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# Antioxidant defense and oxidative stress in children with acute hepatitis A

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**BACKGROUND AND OBJECTIVES:** Published data on oxidative stress in children with acute hepatitis A are still very scarce. This study aims to evaluate the oxidant/antioxidant status of these patients.

**DESIGN AND SETTING:** Prospective, case-control study, over 2.5 years in patients under hospitalized and ambulatory care.

**PATIENTS AND METHODS:** The levels of a whole-blood antioxidant, reduced glutathione; and plasma antioxidants, β-carotene, retinol, ascorbic acid, α-tocopherol; and the biomarker of oxidative stress, malondialdehyde, were evaluated in 50 pediatric patients (age range, 5-16 years; 29 males and 21 females) with acute hepatitis A and in 50 healthy children as control subjects (age range, 5-16 years; 25 males and 25 females).

**RESULTS:** Plasma levels of reduced glutathione, β-carotene, retinol, α-tocopherol and ascorbic acid were significantly lower, while malondialdehyde plasma levels were significantly increased in the patient group when compared to the controls ($P<.0001$ for all parameters).

**CONCLUSIONS:** Our findings show that pediatric patients with acute hepatitis A were influenced by oxidative stress, resulting in significantly lower levels of plasma antioxidants and increased lipid peroxidation. In the absence of other therapeutic options, antioxidant vitamin supplements could be added to the therapy for these patients to help reestablish the oxidant status balance. Further investigations to confirm this suggestion are recommended.

Hepatitis A is an acute, necroinflammatory disease of the liver which results from the infection by the hepatitis A virus (HAV). The mean incubation period is approximately 30 days. Although the disease is usually self-limited, the severity of illness is age-dependent. In children, hepatitis A is usually asymptomatic while in adults symptomatic infection is characteristic and jaundice is common.[1] High prevalence is associated with poor socioeconomic conditions and diverse epidemiological patterns.[2,3] Serbia is considered to be an intermediate anti–HAV-endemic country.[4,5] Relapses of hepatitis A can occur in as many as 20% of patients. Approximately 70% of pediatric patients younger than 6 years of age infected with hepatitis A are asymptomatic and serve as a pool for infection among adults.[6-8]

Oxidative stress plays an important role in the pathogenesis and progression of various liver diseases.[9] Radicals, such as reactive oxygen species (ROS), are highly reactive molecules, naturally occurring as the products of normal metabolic processes. When antioxidant levels are not high enough or the amount of ROS increases, as in some diseases, they may react with DNA, lipids and proteins to cause damage. Oxidative stress is something which can happen if reactive radicals outbalance good antioxidants in the human organism. Reactive oxygen species–mediated liver injury may trigger the following three main mechanisms: lipid peroxidation, cytokine induction and Fas ligand induction. Cytotoxic products of lipid peroxidation, such as malondialdehyde (MDA) and 4-hydroxynonenal, may impair cellular functions, including nucleotide and protein synthesis, which may play a role in hepatic fibrogenesis, and hepatocyte death.[10,11] The main sources of ROS in hepatocytes in acute or chronic disease are mitochondria and cytochrome P450 enzymes, Küpffer

cells and neutrophils.[12] Oxidative stress, as the consequence of increased intracellular ROS concentrations, can be reduced by antioxidants. Antioxidants transform free radicals into less reactive and less harmful species. They include the enzymatic subgroup of antioxidants, such as superoxide dismutase, catalase, glutathione peroxidase; and the nonenzymatic subgroup, comprised of vitamin A, vitamin C, vitamin E and reduced glutathione (GSH).[13]

Published data on oxidative stress in children with acute hepatitis A are still very scarce. There are much more data about oxidative stress related to chronic liver diseases in adults. On the other hand, adult patients with hepatitis A are treated with hepatoprotective herbal drugs, which are usually contraindicated in pediatric patients. Therefore, maintaining antioxidant defense system in children with acute hepatitis A by the supplementation with antioxidant vitamins appears worthwhile. Therefore, we evaluated the antioxidant defense and oxidative stress, measuring serum antioxidants, vitamin A (retinol), provitamin A (β-carotene), vitamin E (α-tocopherol), vitamin C (ascorbic acid); GSH; and a serum lipid peroxidation product, MDA, in children with hepatitis A, in accordance with the aim of our study.

## PATIENTS AND METHODS

The two groups studied consisted of pediatric patients with acute hepatitis A who were admitted to the clinic. The diagnosis of hepatitis A was based on clinical, biochemical [acute hepatitis syndrome, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels 20-50 times greater than the upper normal limit] and serological criteria [IgM and total anti-HAV antibodies were detected by enzyme-linked immunosorbent assay (ELISA)]. A control group consisted of healthy children, anti-HAV IgM and IgG negative who were not taking vitamin supplementation or medication at the time, and had no history of any recurrent or recent infection. The parents of both patients and controls signed the consent form, after being informed about the methods and the purpose of the research.

In this study, whole-blood GSH, plasma levels of MDA, β-carotene, retinol, α-tocopherol, ascorbic acid; serum ALT, AST and total bilirubin levels were determined in all the subjects. Fasting venous blood samples for biochemical analysis were taken from a peripheral vein (v. cubitalis) of each child, of both patient and control groups, and transferred to heparinized and normal tubes. Blood samples were immediately centrifuged at 2500g. Plasma and whole-blood fractions were stored at −80°C until the analyses. Plasma levels of ascorbic acid, α-tocopherol, retinol, β-carotene, and GSH in whole blood were measured by commercial HPLC (high performance liquid chromatography) kits provided by Chromsystems (Munich, Germany). Preparation of the samples was done according to the manufacturer's instructions. Antioxidants were separated on a C18 column by UV (ascorbic acid, 245 nm; α-tocopherol, 295 nm; retinol, 325 nm; β-carotene, 453 nm) and fluorescence detection (GSH, 385 and 510 nm), using a Waters HPLC system (Waters, Eschborn, Germany) with a pump (model 515; Waters), an autosampler (model 717plus; Waters), an UV detector (model 2487; Waters) and a fluorescence detector (model 1100; Hewlett Packard, Boblingen, Germany). Plasma antioxidant capacity was measured by a commercial ELISA kit provided by Immundiagnostik (Bensheim, Germany). The quantity of antioxidants was measured performing the reaction of exogenous peroxide with antioxidants present in the sample. The quantity of unreactive peroxides was determined by a peroxidase-catalysed reaction. The reaction was stopped by adding the acid, which made a colorimetric end point that was read spectrophotometrically at 450 nm by the microplate reader Sunrise (Tecan, Crailsheim, Germany). Quantification was performed by external calibration. Preparation of samples was done according to the manufacturer's instructions. Malondialdehyde in plasma was measured by a commercial HPLC kit (Chromsystems, Munich, Germany). Samples were prepared in accordance with the manufacturer's recommendations. The HPLC system consisted of a pump (model 2150; Pharmacia Biosystems, Freiburg, Germany), an autosampler (model AS-100; BioRad, Munich, Germany) and a fluorescence detector (model 1100; Hewlett Packard, Boblingen, Germany). Serum ALT, AST and total bilirubin were measured in an autoanalyzer (Roche). The same parameters were studied in both groups (in patients and in control subjects).

Statistical analysis was performed using SPSS 10 for Windows. The results were represented as mean and standard deviation. The *t* test was used to compare the mean values of the determined biochemical parameters between the patients with acute hepatitis A and controls. In all data analyses, a value of $P<.05$ was considered to be statistically significant.

## RESULTS

We enrolled 50 children with acute hepatitis A (29 [58%] males and 21 [42%] females) with a mean (SD) age of 10.7 (3.75) years and age range of 5-16 years and 50 healthy children as controls (25 females, 25 males

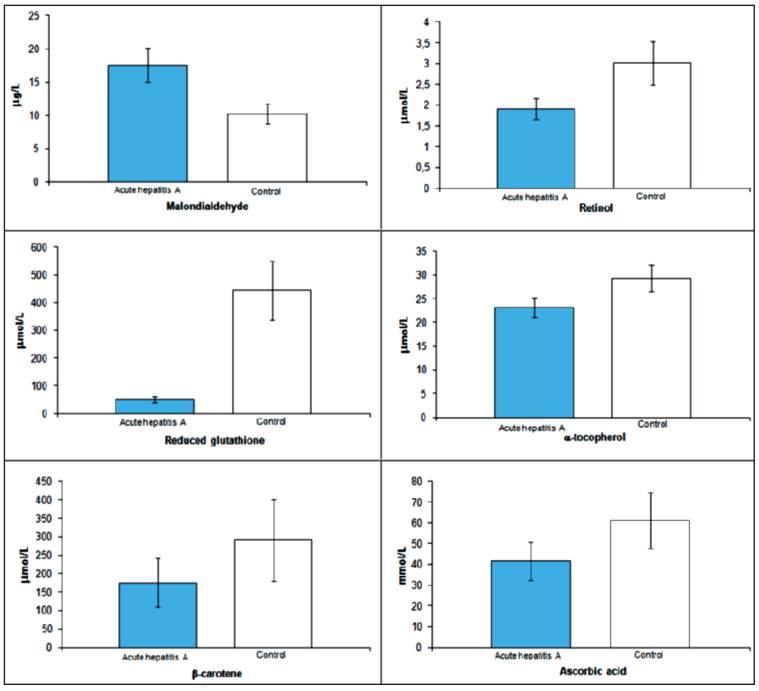


**Figure 1.** Plasma antioxidants and levels of the lipid peroxidation product in study subjects. All differences were statistically significant (*P*<.0001).

**Table 1.** Frequency of physical findings and clinical manifestations in children with acute hepatitis A.

| Clinical parameters | Frequency |
|---|---|
| Jaundice | 38 (75) |
| Low-grade fever | 48 (96) |
| Mild right upper quadrant pain | 42 (84) |
| Hepatomegaly | 45 (90) |
| Palpable spleen | 7 (14) |
| Posterior cervical adenopathy | 5 (10) |
| Palpable ascites | 0 (0) |
| Light-colored stools | 30 (60) |
| Dark-colored urine | 39 (78) |
| Diarrhea | 20 (40) |
| Anorexia | 47 (94) |
| Nausea | 48 (96) |
| Vomiting | 45 (90) |
| Myalgia | 25 (50) |
| Malaise | 35 (70) |

Data are expressed as n (%).

**Table 2.** Serum markers of hepatic injury in 50 children with acute hepatitis A and 50 healthy controls.

| Variable | Acute hepatitis A patients | Healthy controls |
|---|---|---|
| Alanine aminotransferase (IU/L) | 2001 (907) | 19.3 (5.8) |
| Aspartate aminotransferase (IU/L) | 1506 (979) | 21.3 (6.2) |
| Total bilirubin (mg/dL) | 5.01 (1.6) | 0.8 (0.3) |

Data are expressed as mean (standard deviation). All differences were statistically significant (*P*< .0001).

with a mean (SD) age of 9.7 (3.4) years and age range of 5-16 years, thus being comparable in age and gender (*P*>.05). Clinical manifestations and physical findings of the patients are displayed in **Table 1**. Serum markers of hepatic injury (ALT, AST, total bilirubin) of both groups are presented in **Table 2**. Plasma levels of MDA and GSH, β-carotene, retinol, α-tocopherol and ascorbic acid in both groups are presented in **Figure 1**. The difference between the groups for all parameters was statistically highly significant (*P*<.0001, for all parameters).

## DISCUSSION

While investigating hepatitis and oxidative stress, we noticed that the majority of scientific reports refer to the alteration of oxidant status in adults, mainly affected by chronic liver pathology. However, from the results of reports that also discussed acute forms, it could be construed that both acute and chronic forms have similar pathways in the pathogenesis of oxidative stress in the liver.We thus decided that it was worthwhile to attempt to investigate the parameters of these pathways in the pediatric population with hepatitis A.

Oxidative stress can be defined as an increase in oxidants and/or a decrease in antioxidant capacity.[14] Reactive oxygen intermediates have been implicated in the induction of hepatocyte apoptosis that results from a variety of forms of liver injury. Exogenous oxidants induce hepatocyte apoptosis and may mediate death during inflammatory liver injury. Lethal levels of intracellularly generated ROS resulting from hepatotoxin metabolism, or the induction of enzymes in the cytochrome P450 family, are also important inducers of apoptosis. In addition, ROS production may mediate death from a number of diverse factors, including tumor necrosis factor-α, bile acids, ischemia and transforming growth factor-β1 (TGF-beta).[15] The oxidant-induced liver in-

jury is mediated by the direct effects of reactive oxygen species on signal transduction pathways.[16]

Oxidative stress caused by HAV shares identical genes and enzymes, which participate in apoptosis in various liver diseases and are responsible for the same process in acute hepatitis A.[17,18] The study by Cemek et al reports an increased MDA level and decreased GSH, β-carotene, retinol, α-tocopherol and ascorbic acid levels in acute hepatitis A pediatric patients. The depletion of GSH levels in both plasma and erythrocytes is known to be the main intracellular mechanism against oxidative stress and appears to be related to the activity of the liver disease.[19]

Besides the scientific reports that relate to chronic liver diseases, there are articles dealing with acute hepatic illnesses in which some of the parameters determined in our report are mentioned. Malondialdehyde, ALT, AST and total and direct bilirubin levels of the patients with acute viral hepatitis B and chronic viral hepatitis B before treatment were significantly higher ($P<.001$) whereas GSH and β-carotene levels were lower ($P<.001$) than those of the controls.[20,21] There is a relationship between systemic parameters of oxidative stress, insulin resistance, the degree of steatosis and fibrosis in chronic viral hepatitis C.[22] Hepatic steatosis was associated with an increase of MDA ($P<.05$), correlated with disease severity, and consequential decrease of GSH ($P<.05$).[23,24]

The effects of various antioxidants in plasma are additive, and the cooperation of antioxidants in human serum provides protection to an organism against attacks by free radicals.[25-27] Determination of oxidants along with antioxidants is more useful in rendering information about the oxidant status of the organism,[28] and that idea was followed in our research.

Alterations of plasma oxidative status parameters in children are much more frequently mentioned in the studies of various other infective diseases as comapred to those of hepatitis A. Levels of the antioxidant vitamins A, C, E were significantly lower in children with malaria in comparison to healthy controls ($P<.001$ for each vitamin).[29] There were also reduced serum concentrations (with a statistical difference of $P<.001$) of retinol and α-tocopherol in children with schistosomiasis, which was explained as an increased oxidative stress often observed as a part of acute-phase response to infection.[30] Plasma vitamin C levels were significantly ($P<.05$) reduced in septic children, which may be due to active phagocytosis and due to its role as a free radical scavenger.[31] Our findings agree with the previously mentioned facts that parameters of oxidative stress in acute/chronic liver diseases or various acute infectious diseases in children are significantly increased in contrast to decreased antioxidant levels.

To conclude, our research points out that HAV causes oxidative stress in children with hepatitis A. Antioxidant vitamin supplements could be added to the therapy for these patients to help reestablish the oxidant status balance. However, further investigations to confirm this suggestion are recommended.

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