Essential Fatty Acids Status in Infants and Children with Chronic Liver Disease

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Summary The liver plays a central role in the metabolism of essential fatty acids; (EFAs; linoleic, linolenic and arachidonic acids). In this work the relationship between EFAs status and the degree of hyperbilirubinemia as well as oxidant stress in infants and children with chronic liver diseases were evaluated. The study was conducted in 30 subjects with chronic cholestasis, 30 subjects with liver cirrhosis and 30 healthy subjects used as control group. Compared with control subjects, both patient groups, cholestatic and cirrhotic groups had significant decrease in EFAs levels (p< 0.01), significant elevation of total bilirubin (p< 0.0001). In cholestatic group, direct bilirubin was> 20% of total bilirubin levels. As regards oxidant stress parameters, both patient groups had significant elevation of thiobarbituric acid reactive substances; (TBARs; p< 0.0001 in both groups ), significant decrease in retinol, α-tocopherol, and α-tocopherol/total lipids ratio (p< 0.0001 in both patient groups for the three mentioned parameters). In cholestatic group, there was a significant inverse correlation between the EFAs level and total and direct bilirubin level. In both patients groups, there was a significant positive correlation between the EFAs level and the levels of retinol, α-tocopherol and α-tocopherol/total lipids ratio, whereas there was a significant inverse correlation between the EFAs levels and the TBARs levels. In conclusion, patients with chronic liver diseases are at a high risk of EFAs deficiency which is correlated with progressive elevation of serum bilirubin and progressive deterioration of oxidant status.

Key Words: essential fatty acids, hyperbilirubinemia, oxidant stress, infants, children, chronic liver diseases

Introduction

Essential fatty acids (EFAs) are essential components of structural phospholipids in all tissues and modulate cell membrane fluidity and functions. The availability of long chain poly unsaturated fatty acids (PUFA) (>18 carbon atoms), such as arachidonic and docosahexaenoic acids, is important for early human growth and development of membrane rich tissues such as brain and retina [7]. Moreover, ω-6 and ω-3 PUFA serve as precursors of eicosanoids with important biological roles as mediators of immune and vascular functions as well as platelet aggregation [2, 3]. It was hypothesized that children with cholestatic liver disease have a poor PUFA status, because bile acids contribute to efficient PUFA absorption from the gut and because long chain polyunsaturated (LCP) fatty acids are synthesized from their precursors, mainly in the liver by desaturation and elongase systems that are adversely affected.
in chronic liver diseases. Furthermore, PUFA depletion of plasma lipid fractions was reported in adult patients with cirrhosis in whom it was associated with protein energy malnutrition and the occurrence of encephalopathy [4-8]. In contrast, there is only limited information on the essential fatty acid status in infants and children with cholestasis and other chronic liver diseases such as autoimmune hepatitis, chronic hepatitis, glycogen storage diseases and others.

The aim of this work is to study the relationship between EFAs status (linoleic, linolenic and arachidonic acids) and serum bilirubin as well as oxidant status in infants and children with various chronic liver diseases such as chronic hepatitis, autoimmune hepatitis, biliary atresia and others.

Subjects and Methods

The present study was conducted on 90 subjects (60 with chronic liver diseases and 30 control subjects). All subjects were selected from Hepatology Clinic, Faculty of Medicine, Ain Shams University.

The patients group included

- Ten children with autoimmune hepatitis (6 boys and 4 girls), their age ranged from 10 to 12 years old.
- Twenty-five children with chronic hepatitis (20 boys and 5 girls), their age ranged from 3 to 9 years old.
- Five children with glycogen storage disease type I (3 boys and 2 girls), their age ranged from 6 to 7 years old.
- Six children with portal vein thrombosis (2 boys and 4 girls), their age ranged from 7 to 10 years old.
- Four children with Wilson disease (3 boys and 1 girl), their age ranged from 4 to 5 years.
- Four children with congenital biliary atresia (1 boy and 3 girls), their age ranged from 2 to 3 years old.
- Three girls with alpha 1 antitrypsin deficiency, their age ranged from 1 to 2 years old.
- Three boys with veno-occlusive disease, their age ranged from 11 to 14 years old.

The patients group was subdivided into

Group (A): Included 30 patients with cholestasis (4 cases with biliary atresia, 6 cases with autoimmune hepatitis and 20 cases with chronic hepatitis), the diagnostic criteria of cholestatic liver disease were: elevation of direct bilirubin>20% of total bilirubin, i.e. including all preclinical or subclinical jaundice [9, 10], dark urine and pale stools, all patients were not cirrhotic and had compensated liver.

Group (B): Included the other 30 cases of the patients group. They did not have cholestasis, but they had liver cirrhosis, and they had compensated liver.

Furthermore, 30 clinically free children (20 boys and 10 girls), with no history or clinical evidence of liver diseases or any other diseases were chosen as control subjects, their age ranged from 1 to 14 years old.

The following parameters were evaluated for each subject

- Full medical history and thorough clinical examination.
- Abdominal ultrasonography.
- Liver biopsy.
- Specific diagnostic tests for some cases such as: hepatitis markers, upper gastro intestinal track (GIT) endoscopy, liver scanning, 24 h urinary copper and serum ceruloplasmin, serological tests such as antinuclear antibodies, antismooth muscles antibodies and anti mitochondrial antibodies.
- Liver function tests: aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum total bilirubin and direct bilirubin [11].
- Total lipids [12].
- EFAs levels by gas liquid chromatography (GLC). In brief, total lipids were extracted from serum with chloroform/methanol (2 : 1, v/v). The residual protein precipitate was removed by centrifugation and the extract was washed with methanol water, fatty acids were separated from total lipids as saponifiable fraction using 0.5 M alcoholic KOH according to the method described by Folch et al. [13]. Fatty acids were then recovered by acidification and methyl esterification according to the method decribed by Balint [14]. The extract was analyzed by GLC (Varian) programmed at 160–260°C, 4°C/min, using a glass column (Supelco Inc., Bellefonte, PA). Nitrogen was used as the carrier gas at a flow rate 30 ml/min and detection of fatty acids was done by flame ionization detector.
- Plasma retinol and α-tocopherol levels by high performance liquid chromatography (HPLC) [15].

Serum levels of thiobarbituric acid reactive substances (TBARs) i.e. malondialdehyde, by HPLC by the method of Steven et al. [16]. Results were described as mean±SD. Possible correlations were tested by the Spearman Rank Correlations and plotted as linear regressions. Differences and regressions were regarded as statistically significant at $p<0.05$ [17].

**Results**

The results are summarized in the following tables (Tables 1 and 2) and comments:

- EFAs exhibited significant decrease in their levels in both patient groups, cholestatic and cirrhotic groups as compared with control subjects ($p<0.01$).
- Retinol, α-tocopherol and α-tocopherol/total lipids showed significant decrease in their levels in both patient groups as compared with control subjects.
- Serum levels of TBARs, AST, ALT, total bilirubin and direct bilirubin were significantly elevated in both cholestatic and cirrhotic groups as compared with control subjects. Whereas, in cholestatic group, serum total and direct bilirubin level was significantly elevated as compared with cirrhotic group ($p<0.0001$) and direct bilirubin was >20% of total bilirubin.
- Statistical comparison between cholestatic and cirrhotic groups as regards the other biochemical parameters studied, revealed no significant differences.
- In cholestatic group, there was a significant inverse correlation between the levels of EFAs and total bilirubin.

![Table 1](image1.png)

<table>
<thead>
<tr>
<th></th>
<th>Control subjects ($n=30$)</th>
<th>Cholestatic group ($n=30$)</th>
<th>p1</th>
<th>Cirrhotic group ($n=30$)</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>6.26±3.9</td>
<td>6.5±3.34</td>
<td>N.S.</td>
<td>6.3±3.79</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sex male/female</td>
<td>10/5</td>
<td>5/7</td>
<td>N.S.</td>
<td>11/2</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>AST (IU/liter)</td>
<td>17.4±3.34</td>
<td>104.08±28.4</td>
<td>&lt;0.01</td>
<td>96.15±26.87</td>
<td>&lt;0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>ALT (IU/liter)</td>
<td>12.5±4.88</td>
<td>96.16±18.03</td>
<td>&lt;0.01</td>
<td>94.76±24.6</td>
<td>&lt;0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.81±0.08</td>
<td>3.36±0.83</td>
<td>&lt;0.0001</td>
<td>1.39±0.09</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.1±0.08</td>
<td>0.73±0.18</td>
<td>&lt;0.0001</td>
<td>0.13±0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Linoleic acid (μmol/liter)</td>
<td>94.4±13.3</td>
<td>81.47±5.69</td>
<td>&lt;0.01</td>
<td>78.31±3.19</td>
<td>&lt;0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>Linolenic acid (μmol/liter)</td>
<td>3.93±0.69</td>
<td>2.52±0.27</td>
<td>&lt;0.01</td>
<td>2.47±0.18</td>
<td>&lt;0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>Arachidonic acid (μmol/liter)</td>
<td>2.9±0.18</td>
<td>1.87±0.087</td>
<td>&lt;0.01</td>
<td>1.9±0.06</td>
<td>&lt;0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>Retinol (μg/dl)</td>
<td>34.36±4.68</td>
<td>17.44±3.37</td>
<td>&lt;0.0001</td>
<td>26.6±3.74</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>α-Tocopherol (mg/dl)</td>
<td>10.14±1.87</td>
<td>2.6±0.98</td>
<td>&lt;0.0001</td>
<td>3.76±1.05</td>
<td>&lt;0.0001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>α-Tocopherol/total lipids</td>
<td>0.65±0.06</td>
<td>0.226±0.07</td>
<td>&lt;0.0001</td>
<td>0.305±0.037</td>
<td>&lt;0.0001</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>TBARs (μmol/liter)</td>
<td>0.492±0.054</td>
<td>5.054±0.814</td>
<td>&lt;0.0001</td>
<td>5.41±0.744</td>
<td>&lt;0.0001</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

p 1, statistical comparison between patient groups and control group; p 2, statistical comparison between cholestatic group and cirrhotic group.

![Table 2](image2.png)

<table>
<thead>
<tr>
<th>Cholesterol group ($n=30$)</th>
<th>Cirrhotic group ($n=30$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic</td>
<td>Linolenic</td>
</tr>
<tr>
<td>$r$</td>
<td>$p$</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>$-0.987 &lt;0.001$</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>$-0.913 &lt;0.001$</td>
</tr>
<tr>
<td>TBARs</td>
<td>$-0.900 &lt;0.001$</td>
</tr>
<tr>
<td>Retinol</td>
<td>$0.814 &lt;0.001$</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>$0.933 &lt;0.001$</td>
</tr>
<tr>
<td>α-Tocopherol/total lipids</td>
<td>$0.972 &lt;0.001$</td>
</tr>
</tbody>
</table>

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and the levels of total and direct bilirubin.
In both patient groups, there was a significant inverse correlation between the EFAs levels and the levels of TBARs. On the other hand, there was a significant positive correlation between the EFAs levels and the levels of retinol, α-tocopherol and α-tocopherol/total lipids.

Discussion

EFAs deficiency has been reported to occur in advanced liver cirrhosis and other liver diseases such as hepatitis, cholestatic liver diseases and others [5, 18, 19]. In this study, plasma levels of linoleic [C 18: 2, ω-6], linolenic [C 18: 3, ω-3] and arachidonic [C 20: 4, ω-6] acids were found to be significantly reduced in both cholestatic and cirrhotic patient groups as compared with control subjects (p<0.01). EFAs deficiency in those patients could be attributed to many factors: impairment of hepatic metabolism; i.e. impairment of desaturase and elongase systems, fat malabsorption due to impaired bile acid synthesis or secretion, enhanced lipid peroxidation due to increased oxidative stress as well as the presence of malnutrition in those patients.

Furthermore, α-tocopherol, α-tocopherol/total lipids and retinol exhibited significant reduction in their plasma levels in both cholestatic and cirrhotic patient groups as compared with control subjects (p<0.001). There was also a significant positive correlation between the EFAs levels and the levels of α-tocopherol, α-tocopherol/total lipids and retinol in both patient groups. On the other hand, TBARs showed significant elevation of their levels in both patient groups confirming the presence of oxidative stress in those patients. Increased levels of MDA and peroxidation products with thiobarbituric acid connected with hepatocyte injury have been confirmed in both clinical and experimental studies [19–21]. Furthermore, there was also a significant inverse correlation between plasma levels of TBARs and EFAs levels in both patient groups (p<0.001). The presence of oxidative stress could explain the reduced levels of EFAs by enhancing their susceptibility EFAs to lipid peroxidation. These findings agreed with those reported by Socha et al. [22, 23] who attributed the decrease in PUFA levels in cholestatic liver disease cases to several factors: enhanced lipid peroxidation, reduced dietary intake, as well as fat malabsorption that occurs frequently in patients with liver disease due to impaired bile salts synthesis or secretion. Impaired fat absorption could also explain the observed decrease in α-tocopherol and retinol plasma levels.

In both cholestatic and cirrhotic patients groups, serum levels of total and direct bilirubin showed significant elevation as compared with control subjects, whereas, in cholestatic group, serum levels of total and direct bilirubin showed significant elevation as compared with cirrhotic group and direct bilirubin was >20% of total bilirubin. Furthermore, our results demonstrated significant inverse correlation between the EFAs levels and the serum total and direct bilirubin levels in cholestatic group. Similar findings were reported by Babin et al. [24] and Dupont et al. [25]. They found depletion of linoleic and arachidonic acids from total plasma fatty acids in children with paucity of intralobular bile ducts, biliary atresia and Alagille syndrome. Yamashiro et al. [26] confirmed the metabolic benefit of ursodeoxycholic acid treatment on EFAs deficiency in patients with biliary atresia.

However, there is no indication of a direct relationship between hyperbilirubinemia and the activity of hepatic microsomal desaturase/elongase systems used for LCP synthesis [27–29]. Microsomal membrane lipid peroxidation might contribute to the disturbed LCP synthesis, and children with cholestasis seem to be more vulnerable to oxidative damage, as indicated by high plasma TBARs concentrations in our patients as well as in others and in an experimental model [30, 31]. Lemonnier et al. [30] also found a significant correlation of plasma TBARs concentration with bilirubin concentration in children with biliary atresia and a paucity of intralobular bile ducts.

Several investigators proposed that direct supplementation with LCP could provide a unique advantage in the correction of EFAs deficiency in patients with chronic liver diseases as well as end stage liver diseases [29, 32, 33]. Lepage et al. [34] reported that ursodeoxycholic acid could improve the hepatic metabolism of EFAs and retinol in children with cystic fibrosis associated with liver disease as well as in cases of chronic hepatitis.

From this work, we can conclude that infants and children with various chronic liver diseases are at a high risk of EFAs deficiency that probably leads to a wide array of both cellular and clinical consequences including poor neurological, visual and psychomotor development and in addition vitamin E deficiency, might be a further factor that contributes to neurological impairment in children with chronic liver dis-

eases. Reduced arachidonic acid availability may contribute to a disturbed eicosanoids balance and may be one factor in the pathogenesis of altered coagulation, immunological response and renal functions [35]. A positive correlation between arachidonic acid status and growth was found in preterm infants and in animal models [36] and it was reported that poor arachidonic acid status might also contribute to growth disturbances observed in children with cholestasis [37]. Furthermore, EFAs supplementation in these patients needs extensive investigations as regards, the route, dosage, and safety of supplementation. The use of antioxidants as well as the use of bile salts should be justified for those subjects.

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