RAPID COMMUNICATION

Relationship between antioxidant capacity and oxidative stress in children with acute hepatitis A

Mustafa Cemek, Semih Dede, Fahri Bayiroğlu, Hüseyin Çaksen, Fatma Cemek, Nihat Mert

Abstract

AIM: To investigate in children with acute hepatitis A. According to our knowledge, there are no data about the blood levels of malondialdehyde (MDA, an indicator of oxidative stress) and nonenzymic antioxidants in children with acute hepatitis A.

METHODS: Whole blood MDA and reduced glutathione (GSH), serum β-carotene, retinol, vitamin E and vitamin C levels were studied in 19 (10 females, 9 males) children with acute hepatitis A and in 29 (13 females, 16 males) healthy control subjects.

RESULTS: There was a statistically significant difference between patients and controls for all parameters (P<0.05). Lipid peroxidation marker MDA was significantly elevated (P<0.001), while antioxidants β-carotene, retinol and GSH were significantly decreased (all P<0.001) in patients compared to healthy subjects. In addition, α-tocopherol and ascorbic acid levels were significantly lower in patients when compared to age and sex matched controls (P<0.05, P<0.01, respectively).

CONCLUSION: Our study shows that hepatitis A virus induces oxidative stress in children with hepatitis A. This finding could be taken into consideration to improve the therapeutic approach in acute hepatitis A.

© 2006 The WJG Press. All rights reserved.

Key words: Antioxidant; Oxidative stress; Hepatitis A; Child

Cemek M, Dede S, Bayiroğlu F, Çaksen H, Cemek F, Mert

N. Relationship between antioxidant capacity and oxidative stress in children with acute hepatitis A. World J Gastroenterol 2006; 12(38): 6212-6215

http://www.wjgnet.com/1007-9327/12/6212.asp

INTRODUCTION

Acute viral hepatitis is one of the most common infectious diseases while hepatitis A is the most prevalent form of acute viral hepatitis in many parts of the world. In developing countries located in Africa, Asia, and Latin America, seroprevalence rates approach 100% and most infections occur by 5 years of age. By contrast, seroprevalence rates in the USA, Western Europe, and in several Mediterranean countries, have been falling during the past few decades[1]. Viral hepatitis is also a major health problem in Turkey. The most important causes of spreading the disease are low levels of socioeconomical status and poor hygiene conditions, particularly in Eastern Turkey[2,3]. Hepatitis A mostly occurs in the context of community-wide epidemics during which infection is transmitted from person to person by the fecal-oral route[4]. The highest rates of disease are seen among children and young adults, and asymptomatic infection among young children is common[5].

Mitochondria and cytochrome P450 enzymes are the main sources of reactive oxygen species (ROS) in hepatocytes acutely and/or chronically exposed to a “toxic” injury (e.g., environmental drugs, alcohol, therapeutic drugs, viruses). ROS also derive from Kupffer and inflammatory cells, in particular neutrophils[5]. Oxygen-containing free radicals (such as the hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite) are highly reactive species, capable in the nucleus and in the membranes of cells of damaging biologically relevant molecules such as DNA, protein, carbohydrates, lipids[7] and are produced in physiological and pathological conditions in living organisms. Lipid peroxidation (LPO) is an autocatalytic process which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer, toxicity of xenobiotics and aging. Polyunsaturated fatty acids of the membrane are peroxidized by free radical-mediated reactions. Malondialdehyde (MDA) is one of the end-products in
the LPO process\cite{8,9}. Oxidative stress is a reflection of excess intracellular concentrations of ROS\cite{10} and one of the important indicators of cellular damage. Antioxidants transform free radicals into less reactive species, thereby limiting their toxic effects. There are several endogenous antioxidant systems to deal with the production of ROS. These systems can be divided into enzymic and nonenzymic groups. The enzymic subgroup includes superoxide dismutase, catalase and glutathione peroxidase. The nonenzymic group includes a variety of biologic molecules, such as vitamins E, A, and C and reduced glutathione (GSH)\cite{11}.

According to our knowledge, there are no data concerning the effects of oxidative stress on blood nonenzymic antioxidant status in children with hepatitis A. Hence, in the present study, we measured lipid peroxidation levels and antioxidant status such as fat-soluble (vitamin A and vitamin E) and water-soluble (vitamin C and GSH) in children with hepatitis A.

**MATERIALS AND METHODS**

The investigation included 19 children with acute hepatitis A and 29 control subjects who were admitted to Yüzüncü Yıl University, Faculty of Medicine Department of Pediatrics. The definition of the patients was based on clinical, biochemical and serological criteria. The determination of hepatitis A was made by a pediatrician after taking a detailed history and examining the children for signs of infection. The clinical and biochemical criteria are acute illness compatible with hepatitis and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels greater than 20-50 times the normal upper limit. However, the diagnosis of hepatitis A was confirmed by serologic tests (IgM and total anti-HAV antibodies were detected by enzyme-linked immunosorbent assay). The control group consisted of healthy children with normal physical examination. None of them had a history of recurrent or recent infection.

Parents of the children, who were enrolled in the study, were informed about the purpose of the study and their consent was obtained. In the present study, whole blood MDA and GSH, and serum β-carotene, retinol, α-tocopherol, ascorbic acid, ALT, AST and total bilirubin levels were measured in all of the subjects. Fasting venous blood samples for the biochemical analysis were taken from each person and transferred to heparinized and normal tubes. Whole blood was collected into heparinized tubes and whole blood MDA and GSH levels were studied on the same day of admission. MDA, which is an important indicator of lipid peroxidation, was determined by spectrophotometry of the pink-colored product of the thiobarbituric acid-malondialdehyde complex formation\cite{12}. Whole blood GSH concentration was measured by a spectrophotometric method\cite{13}. Serum was obtained by centrifugation at 2000 g for 10 min, of blood samples taken without anti-coagulant which was allowed to clot for 30 min at room temperature, and stored at -20°C until analysis date. Hemolysed samples were excluded. To avoid isomerization of vitamin A and vitamin E, blood samples were protected from light as soon as they were drawn. The levels of β-carotene at 425 nm and vitamin A (retinol) at 325 nm were detected after the reaction of serum: ethanol: hexane at the ratio of 1:1:3, respectively\cite{14}. Vitamin E (α-tocopherol) was analyzed colorimetrically with 2,4,6-tripridyl-s-triazin and ferric chloride after extraction with absolute ethanol and xylene\cite{15}. Serum vitamin C (ascorbic acid) level was determined after derivatization with 2,4-dinitrophenylhydrazine\cite{16}. Serum ALT, AST and total bilirubin levels were measured in an autoanalyzer (Roche). The same parameters were also studied in the control subjects.

The results are expressed as mean ± SD. Student’s t-test was used to compare the mean values of different biochemical parameters between hepatitis A and control groups. In all data analysis, a value of $P < 0.05$ was considered statistically significant.

**RESULTS**

Our study included 19 (53% female, 47% male) children with acute hepatitis A and 29 (45% female, 55% male) healthy control subjects. The age ranged from 2-9 years (4.42 ± 1.77 years) and 2-12 years (4.89 ± 2.22 years) in the study and control groups, respectively. The two groups were comparable in terms of age and gender ($P > 0.05$). Serum enzyme markers of hepatic injury (ALT, AST, and total bilirubin) were significantly raised in the study group. The ALT level ranged from 907 to 4051 IU/L (1869.22 ± 939.3 IU/L, normal values ≤ 40 IU/L), the AST level ranged from 352 to 4690 IU/L (1827.11 ± 1251.5 IU/L, normal values ≤ 40 IU/L) and the total bilirubin level ranged from 1.15 to 8.05 mg/dL (4.98 ± 1.4 mg/dL, 0.2-1.2 mg/dL) in the patients’ group.

Whole blood MDA and GSH levels and serum β-carotene, retinol, α-tocopherol and ascorbic acid levels in the patient group and healthy subjects are presented in Table 1. As seen in the Table, there were statistically significant differences between the groups for all parameters ($P < 0.05$). MDA (as indicator of the lipid peroxidation) was markedly increased in the children with acute hepatitis A when compared to the control group ($P < 0.001$). However, nonenzymic antioxidant status such as fat-soluble (vitamin A and vitamin E) and water-soluble (vitamin C and GSH) were found to be decreased in the

| Table 1 Serum antioxidant vitamins and whole blood MDA and GSH levels in the patient and control groups (mean ± SD) |
|-----------------|-----------------|-----------------|
|                 | Acute Hepatitis A | Control |
| **MDA (nmol/mL)** | 1.92 ± 0.14 | 1.11 ± 0.11 | < 0.001 |
| **GSH (mg/dL)** | 3.89 ± 1.59 | 34.38 ± 1.41 | < 0.001 |
| **β-carotene (μg/dL)** | 10.52 ± 1.19 | 17.44 ± 1.78 | < 0.001 |
| **Retinol (μg/dL)** | 22.67 ± 1.42 | 32.81 ± 2.13 | < 0.001 |
| **α-tocopherol (mg/dL)** | 0.56 ± 0.31 | 0.88 ± 0.17 | < 0.001 |
| **Ascorbic acid (mg/dL)** | 1.92 ± 0.14 | 1.11 ± 0.11 | < 0.001 |

$P$ values were calculated using the Student’s t-test. MDA: Malondialdehyde; GSH: Reduced glutathione; Retinol: Vitamin A; α-tocopherol: Vitamin E; Ascorbic acid: Vitamin C.
study group. Serum β-carotene and retinol, and whole blood GSH levels were significantly lower in children with hepatitis A than in the control group (all \( P < 0.001 \)). Nevertheless, serum α-tocopherol and ascorbic acid levels were also significantly decrease in patient group (\( P < 0.05 \), \( P < 0.01 \), respectively).

**DISCUSSION**

The normal liver is a well equipped organ in terms of either enzymic or nonenzymic antioxidants although most of the hepatic antioxidant defenses are essentially confined to parenchymal cells. Kupffer cells, hepatic stellate cells or endothelial cells are potentially more exposed or sensitive to oxidative stress-related molecules. Published experimental evidence clearly indicates that hepatic as well as plasma antioxidant defenses (in particular, GSH and α-tocopherol) are often significantly decreased in several liver disease\([27,28]\).

Oxidative stress is a physiologic consequence of aerobic metabolism. The intermediate components formed in aerobic organisms, such as superoxide and hydrogen peroxide, lead to the further production of ROS that can oxidize membrane lipids and disrupt metabolic processes. GSH is essential for protection against these toxic products of oxygen metabolism, especially as a substrate for GSH peroxidases\([19]\). Inflammation, oxidative stress and medications that are detoxified through GSH consuming products of oxygen metabolism, especially as a substrate for GSH peroxidases\([19]\). Inflammation, oxidative stress and medications that are detoxified through GSH consuming pathways will all result in GSH loss and excessive cysteine catabolism\([29]\).

GSH depletion in lymphoid cells may interfere with the immunological mechanisms involved in viral clearance, thus facilitating viral replication and enhancing liver damage because of the oxidative stress. Lymphocyte activation \textit{in vitro} can be inhibited completely by decreasing GSH by 10% to 40%\([21]\) and Barbaro et al.\([20]\) reported the depletion of GSH in chronic hepatitis C (CHC) patients was associated with a mean 12.7% reduction of peripheral blood mononuclear cells cytoxic activity compared to controls\([22]\).

ROS are well characterized mediators of cell and tissue injury. Generally, cells defend themselves from ROS and other toxic oxygen species by a variety of mechanisms including nonenzymic and enzymic defence systems. An ineffective scavenging capacity of antioxidant systems may play a relevant role in determining the degree of oxidative stress\([23,25]\).

The increase in serum MDA concentration in CHC patients may very well fit in with the recently reported glutathione depletion observed in hepatic tissue, plasma, and peripheral blood mononuclear cells of CHC patients\([26]\). Hepatitis C virus (HCV) infection is associated with reduced GSH levels in both plasma and erythrocytes known to be the main intracellular mechanism against oxidative stress, and this depletion appears to be related to the activity of the liver disease\([26,27]\). Likewise, the present study also reports an increased MDA and decreased GSH levels in the acute hepatitis A patients.

The decreased content of cellular and plasma GSH, most likely mediated by HCV, or in the present paper's case by hepatitis A virus, may render biological structures more susceptible to oxidative attack and this condition may expose circulating lipids and proteins to oxidative modifications with consequent loss of their biological functions. Several authors’ findings are consistent with each other, in which it is reported that a reduction of hepatic GSH stores might be partly responsible for the cytopathic effect induced by HCV\([28,29]\); it is also conceivable that the observed GSH depletion in lymphoid cells\([26]\) might interfere with the immunological mechanism involved in viral clearance, thus facilitating viral replication and enhancing, with time, liver damage through a greater oxidative stress\([30]\).

Free oxygen radicals take part in pathogenesis of chronic hepatitis of B and C type in children as they decrease the antioxidant barrier efficiency diminishing catalase and superoxide dismutase levels\([31]\). In the same manner, it is likely that free oxygen radicals can take role in the pathogenesis of the acute hepatitis A since antioxidant vitamin levels were decreased in our study. As mentioned above, the reason might be the altered antioxidant capacity because of the decreased antioxidant vitamins in blood.

As expected from the other hepatitis cases, in acute hepatitis A, nonenzymic antioxidant levels such as GSH and β-carotene, retinol, vitamin E, vitamin C were found to have decreased in this study.

There are several possible explanations for the low serum retinol levels seen in chronic liver disease. Defective synthesis of retinol binding protein prevents the customary mobilization of retinol from the liver to the periphery. This would suggest that retinol exerts its antiproliferative effect in a paracrine or systemic manner rather than by an immediate local effect. Another possibility is that the absorption of dietary retinol is impaired, which may also explain why levels are even lower in patients with cholestatic liver disease. Whilst other groups have suggested that serum retinol levels are a reflection of nutritional status\([32]\), the other authors did not report any association between decreasing body mass index and lower retinol levels within the cohort of patients studied. In conformity with our results, von Herbay et al.\([33]\) showed that vitamin E plasma levels were significantly lower (\( P < 0.01 \)) compared to the control group. They suggest that the decreased vitamin E levels in patients with acute or chronic viral hepatitis with high activity of disease may be due to free radical-mediated liver injury.

Vitamin C, the major water-soluble antioxidant, is beneficial in reducing oxidative stress, but is harmful depending on the sensitive balance of its concentration. Ascorbic acid has been shown to efficiently scavenge superoxide, hydrogen peroxide, hypochloride, hydroxyl radicals, and peroxyl radicals\([34]\) and to restore the antioxidant properties of fat-soluble α-tocopherol, therefore; it interrupts the radical chain reaction of lipid peroxidation\([35]\). In this study, vitamin C levels were found to be significantly lower in the hepatitis A group than in the controls (Table 1). To our knowledge, there is no data concerning the levels of serum vitamin C in children with hepatitis A. The reduction in serum concentration of vitamin C might be due to increased rate of ascorbic acid oxidation in oxidative stress.

In conclusion, this study shows that hepatitis A virus
induces oxidative stress in children with hepatitis A. This finding could be taken into consideration to improve the therapeutic approach in acute hepatitis A.

REFERENCES

1 Koff RS. Hepatitis A. Lancet 1998; 351: 1643-1649
2 Erdoğan MS, Otkun M, Tatman-Otkun M, Akata E, Türe M. The epidemiology of hepatitis A virus infection in children, in Edirne, Turkey. Eur J Epidemiol 2004; 19: 267-273
3 Tosun S, Ertan P, Kasirga E, Atman U. Changes in seroprevalence of hepatitis A in children and adolescents in Manisa, Turkey. Pediatr Int 2004; 46: 669-672
4 Bell BP, Shapiro CN, Alter MJ, Moyer LA, Judson FN, Mottram K, Fleener M, Ryder PL, Margolis HS. The diverse patterns of hepatitis A epidemiology in the United States-implications for vaccination strategies. J Infect Dis 1998; 178: 1579-1584
5 Venczel LV, Desai MM, Vertz PD, England B, Hutin YJ, Shapiro CN, Bell BP. The role of child care in a community-wide outbreak of hepatitis A. Pediatrics 2001; 108: E78
6 Loguercio C, Federico A. Oxidative stress in viral and alcoholic hepatitis. Free Radic Biol Med 2003; 34: 1-10
7 Hercberg S, Galan P, Preziosi P, Alfaroz MJ, Vasquez C. The potential role of antioxidant vitamins in preventing cardiovascular diseases and cancers. Nutrition 1998; 14: 513-520
8 Kurata M, Suzuki M, Agar NS. Antioxidant systems and erythrocyte life-span in mammals. Comp Biochem Physiol B 1993; 106: 477-487
9 Hagihara M, Nishigaki I, Maseki M, Yagi K. Age-dependent changes in lipid peroxide levels in the lipoprotein fractions of human serum. J Geronol 1984; 39: 269-272
10 Wattanapitayakul SK, Bauer JA. Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications. Pharmacol Ther 2001; 89: 187-206
11 Bast A, Haenen GR, Doelman CJ. Oxidants and antioxidants: state of the art. Am J Med 1991; 91: 25-135
12 Jain SK, McVie R, Duett J, Herbst JJ. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 1989; 38: 1539-1543
13 Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 1980; 106: 207-212
14 Suzuki J, Katoh N. A simple and cheap methods for measuring serum vitamin A in cattle using only a spectrophotometer. Nihon Juigaku Zasshi 1990; 52: 1281-1283
15 Martinek rg. Method for the determination of vitamin e (total tocopherols) in serum. Clin Chem 1964; 10: 1078-1086
16 Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymod 1979; 62: 3-11
17 Inoue M. Protective mechanisms against reactive oxygen species. In: Arias IM, Fausto N, Jakoby WB, Schachter D, Shafritz DA, editors. The liver: biology and pathobiology. New York: Raven, 1994: 443-459
18 Parola M, Robino G. Oxidative stress-related molecules and liver fibrosis. J Hepatol 2001; 35: 297-306
19 DeLeve LD, Kaplowitz N. Importance and regulation of hepatic glutathione. Semin Liver Dis 1990; 10: 251-266
20 Orskov ER, Fraser C. The effects of processing of barley-based supplements on rumen pH, rate of digestion of voluntary intake of dried grass in sheep. Br J Nutr 1975; 34: 493-500
21 Staal FJ, Ela SW, Roederer M, Anderson MT, Herzenberg LA, Herzenberg L. Glutathione deficiency and human immunodeficiency virus infection. Lancet 1992; 339: 909-912
22 Barbaro G, Di Lorenzo G, Ribersani M, Soldini M, Giancaspro G, Bellomo G, Belloni G, Grisorio B, Barbarini G. Serum ferritin and hepatic glutathione concentrations in chronic hepatitis C patients related to the hepatitis C virus genotype. J Hepatol 1999; 30: 774-782
23 Cochrane CG. Cellular injury by oxidants. Am J Med 1991; 91: 235-305
24 Kakkar R, Kalra J, Mantha SV, Prasad K. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. Mol Cell Biochem 1995; 151: 113-119
25 Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. Lab Invest 1982; 47: 412-426
26 Barbaro G, Di Lorenzo G, Soldini M, Parrotto S, Bellomo G, Belloni G, Grisorio B, Barbarini G. Hepatic glutathione deficiency in chronic hepatitis C: quantitative evaluation in patients who are HIV positive and HIV negative with correlations with plasmatic and lymphocytic concentrations and with the activity of the liver disease. Am J Gastroenterol 1996; 91: 2569-2573
27 Belouqui O, Prieto J, Suárez M, Gil B, Qian CH, García N, Civeira MP. N-acetyl cysteine enhances the response to interferon-alpha in chronic hepatitis C: a pilot study. J Interferon Res 1993; 13: 279-282
28 Vendemiale G, Grattagliano I, Portincasa P, Serviddio G, Palasciano G, Altomare E. Oxidative stress in symptom-free HCV carriers: relation with ALT flare-up. Eur J Clin Invest 2001; 31: 54-63
29 Suarez M, Belouqui O, Ferrer J, Gil Qian C, Garcia N, Prieto M. Glutathione depletion in chronic hepatitis C. Int Hepatol Commun 1993; 1: 215-221
30 Suthanthiran M, Anderson ME, Sharma VK, Meister A. Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD9 antigens. Proc Natl Acad Sci USA 1990; 87: 3343-3347
31 Chrobok AM, Szafarska-Saczepanik A, Drewa G. Antioxidant defense in children with chronic viral hepatitis B and C. Med Sci Monit 2000; 6: 713-718
32 Black DA. Heduan E, Mitchell D. Hepatic stores of retinol and retinyl esters in elderly people. Age Ageing 1998; 17: 337-342
33 von Herbay A, Stahl W, Niederau C, von Laar J, Strommeyer G, Sies H. Diminished plasma levels of vitamin E in patients with severe viral hepatitis. Free Radic Res 1996; 25: 461-466
34 Bendich A, Machlin LJ, Scandura O, Burton GW, Wayner DDM. The antioxidant role of vitamin C. Adv Free Radic Biol Med 1986; 2: 419-444
35 Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch Biochem Biophys 1993; 300: 535-543