHC patients and healthy subjects.

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), fasting blood glucose, uric acid, and bilirubin were significantly higher in the group of CHC patients than in the control group ($p<0.05$).

Serum levels of AOPP and MDA were also significantly higher in the CHC group ($p<0.001$), but there was no significant difference between the two groups in terms of TRAP value ($p>0.05$). The ratio between antioxidant capacity and OS described as the TRAP/AOPP index was significantly lower in CHC patients than in healthy controls ($p<0.001$) (Table 1).

Patients were divided into four groups according to steatosis percentage of liver biopsy specimens (Group 1: <5%, Group 2: 5-10%, Group 3: 11-20%, Group 4: >20%). There were 12 patients in Group 1, 5 patients in Group 2, 7 patients in Group 3, and 5 patients in Group 4. No significant association was found between AOPP, MDA, TRAP, or TRAP/AOPP index and steatosis percentage ($p>0.05$).

There was no correlation between serum AOPP levels or MDA levels and necroinflammatory activity, fibrosis or liver transaminase levels ($p>0.05$).

In CHC patients, MDA and TRAP values were positively correlated to AOPP (Figures 1, 2), and the TRAP/AOPP ratio decreased as the AOPP value increased ($p<0.05$) (Table 2). There was no statistically significant correlation between MDA, TRAP and AOPP levels in the healthy control group. A statistically significant positive correlation was observed between MDA level and TRAP level in CHC patients; however, no such correlation was found in the healthy control group (Table 3).

DISCUSSION

Advanced oxidation protein products (AOPP) is a new OS indicator; it shows the oxidation-mediated protein damage and plays a role as an inflammatory mediator (14). In recent times, the role of AOPP in uremia, coronary artery disease and diabetes mellitus has attracted considerable attention (14-16). AOPP is generated by an effect of the

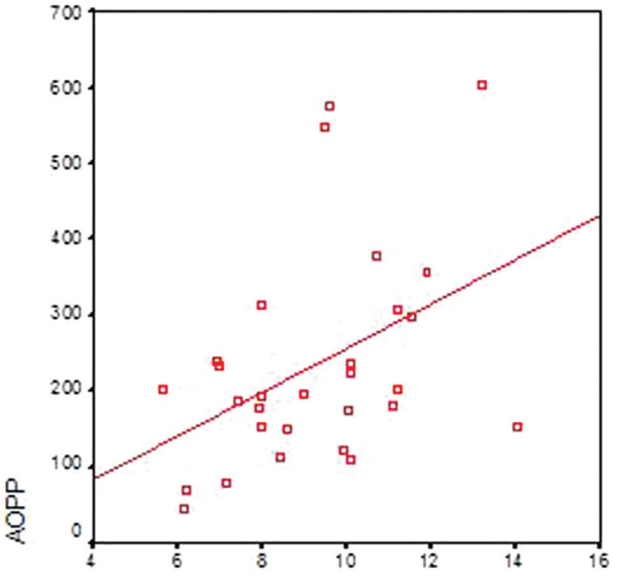

Figure 1. Graphic depicting the relationship between AOPP and MDA levels. In CHC patients, MDA and TRAP values were positively correlated with AOPP.

Table 1. Parameters of oxidative stress, antioxidant system and biochemical findings

	CHC patients (n:29)	Control group (n:46)	P value
ALT IU/L	59.4±27.9	17.7±7.2	<0.001
AST IU/L	43.6±15.6	18.3±3.9	<0.001
GGT IU/L	52.1±37.4	18.6±8.3	<0.001
Fasting blood glucose mg/dl	94.6±9.3	90.3±7.5	<0.05
Uric acid mg/dl	4.6±1.9	3.7±1.2	<0.05
Bilirubin mg/dl	0.8±0.6	0.6±0.3	<0.05
AOPP µmol/L	235.0±142.8	116.7±79.5	<0.001
MDA µmol/L	9.3±2.1	6.5±1.1	<0.001
TRAP µmol/L	1511.7±396.8	1477.6±386.5	>0.05
TRAP/AOPP (index)	8.3±4.1	16.5±9.5	<0.001

SD: Standard deviation. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. GGT: Gamma-glutamyl transferase. AOPP: Advanced oxidation protein products. MDA: Malonyldialdehyde. TRAP: Total radical-trapping antioxidant parameter. All values are expressed as mean ± standard deviation (Mean ± SD)

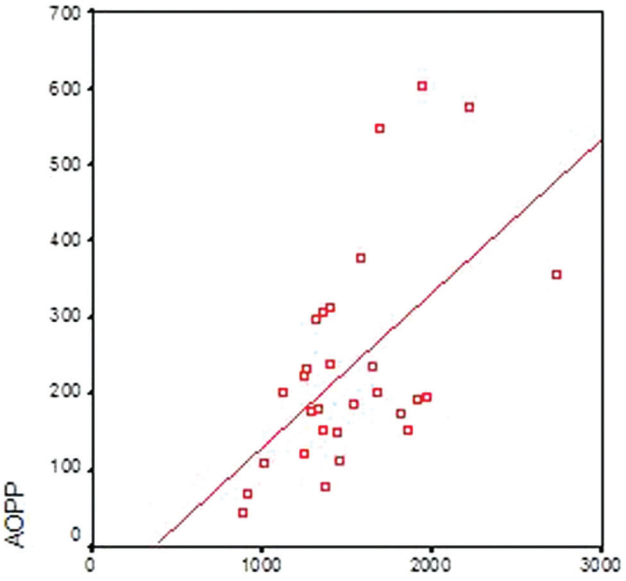

Figure 2. Graphic depicting the relationship between AOPP and TRAP levels. In CHC patients, MDA and TRAP values were positively correlated with AOPP.

chloramine oxidants from the active neutrophil myeloperoxidase enzyme (predominantly hypochlorase, acid and chloramines) during OS. These are defined as cross- bonded protein products including di-tyrosine, and are accepted as the trusted indicators of protein oxidative modifications (11,17).

Our study showed that serum AOPP level is statistically significantly higher in CHC patients when compared with the healthy control group.

Table 2. The relation between AOPP and other parameters

Group		MDA	TRAP	TRAP/AOPP
CHC	R	0.429	0.559	-0.760
(n=29)	p	0.020	0.002	0.000
Healthy Control	R	0.230	0.227	-0.613
(n=46)	p	0.125	0.129	0.000

AOPP: Advanced oxidation protein products. MDA: Malonyldialdehyde. TRAP: Total radical-trapping antioxidant parameter. CHC: Chronic hepatitis C.

Table 3. The relation between MDA and TRAP level

Group		TRAP	TRAP/AOPP
CHC	R	**0.514**	-0.336
(n=29)	p	**0.004**	0.074
Healthy Control	R	0.145	-0.228
(n=46)	p	0.336	0.128

AOPP: Advanced oxidation protein products. TRAP: Total radical-trapping antioxidant parameter. CHC: Chronic hepatitis C.

This finding supports the hypothesis that OS plays a role in CHC pathogenesis (18,19).

Malonyldialdehyde (MDA) is the end-product of lipid peroxidation and forms by degradation of the polyunsaturated lipids by ROS. It has previously been reported that MDA level in CHC patients increases in serum and in liver tissue (20,21). Our study has shown that the MDA level in CHC patients was statistically significantly higher when compared to the healthy control group. This finding is compatible with previous reports.

Total radical-trapping antioxidant parameter (TRAP) is used to measure the antioxidant capacity of a plasma sample. Reduced TRAP levels reflect the reduced antioxidant activity in type 2 diabetes mellitus patients (10). When we compared the CHC patients with the control group in our study, no significant difference was found in terms of TRAP. However, there was a positive correlation between TRAP levels and AOPP and MDA levels in the CHC group, but not in the control group.

In addition, we used the TRAP/AOPP index, which determines the balance between the antioxidant system and OS (10). The TRAP/AOPP index of CHC patients was significantly lower when compared to the healthy control group. Despite the increase in TRAP level, parallel to the increase in AOPP and MDA levels, a low TRAP/AOPP index in CHC patients suggests that the magnitude of TRAP increment is relatively small when compared with the increase in AOPP.

Recently, Venturini et al. (22) evaluated the relationship of OS with iron status and disease activity markers in untreated CHC patients. Differing from our findings, the investigators reported lower values in TRAP; however, TRAP was determined by measuring the chemiluminescence inhibition time induced by 2,2-azobis (2-amidinopropane), and AOPP and TRAP/AOPP index were not evaluated in that study.

Oxidative stress (OS) contributes to lipid accumulation in the liver (steatosis), where it plays a major role in terms of necroinflammation and hepatic cell necrosis (20,23,24). The lipid accumulation in the liver in turn increases the potential of OS triggering the lipid peroxidation. Steatosis and lipid peroxidation in both alcoholic and non-alcoholic hepatitis cause stellate cell activation, which in turn causes fibrogenesis and, consequently, cirrhosis of the liver (1,18).

In our study, steatosis percentage was correlated neither to AOPP levels nor to MDA levels. As mentioned above, increased OS is expected to be associated with increased steatosis. This discrepancy may be explained by the small sample size of our study.

According to the HCV genotype, the mechanism of lipid accumulation in the liver varies. Patients infected with HCV genotype non-3 demonstrate a general "metabolic type" steatosis, whereas patients infected with HCV genotype 3 demonstrate a "viral type" steatosis directly due to the cytopathic effects of the virus (25,26). HCV genotype 1b was determined in all of our patients. This finding is not surprising; 97.1% of CHC patients in Turkey are infected with HCV genotype 1b (27).

Through the studies carried out in humans and experimental models, the mechanism for the "viral type" steatosis has been established as the interaction of viral proteins with lipoprotein generation and secretion processes (19,28). The mechanism of metabolic type steatosis of HCV is complex and is not yet fully understood. Recent studies have shown that insulin resistance, hyperhomocysteinemia and dysregulation of adipocytokines may be responsible (29-31).

Recently, Vidali et al. (19) studied the relation between OS, insulin resistance, steatosis, and fibrosis in CHC. The results of that study pointed out that OS frequently occurs in CHC patients and plays a role in the development of HCV-related "metabolic type" steatosis. In our study, however, we did not find any significant correlation between OS and the steatosis percentage. This may be due to the difference in methods for the assessment of OS and different sample sizes in the above-mentioned study and ours.

A limitation of our study is that the relationship between markers of metabolic syndrome and insulin resistance were not compared to markers of OS. The small sample size is another limitation.

In conclusion, we noted that OS increases in CHC patients as shown by increases in AOPP and MDA. However, antioxidant defense mechanisms represented by TRAP may not increase sufficiently and accordingly, therefore, the balance destabilizes in favor of OS. To the best of our knowledge, this is the first study to evaluate serum AOPP levels in CHC patients.

When CHC patients are compared with the control group, no significant difference is present in terms of BMI; however, fasting blood glucose and uric acid levels were found to be higher when compared with the control group. These may be considered as indirect findings of metabolic syndrome and insulin resistance. However, further studies with larger numbers of patients are required to verify these findings.

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