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Original Article

Biochemistry Section

Anti-oxidant Status in Patients with Chronic Hepatitis C in Rajasthan, India

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ABSTRACT

Background/Aims: Hepatitis C is a global disease and being endemic in India, it is one of the most important causes of chronic liver disease and furthermore, it is related to carcinogenesis. The pathogenesis of the Hepatitis C disease includes both direct virus induced liver damage, immunological liver damage and oxidative stress. Vitamin E and A have important roles in the Anti-oxidant defense system and they reduce oxidative stress. Our aims were to estimate the levels of the Anti-oxidants, vitamin A, vitamin E and vitamin C in the serum of Chronic Hepatitis C (CHC) patients and to compare them with the levels in normal healthy controls.

Material and Methods: A retrospective study was performed at the Department of Gastroenterology and Biochemistry at SMS Medical College, Jaipur, India. In 20 patients of CHC, the serum levels of vitamin A, E and C were estimated by spectrophotometry. Twenty healthy controls were also included in the study and the serum levels of these vitamins were measured in them also.

Statistical analysis: It was performed by using the Student's t-test and the correlation between the variables was studied by using the Pearson's correlation coefficient test.

Results: The serum vitamin A levels were significantly lower in the patients than in the controls ($p<0.001$) and the serum vitamin E and vitamin C levels were also significantly decreased ($p<0.001$).

Conclusions: The increased oxidative stress in the Chronic Hepatitis C patients is evidenced by decreased serum Vitamin A, E and C levels and so, further studies on the liver levels of these Anti-oxidants and the management of the dietary Anti-oxidants may help in the management of CHC.

Key Words: Chronic Hepatitis C, Anti-oxidant, Vitamin A, Vitamin E, Vitamin C

INTRODUCTION

The global prevalence of the Hepatitis C virus (HCV) infection is around 2%, with 170 million persons being chronically infected with the virus and 3 to 4 million persons being newly infected each year [1]. In India, hepatitis c is an endemic disease leading to acute hepatitis, which may result in chronic liver disease in 50-70% of the cases [2]. Hepatitis C is an emerging infection in India and the Hepatitis C virus is an important pathogen which causes liver disease in India. The high risk of chronicity of this blood-borne infection and its association with hepatocellular carcinoma underscores its public health importance [3]. Several studies have looked at the prevalence of hepatitis C in chronic liver disease in India. The prevalence of hepatitis C has ranged from 10.8% to as high as 48.5% [4-6]. India's blood-banking system has serious shortcomings. Professional blood donation continues to flourish despite the presence of a law which condones this. Another malaise in our health system is the reuse of improperly sterilized needles. Both these factors are potential sources for the spread of hepatitis C in India [3]. The exact reason behind the liver injury and fibrosis in chronic hepatitis C (CHC) is not fully known, but some studies have suggested that immunological liver damage and oxidative stress may be involved in its pathogenesis [7-9].

If a homeostasis is not maintained between the rate of formation of the free radicals and the rate of their neutralization, oxidative damage accumulates, which is known as oxidative stress [10]. Oxidative stress, which is imposed either directly by the virus or by the host-immune response, is a potentially important pathogenic mechanism in the hepatitis C virus disease, as well as in other chronic liver diseases, which can initiate and promote multistage carcinogenesis also [11, 12]. Some vitamins play an important role in the anti-oxidant defense system and they reduce oxidative stress [13, 14]. To the best of our knowledge, only very few studies have been performed with respect to the estimation of the serum anti-oxidant levels in patients with CHC and their role in the prevention and treatment of chronic hepatitis C. In the light of these explanations, the present study was undertaken to find out the levels of the anti-oxidants, vitamin A, vitamin E and vitamin C in the serum of the CHC patients in Rajasthan (India).

MATERIAL AND METHOD

This study was conducted in the Department of Biochemistry and Gastroenterology, SMS Medical College, Jaipur, Rajasthan. Twenty patients who were seropositive for hepatitis C were included in the study. A detailed history was obtained from all the patients regarding the demographics, the history of drug abuse, previous blood transfusion, haemodialysis and alcohol or tobacco abuse. A thorough physical examination was carried out on all the patients. Routine haematological and radiological investigations were also done. Twenty age and sex matched controls were selected from the general population of Jaipur, who were non-smokers, non-alcoholics, free from any abnormality on routine clinical examination, without any Anti-oxidant supplementation prior to the study and who had no history of taking drugs that could affect the Anti-oxidant status. An informed written consent was taken from both the patients and the controls. This study was also approved by the Institutional Ethical Committee, SMS Medical College, Jaipur.

5 ml of fasting (in the morning) venous blood samples were collected for the study, in plain vials (without anti-coagulant). The serum was seperated by centrifuging the blood at 3000 rpm for 10 min. It was stored at -20ºC to estimate the levels of vitamin A, vitamin E and vitamin C.

Serum vitamin A was estimated by using Trifluoroacetic acid [15]. Serum Vitamin E was estimated by using bathophenonthroline [16]. Serum vitamin C was estimated by using 2, 4 – dinitrophenylhydrazine and a spectrophotometer [17].

The results were presented as mean±S.D. Statistical analysis was performed by using the Student's t-test and the correlation between the variables was studied by using the Pearson's correlation coefficient test.

RESULT

The data of the patient's demographics and laboratory investigations are presented in [Table/Fig-1]. The serum vitamin A level in the CHC patients was 34.7 ± 7.5 µg/dl, which was significantly lower than that of the controls (54.6 ± 10.5 µg/dl, p<0.001). The serum vitamin E (α-tocopherol) levels were also significantly decreased in the CHC patients as compared to the controls (0.66 ± 0.14 v/s 1.04 ± 0.17 mg/dl, p<0.001). The serum vitamin C levels were also significantly decreased in the CHC patients as compared to the controls (0.74 ± 0.19 v/s 1.36 ± 0.32 mg/dl, p<.001) [Table/Fig-2].

DISCUSSION

The HCV infection is characterized by increased markers of oxidative stress [18]. The lipid peroxidation products are found to be increased in the serum, peripheral blood mononuclear cells, and the liver specimens of the hepatitis C patients. 4-Hydroxynonenal and 8-hydroxyguanosine, markers of oxidative DNA damage, are elevated in the HCV infection. Oxygen derived free radicals play a role in liver injury because of hepatitis C and other liver disorders. The increase in free radical formation is manifested by the increased hepatic and serum levels of the lipid peroxidation products [8, 19]; these have also been reported in subjects with the HCV infection.

Demographic profile:			
Parameter	**Details**		
Number of patients	20		
Males/Females	14/6		
Age (Years)	35–60		
Laboratory data:			
Parameter	**Mean ± SD**	**Range**	**Normal Range**
WBC (1000/cumm)	6.93 ± 1.95	3.54-10.92	4.3-10.0
ALT (U/L)	112.7 ± 18.8	82-136	5-36
AST (U/L)	107.9 ± 16.8	85-135	0-40
Total Bilirubin (mg/dl)	.56 ± .22	.2-.9	< 1
ALP (IU/L)	397.2 ± 73.9	296-520	105-390
Albumin (g/dl)	4.21 ± .41	3.6-4.9	3.5-5.0

[Table/Fig-1]: Demographic profile and Laboratory data of CHC patients

	Controls (n=20)	CHC Patients (n=20)
Serum Vitamin A(µg/dl)	54.6 ± 10.5	34.7 ± 7.5*
Serum Vitamin E(mg/dl)	1.04 ± 0.17	0.66 ± 0.14*
Serum Vitamin C(mg/dl)	1.36 ± 0.32	0.74 ± 0.19*

[Table/Fig-2]: Serum levels of Antioxidant in Controls and CHC patients Values are expressed as mean ± SD; *p<0.001.

The increased oxidative stress in hepatitis C may be explained on the basis of chronic inflammation, and the continued generation of reactive oxygen species. The reactive nitrogen species may be explained by NAD(P)H oxidase (Nox 2 protein) of the Kupffer cells and the polymorphonuclear cells in the liver [20]. In previous studies which were conducted on CHC patients, the oxidative status of the subjects was determined by using the measurements of Malondialdehyde (MDA) [9] and the 8-isoprostane levels [21].

The reduced levels of the lipid soluble vitamins in plasma and the liver tissue of cirrhotic patients, mainly alcohol related, have been reported previously [22, 23]. Only few studies have looked at the levels of Anti-oxidants in the serum of patients with chronic hepatitis [8, 23].

The present study showed significant reduction in the serum vitamin A levels in the CHC patients as compared to the controls, which was similar to the reports of previous studies [9]. The reported normal range of vitamin A is 40-80 µg/dl [15]. The observed values for this vitamin for the control group were within this range. Having been identified in 1913, vitamin A was the first fat-soluble vitamin which has been discovered. Being also known as retinol, vitamin A has been called the "anti-infective" vitamin due to its role in supporting the immune system, but it rarely receives much attention. The amount of circulating retinol depends on the specific hepatocyte function, such as the de-esterification of stored retinol and the retinol binding protein synthesis, which are possibly affected in chronic hepatitis. That is why with preserved or increased liver levels, the serum retinol level is decreased [24].

Vitamin E (alpha Tocopherol) is a lipid soluble vitamin and it helps in cellular growth and in the maintenance of membrane permeability. It is an efficient Anti-oxidant and a modulator of the immune system [14]. The finding of decreased serum levels of vitamin E in the CHC patients in the present study, was also supported by the findings of previous studies [9, 21]. The reported normal range of Vitamin E is 0.8-1.2 mg/dl [17]. The observed values for vitamin E for the control group were within this range. Vitamin E supplementation may increase the Anti-oxidant protective effect against both plasma lipid peroxidation and DNA damage [14].

In the study by Jain et al (2002) [21], vitamin C (ascorbic acid) which is the most effective water soluble vitamin, was also found to decrease significantly (P<0.001) in the CHC patients as compared to the controls. Ascorbic acid exists in blood in the oxidized (DHAA) and reduced forms (RAA) and its transportation across the cell membranes is in the form of DHAA, which is less ionized at physiological pH and it has more membrane permeability [25]. Ascorbate is an excellent reducing agent (Terminal Small-Molecule Anti-oxidant). It readily undergoes two consecutive, yet reversible, one-electron oxidation processes to form the ascorbate radical (Asc•–) as an intermediate. Because Asc•– has its unpaired electron in a highly delocalized ϖ-system, it is a relatively unreactive free radical. These properties make ascorbate a superior biological, donor Anti-oxidant [26, 27].

The increased inflammation of neutrophils by the formation of lipid peroxides leads to oxygen mediated injury in the liver [28]. Damaged hepatocytes, inflammatory cells and cytokines, by generating superoxide radicals, peroxy radicals and singlet oxygen, contribute to oxidative stress [29-31]. Vitamins E and C, with other Anti-oxidants, is believed to act against superoxide radicals, peroxy radicals and singlet oxygen [16]. So, their decreased levels may indicate their use in fighting again oxidative stress.

Whereas the antioxidants/reductants might be useful at improving HCV-associated diseases, whether these compounds suppress, enhance, or have no effect on HCV remains to be studied further. With regards to the Anti-oxidant therapy, it should also be noted that ascorbic acid (vitamin C) can in fact promote hydroxyl radical production in the presence of free irons [32]. Thus, some Anti-oxidants can act as pro oxidants rather than Anti-oxidants in the hepatitis C patients with excess iron deposition in the liver.

In conclusion, increased oxidative stress in Chronic Hepatitis C is evidenced by decreased serum vitamin A, E and C levels. A dietary supplement of Anti-oxidants may help in the management of CHC.

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