SERUM CERULOPLASMIN AND ALPHA-1 ANTITRYPSIN LEVELS AS MARKERS OF RECOVERY IN ACUTE VIRAL HEPATITIS

BINITA GOSWAMI2, MOUSHUMI LODH1, SANDHYA LAL3 AND MD.ASHIF ALI AHMED4

1Department of biochemistry, IQ City Medical College and Narayana Multispeciality Hospital, Durgapur
2Department of biochemistry, Lady Hardinge Medical College & associated Hospitals, New Delhi
3Department of Gastroenterology, The Mission Hospital, Durgapur.

ABSTRACT

Background: Acute viral hepatitis is a common problem in developing countries due to poor hygiene and inadequate sanitation. Biochemical alterations in acute hepatitis have been well documented in literature. There has been a growing body of evidence in support of the role of inflammation and Reactive oxygen species (ROS) in the pathogenesis of hepatic lesions. Ceruloplasmin is an important chain breaking antioxidant while alpha 1antitrypsin is an acute phase reactant. The present study was undertaken to ascertain the association, if any, between the biochemical parameters of hepatitis and markers of inflammation and antioxidant such as alpha 1antitrypsin and ceruloplasmin respectively.

Methods: Patients presented with acute liver disease over a period of one month during December-January were evaluated prospectively. Fifty seven patients were enrolled after written informed consent.

Result: Hepatitis A cause detected in 18 (31.5%), hepatitis B in 8 (18.0%) and hepatitis E in 9 (7.01%) patients. 30 were virally seropositive cases. 27 cases were non seropositive and there were no HIV or HCV positive cases. Hepatitis A was the most common and afflicted, particularly young adult teens and children, whereas hepatitis B and E were more pronounced among older age group. α1AT & ceruloplasmin levels were found to be higher in patients compared to controls & values were statistically significant (p<0.05).

Conclusion: Significantly increased levels of ceruloplasmin and alpha -1 antitrypsin point towards the prognostic role of these markers in viral hepatitis.

KEYWORDS: Acute hepatitis, hepatitis(A,B,C,D,E)ceruloplasmin, alpha -1 antitrypsin

BINITA GOSWAMI
Department of biochemistry, Lady Hardinge Medical College & associated Hospitals, New Delhi.
INTRODUCTION

Acute hepatitis remains a public health problem in India despite improving sanitations, health awareness, socioeconomic conditions. Hepatitis E is the commonest cause of acute hepatitis in adults and hepatitis A in the commonest cause in paediatrics age group. India is hyperendemic for hepatitis A & E. 1-2 It has been documented that viral hepatitis leads to increased reactive oxygen species (ROS) and consequent oxidative damage.3 Oxidative stress refers to an imbalance between the production of pro-oxidant species through various endogenous and exogenous processes and the extensive anti-oxidant defense mechanisms in the body. There are two sources of free radicals, endogenous and exogenous. The endogenous free radicals are derived from normal cellular metabolism (ETC), oxidative burst in phagocytic cells and the reactions of certain enzymes such as oxidases, cyclooxygenases, lipooxygenases, etc. to name a few. The exogenous sources of free radicals include tobacco smoke, air pollutants, organic solvents, anesthetics, pesticides, drugs, and radiation. These reactive oxygen species carry out disastrous modifications in bio-molecules (lipids, protein or DNA), leading to deleterious consequences. 4-7 The liver diseases produce reactive oxygen species that are involved in the transcription and activation of a large series of cytokines and growth factors which, in turn, can contribute to further production of free radicals. The extent of cellular damage is manifested by either an increase in the oxidation products or a decrease in the antioxidant levels or both.8-10 Hence, by measuring the oxidative stress parameters, the extent of liver damage can be assessed. It has been reported that ROS directly affect the functioning of stellate cells and myofibroblasts by up-regulating the expression of critical fibrosis associated genes such as COL1A1, COL1A2, MCP1, and TIMP1 through Jun Kinase JNK, activator protein-1, and NFxB. Hence a decrease in anti-oxidant levels along with co-existent elevated lipid peroxidation end products leads to a pro-fibrogenic response by enhancing collagen I protein expression. Furthermore, studies have shown that the X gene product of hepatitis B virus increases ROS production through its interaction with ion channels in the mitochondrial membrane.8 ROS also stimulate apoptosis of hepatocytes by activation of the FAS pathway and induction of pro-inflammatory pathway, including tumor necrosis factor-α, bile acids, ischemia and transforming growth factor-β1 (TGF-beta). 9 Studies have proven the putative role of ROS as second messenger for nuclear factor kappa beta (NFKβ) which in turn increases the production of NO through induction of iNOS. NO has a role in phagocytosis, inflammation and tissue injury. In addition, it also produces the highly reactive peroxynitrite species which in turn accelerates pro-oxidant damage.10 Ceruloplasmin is a ferroxidase enzyme that is encoded by the CP gene. Ceruloplasmin binds copper and integrates iron and copper homeostasis. In addition to that, it may have a protective effect as an antioxidant agent through its ability to prevent oxidative damage, using copper (II) centers. Studies have demonstrated that ceruloplasmin is an effective chain-breaking antioxidant for a variety of radicals, independently of its catalytic ferroxidase activity.11-12 Alpha1-Antitrypsin (AAT) is the prototypical member of the SERPIN (an acronym for serine proteinase inhibitor) family of protease inhibitors. Approximately 70-80% of AAT is synthesized by liver cells. During an inflammatory response, AAT levels increase as a result of local synthesis by resident or invading inflammatory cells. 13 Studies have demonstrated the immune-modulatory and anti-inflammatory role.14 This study was conducted to evaluate the association if any between alterations in the parameters of liver function tests and ceruloplasmin & alpha-1 antitrypsin levels in subjects with acute hepatitis.

MATERIALS & METHODS

Settings
The study was commenced after obtaining ethical clearance from the institutional ethical committee. Out of a total of 207 random OPD cases, 57 patients having the symptoms such as jaundice, yellowish discoloration of sclera, skin and urine, pain in abdomen, fever, vomiting, as well as loose stool for few days admitted to the Gastroenterology department were enrolled as cases. Thirty controls were also recruited from healthy volunteers who did not have hepatitis, according to clinical and laboratory findings. All subjects were recruited in the study, after obtaining written consent, post explanation of the study protocol.

Design
Their clinical profile, complete Liver Function Test, Prothrombin Time, viral serology and alpha-1 antitrypsin (A1AT) & ceruloplasmin (CP) levels were recorded and compared with 30 normal healthy age & sex matched controls.

Subjects
The exclusion criteria were:
- Ultrasonographic evidence of biliary obstruction or chronic liver disease
- Causes of liver injury, chronic alcoholics, patients with chronic liver disease, drug induced hepatitis
- Systematic diseases like Malaria, Typhoid, etc

Assay
7 ml of blood was drawn and sample divided into plain vacutainer and citrated vacutainers for Liver Function Test and Prothrombin Time respectively. The tests were carried out on Beckman synchron CX5 fully automated biochemical analyser in the Department. Of Biochemistry in the Mission Hospital, Durgapur. Various parameters were measured by different methods such as total and direct bilirubin is measured by Diazo method, serum ALT, AST, GGT is measured by an enzymatic rate method. Serum ALP is measured by enzymatic rate method using a 2-amino-2methyl-1-propanol (AMP) buffer. TP and Albumin are measured by end point biuret and end point method respectively, a1AT and CP are measured by Turbidimetry.

Statistical analysis
Statistical analyses were done by Windows Microsoft Excel 2007. All the dates were expressed as mean ± S.D. statistical analysis was done using windows MS
Excel 2007. Association between parameters were studied using Z test and association was considered statistically significant if P < 0.05.

RESULTS

A total of 57 cases of acute hepatitis were seen during the period of study. No statistically significant difference was seen between the ages of the cases and controls. The total number of males was 42 compared to 15 females, reflecting the predominance of acute hepatitis in males than females. Figure 1 depicts the age specific incidence of hepatitis among the cases. It is evident that the incidence is quite high in the younger age group. Tables 1 and 2 depict the sero-positivity results and the spectrum of clinical presentation among the cases. Sero-positivity was observed in 30 patients while 27 were non seropositive. Similarly, the common symptoms at the time of presentation to the hospital were loss of appetite, vomiting and yellowish discoloration. Table no 3 illustrates the comparison of biochemical parameters among the cases and controls. As expected the liver function tests are deranged severely in the cases with significantly elevated levels of bilirubin, liver enzymes and prothrombin time. The albumin levels were significantly lower among the hepatitis patients signifying decreased hepatic synthetic function. Serum ceruloplasmin levels as well as 24 hr urinary copper excretion were significantly elevated in the cases as compared to the controls. Alpha -1 antitrypsin level was significantly high in the cases as compared to controls. However, no statistically significant correlations could be obtained between the parameters under study.

Table 1

<table>
<thead>
<tr>
<th>Type of Hepatitis</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis A</td>
<td>18</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>8</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>4</td>
</tr>
<tr>
<td>Total:</td>
<td>30</td>
</tr>
<tr>
<td>Non seropositive</td>
<td>27</td>
</tr>
<tr>
<td>HCV &amp; HIV</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Chief presenting complaints</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Jaundice, yellowish discoloration of sclera, skin and urine</td>
<td>57</td>
</tr>
<tr>
<td>2. Nausea</td>
<td>42</td>
</tr>
<tr>
<td>3. Pain in abdomen/joint pain</td>
<td>45</td>
</tr>
<tr>
<td>4. Fever with chills/seizures</td>
<td>10</td>
</tr>
<tr>
<td>5. Vomiting</td>
<td>57</td>
</tr>
<tr>
<td>6. Loss of appetite</td>
<td>57</td>
</tr>
<tr>
<td>7. Burning sensation</td>
<td>15</td>
</tr>
<tr>
<td>8. Loose stool</td>
<td>20</td>
</tr>
<tr>
<td>9. Anorexia and occasional headache</td>
<td>40</td>
</tr>
</tbody>
</table>
DISCUSSION

In the study, the peak incidence of acute hepatitis was noticed mainly in the age group of 11-20 years, particularly teens and children. Males were predominant as compared to females. The maximum number of patients presented with chief complaints of jaundice, vomiting and loss of appetite. Hepatitis A was predominant, this is an exception to the findings reported by Chadha et al\(^{19}\) and other researchers\(^{16,18}\) who found hepatitis E as the major cause of acute hepatitis in India. In biochemical profile, cases showed higher levels of T.bil, D.bil, I. bil, SGOT, SGPT, GGT, ALP and globulin than controls which is as expected. Urinary copper and serum ceruloplasmin levels in patients showed higher levels than normal and the differences was statistically significant (P<0.05). The same pattern was observed for alpha-1 antitrypsin levels also. Copper (Cu) is an essential metal for living organisms that utilize oxygen for respiration and is required as a cofactor of many enzymes involved in oxidoreductive reactions - such as superoxide dismutase, llysyl oxidase, tyrosinase, and dopamine β-hydroxylase. However, the redox-active property of this metal may have toxic effects on cells due to the generation of harmful reactive oxygen species. Given these circumstances, it is said that cells have a dependable system for Cu homeostasis that efficiently distributes this essential metal to cuproenzymes, thereby preventing damage to proteins, nucleic acids, sugars, and lipids. In particular, influx, efflux, and intracellular distribution with maintenance of the oxidation state of Cu are strictly regulated.

Ceruloplasmin plays an important role in the process of regeneration of damaged tissues. It promotes a switch from pro-inflammatory to anti-inflammatory pathways necessary for the resolution of inflammation. AAT plays a significant role in the resolution of the hepatic inflammation. The significantly higher serum levels of AAT in the patients with acute hepatitis may signify its role in the resolution of the hepatic inflammation.

CONCLUSION

To the best of our knowledge, there are very few published studies on the biochemical profile of acute hepatitis with special reference to ceruloplasmin and alpha-1 antitrypsin in Eastern India. The ceruloplasmin and alpha-1 antitrypsin levels also showed increased levels and thus can serve as markers to determine the severity of acute hepatitis. But further studies over larger population are required to arrive at a concrete conclusion.

CONFLICT OF INTEREST

Conflict of Interest declared as none.
REFERENCES


