Simple and rapid detection of liver cirrhosis in children by tracking serum IgA/transferrin ratio

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Summary

Objective: The aim of this study is to assess the level of immunoglobulin A (IgA) and transferrin of children patients with liver cirrhosis to determine the relation between liver cirrhosis and IgA/transferrin ratio.

Methods: The study involved 32 children classified into liver patients without cirrhosis (n=12), and liver patients with cirrhosis (n=10) as well as a group of normal healthy children (n=10) for comparison. In all of these subjects, serum alanine (ALT) and aspartate (AST) aminotransferase activity, as well as serum IgA and transferrin level, were also determined.

Results: Our results revealed that the mean values of both ALT and AST activities were significantly high in both groups of patients with and without liver cirrhosis; the activity of both enzymes was relatively higher in patients with liver cirrhosis than in those without liver cirrhosis. Furthermore, the amount of IgA showed very significant decreased values in patients without liver cirrhosis while highly significant increased values were obtained in cirrhotic patients as compared to their corresponding values in normal group. The concentration of serum transferrin showed insignificant values in cases of liver cirrhosis, while it showed moderately significant decreased levels in cases of liver cirrhosis. Although the values of IgA/transferrin ratio showed insignificant values in patients without liver cirrhosis, these values were significantly high in cirrhotic patients. In addition, it has been found that in liver cirrhotic patients the mean values of IgA/transferrin ratio nearly reached 2.5 as compared to the ratios in normal or non-cirrhotic patients.

Conclusions: From the present study we have postulated that the determination of IgA and transferrin in serum or plasma may open up a very simple and safe means for the early detection of latent cirrhosis. The value of IgA/transferrin >2.5 ratio was found to be a highly significant increase in latent cirrhosis as compared to children without cirrhosis or in normal children. This value can be considered as an indicator of latent cirrhosis in children.

Introduction

Cirrhosis is the twelfth leading cause of death by disease, killing about 26,000 people each year. Also, the cost of cirrhosis in terms of human suffering, hospital costs, and lost productivity is high. Individuals with most types of cirrhosis of the liver are at an increased risk of developing hepatocellular carcinoma (HCC) [1].

Studies of infection with hepatitis C virus (HCV) progression to HCC are expected to provide new insights on the management of this growing problem and therefore are of great public health interest. The natural progression of HCV infection to chronic hepatitis, cirrhosis, and HCC is slow. Chronic hepatitis develops in ~80% of those infected with HCV [2]. Over the course of ≥20 years, 10-30% of HCV carriers develop cirrhosis, while patients with cirrhosis have an annual risk of 1-2% for developing HCC. The prognosis of patients with HCC remains extremely poor. The currently available systemic therapies demonstrate poor to modest response rates and have not been shown to improve survival in patients with HCC [3, 4].

In chronic hepatitis, inflammatory cells infiltrate the portal tracts and may also collect in small clusters in the parenchyma. The latter instance is usually accompanied by focal liver cell necrosis. The margin of the parenchyma and portal tracts may become inflamed, with liver cell necrosis at this site (interface hepatitis) [5].
Cirrhosis can result from direct injury to the liver cells or from indirect injury via inflammation or obstruction to bile ducts that drain the liver cells of bile. The major etiological risk factors for liver cirrhosis and HCC include chronic alcoholism, chronic viral hepatitis (types B, C, and D), and autoimmune hepatitis. Common causes of indirect injury by way of bile duct damage include primary biliary cirrhosis, primary sclerosing cholangitis, and biliary atresia (a common cause of cirrhosis in infants). Less common causes of cirrhosis include direct liver injury from inherited disease, such as α-1-antitrypsin deficiency, haemochromatosis, Wilson’s disease, galactosemia, cystic fibrosis, and glycogen storage disease [8-10].

The abundant methods available for the diagnosis of liver diseases (laboratory and immunological methods along with liver biopsy, radiology, scintigraphy, endoscopy, sonography, and computer tomography) make a rational planning of their employment essential. Clinical diagnoses are based on laboratory tests in order to determine the extent and activity of the disease and on various instrumental techniques in order to determine the existence of a portal hypertension or the development of a hepatoma in the cirrhosis. The diagnosis may be clarified by a blind liver biopsy or a puncture under laparoscopic viewing. The laboratory findings indicated that when α-fetoprotein in the serum was elevated above 150 mg/ml, the incidence of HCC was shown in 85% of the cases. A reversal of the albumin/globulin ratio and a beta/gamma type in electrophoresis may also enable the diagnosis of cirrhosis [11].

Cirrhosis is a chronic diffuse liver disease that is characterized by fibrosis and nodule formation. The condition results from liver cell necrosis and collapse of hepatic lobules [12]. According to Wight [13], Balistreri and Rej [14], and Conn [15], cirrhosis can be classified depending on the morphological features of nodular size, where the cirrhotic nodules lack normal lobular organization and are surrounded by fibrous bands of variable thickness into micronodular or macronodular and mixed liver cirrhosis. Regeneration in a micronodular cirrhosis results in a macro or mixed appearance [16].

Because of the limitation and risks of liver biopsy, it is no longer considered mandatory as the first line indicator of liver injury, and many markers have been developed as non-invasive alternatives [17]. The aim of our study was to assess the level of IgA and transferrin of children with liver cirrhosis in an attempt to use these parameters in the early diagnosis of latent cirrhosis in children without using the painful biopsy technique or other unspecific markers.

Materials and methods

This study involved 22 Egyptian children and babies ranging in age from 4 months to 10 years, all of whom were suffering from liver disease and had been hospitalized and were undergoing treatment at the pediatric department of the Faculty of Medicine of Ain Shams University. For comparison, 10 normal healthy children were also taken from the same age group and under the same socio-economic conditions as the patients. A written consent was given by parents of all participants, and the study was approved by the ethical committee at the National Research Centre, Egypt.

As shown in Table 1, depending on the entire picture of the disease, including clinical features, laboratory investigations and histological assessment of liver biopsy slides, the patients were assigned to one of the following groups:

Patients without liver cirrhosis: twelve subjects (4 male and 8 female), all met the criteria for liver disease without cirrhosis in biopsy. In all subjects hepatosplenomegaly, jaundice, surrounding portal hepatitis, and intracellular cholestasis were present.

Patients with liver cirrhosis: ten subjects (5 male and 5 female), with post-inflammatory cirrhosis of the liver, all of whom had clinical, biochemical, and histological markers of cirrhosis.

Venous blood samples were collected from all subjects and left to clot. Serum samples were separated by centrifugation at 3000 rpm for 10 min, and unhemolyzed serum samples obtained were either immediately analyzed or kept at −20°C till the time of analysis.

In all patients serum alanine (ALT) and aspartate (AST) aminotransferase activity was assessed by measuring serum activity spectrophotometrically according to the manufacturer’s instructions, using

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reagent kits obtained from Roche Diagnostics (Mannheim, Germany).

Quantitative investigations of IgA and transferrin were carried out by immunodiffusion technique using anti-IgA (1.50 μl/cm² plate area), and anti-transferrin (0.50 μl/cm² plate area) were obtained from Dakkopatt (Copenhagen, Denmark) as described by Mancini et al [18], where the squared diameter of different concentration from normal standard human serum (Dade Behring, Marburg, Germany) are plotted against the amount of antigen applied to the wells, by interpolation on the curve unknown samples can be quantitated. The IgA/transferrin ratio was also calculated.

Statistical analysis
Data were expressed as mean ± standard error (S.E.) and analyzed statistically using a one-way analysis of variance (ANOVA) followed by the Student’s t-test for comparison between groups. Differences were considered significant at P<0.05.

Results
The results presented in Table 2 showed that the activities of ALT and AST were significantly (P<0.05) high in both groups of patients without liver cirrhosis and with liver cirrhosis. The activity of both enzymes showed highly significant increased value (P<0.05) in patients with liver cirrhosis as compared to those without liver cirrhosis.

The data obtained for serum IgA and transferrin (mg/dl) as well as the IgA/transferrin ratio in the analyzed groups as compared to the normal healthy group are given in Figures 1 and 2. The level of IgA showed very highly significant decreased values (P<0.05) in patients without liver cirrhosis, while highly significant increased values (P<0.05) were obtained in cirrhotic patients as compared to their corresponding values in the control group. The concentration of serum transferrin showed insignificant values (P>0.05) in cases without liver cirrhosis, whereas these values showed moderately significant decrease in cases with liver cirrhosis.

The values of IgA/transferrin ratio showed insignificant values (P>0.05) in patients without liver cirrhosis, although these values showed a significantly high level in cirrhotic patients. This ratio ranged between 0.62-1.27 (mean 0.94) in the normal healthy group, but between 1.33-2.77 (mean 2.5) and 0.25-0.95 (mean 0.55) in patients with and without cirrhosis, respectively. Furthermore, it has been found that the mean values of IgA/transferrin ratio in liver cirrhotic patients reached nearly 2.5 as compared to either the normal group or patients without cirrhosis. This obtaining value can be now considered as an indicator of liver cirrhosis in children associated with liver diseases.

Table 1. The clinical data in 12 non-cirrhotic and 10 cirrhotic patients

<table>
<thead>
<tr>
<th>NON-CIRRHOTIC PATIENTS</th>
<th>PN</th>
<th>SEX</th>
<th>AGE</th>
<th>SYMPTOMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>4 m</td>
<td>Hepatosplenomegaly, asthmatic bronchitis</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>4.5 y</td>
<td>Jaundice</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>6 y</td>
<td>Cholestatic hepatitis</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>5 m</td>
<td>Neonatal jaundice, biliary atresia</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>8 m</td>
<td>Hepatoemegaly</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>4 y</td>
<td>Prolonged neonatal jaundice and intracellular cholestasis surrounding portal hepatitis</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>3 y</td>
<td>Chronic hepatitis with moderate portal inflammation and moderate piecemeal necrosis</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>3 y</td>
<td>Mild hepatomegaly, bronchial asthma and progressive abdominal distention</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>4 m</td>
<td>Neonatal jaundice</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>1 y</td>
<td>Hepatoemegaly</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>5 y</td>
<td>Liver fibrosis, dyspnea, diarrhea, vomiting and cough</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>5 y</td>
<td>Neonatal jaundice and hepatomegaly</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>CIRRHOTIC PATIENTS</th>
<th>PN</th>
<th>SEX</th>
<th>AGE</th>
<th>SYMPTOMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>2.5 y</td>
<td>Periportal cirrhosis</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>8.5 y</td>
<td>Mixed portal cirrhosis with mild activity</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>9 y</td>
<td>Cirrhotic changes</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>5 m</td>
<td>Cirrhosis</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>9 y</td>
<td>Liver cirrhosis</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>6 y</td>
<td>Active liver cirrhosis</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>10 y</td>
<td>Cirrhotic changes</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>3 y</td>
<td>Mild cirrhosis and hepatomegaly</td>
<td></td>
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<tr>
<td>9</td>
<td>F</td>
<td>9 y</td>
<td>Liver cirrhosis</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>10 y</td>
<td>Liver cirrhosis</td>
<td></td>
</tr>
</tbody>
</table>

| PN, patient number; F, female; M, male; m, month; y, year. |

Table 2. The activities of serum ALT and AST in the investigated groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Non-cirrhotic</th>
<th>Cirrhotic</th>
</tr>
</thead>
</table>
| ALT (IU/l)   | 8.01 ±  
0.40     | 74.16 ±  
12.38a
b      | 105.00 ±  
66.16a
b      |
| AST (IU/l)   | 7.41 ±  
0.42     | 66.16 ±  
9.55a
b      | 175.20 ±  
55.65a
b      |

Data are expressed as means ± S.E.

a, significantly different from control; b, significantly different from non-cirrhotic group at P<0.05 using Student’s t-test.
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Figure 1. The concentration of serum IgA and transferrin as expressed by mean value ± S.E. in patients with and without liver cirrhosis as compared to controls.

Discussion

It is uncommon for patients to first consult a physician when they are alarmed by a rapid increase in circumference of the abdomen or by a troublesome dyspnea as a result of ascites. Classical liver skin signs such as spider naevi ‘bank note’ skin, together with a high degree of limitation of coagulation function, a reversal of the albumin-globulin ratio, and a beta-gamma type in electrophoresis mostly enable a diagnosis of a real case of cirrhosis. In order to determine, the external and the activity of the disease, clinical diagnoses are based on laboratory tests and on various instrumental techniques, in order to determine the existence of a portal hypertension or the development of a hepatoma in the cirrhosis. When the coagulation findings permit, the etiology may be clarified by a blind liver biopsy or a puncture under laparoscopic viewing, or in the case of suspicion of a hepatoma, a sonographically guided fine needle puncture to obtain cytological material [19].

In the present study, it has been emphasized that the ratio formed from the concentration of serum IgA and transferrin (IgA/transferrin ratio) is already suitable in the pre-clinical phase to clarify whether the risk of a liver cirrhosis exists. In this pre-clinical stage, the formation of mesenchymal tissue is coupled with an intrahepatic infiltration of plasmocytes characteristic of the inflammatory reaction. The plasmocytes synthesize immunoglobulins of the classes IgA and IgG, the elevation of IgA initially predominating [20]. In contrast to this, the plasma concentration of the transferrin synthesized in the liver, a beta-globulin with a transport function for iron decreases. Although the rate of secretion is raised during an inflammatory reaction of the liver cells, the even more elevated catabolic rate results in a reduction of transferrin in the serum. Under these circumstances the mean values of serum IgA was found to be 348.70 mg/dl in 10 cases of children suffering from liver cirrhosis, whereas these values were 116.87 mg/dl in 12 cases without liver cirrhosis and it was 196.90 mg/dl in 10 normal healthy children. The results also revealed that the IgA/transferrin ratio level was greater than 2.5 with a highly significant increase in children with liver cirrhosis as compared to children without liver cirrhosis, indicating a latent cirrhotic process. Since it is very important to diagnose latent cirrhosis in children as early as possible, the cases were selected without concomitant with any other ailments.

The absolute values of the parameters IgA and transferrin are taken into account in interpretation of the ratio between the two, since this value can be influenced by other diseases. In heamochromatosis erythropoietic porphyria (Gunther’s disease), nephritis, malignant tumors, in infections associated with anaemia, and in gastric resection, transferrin is decreased in the blood. An elevation of the serum concentration of transferrin is to be found towards the end of a pregnancy or under oral contraceptives, and in iron deficiency anemia and acute hepatitis [20].

IgA in its turn exhibits higher concentrations in infections, some autoimmune diseases, and in the IgA myeloma. Lowered IgA values are encountered in immunodeficiencies, in the nephrotic syndrome, under immunosuppressive therapy, in leukaemias.
and monoclonal gammopathies of other immunoglobulin classes, and in light chain disease. For this reason, it is therefore necessary to interpret the change in serum IgA concentration within the framework of a completed analysis of the immune system, which should consist of qualitative and quantitative determination of the various classes of immunoglobulins [20].

Moreover, in our investigation we found significant correlation between increasing the serum aminotransferase activity as a result of liver injury and IgA/transferrin ratio in cirrhotic patients. Increase in serum IgA and decrease in the level of transferrin in patients with liver cirrhosis can be explain by the fact that in cirrhosis the marked decrease of transferrin level may be related to the decreased rate of synthesis due to severe injury of liver cells leading to decreased synthesis of protein resulting from the exhaustion of liver cells and the decreased intake of essential amino acids [14]. Furthermore, Sherlock [12] suggested that the highly significant level of α2-macroglobulin in liver cirrhosis is known and could conceivably be part of a general increase in synthesis of plasma globulins, including IgA, IgM, and IgG, to compensate the hypoalbuminaemia which frequently present in cirrhosis [21].

From the present study we have concluded that the determination of IgA and transferrin in serum or plasma may open up a very simple and safe means for the early detection of latent cirrhosis. The value of IgA/transferrin >2.5 ratio was found to be highly significantly increased in latent cirrhosis as compared to patients without cirrhosis or to normal subjects. This value can be considered as an indicator of latent cirrhosis in children associated with liver diseases.

References