Essential fatty acid status in infants and children with chronic liver disease

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الأحماض الدهنية الأساسية في الرضع والأطفال المصابين بأمراض الكبد المزمنة

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الخلاصة: أجريت هذه الدراسة بغرض تقييم العلاقة بين مستوى الأحماض الدهنية الأساسية، ومستوى فرط بيليروبين الدم، ومستوى المؤكسدات بالدم عند الأطفال والرضع المصابين بأمراض كبدية مزمنة. وقد تم فحص 30 مريضاً مصاباً بالركود الصفراوي، و30 مريضاً مصاباً بتشمع كبدي، مع اختيار 30 شخصاً من المتمتعين بالصحة كشواهد. وأظهرت نتائج الدراسة انخفاضاً ملحوظاً في مستوى الأحماض الدهنية الأساسية وارتفاعاً ملموساً في البيليروبين الكلي بين مجموعات المرضى. كما لوحظ فهم ارتفاع في المواد المتفاعلة مع حمض الثيوبارميتورك، وتَرابط هذا الارتفاع ترابطاً عكسياً مع انخفاض مستويات الأحماض الدهنية الأساسية. كما لوحظ انخفاض ملموس في الرتينول، والألفاتوكوفيرول، والألفاتوكوفيرول، وفي نسبة الألفاتوكوفيرول إلى الشحميات الإجمالية. وترابط هذا الانخفاض ترابطاً إيجابياً هاماً إحصائياً مع انخفاض مستوى الأحماض الدهنية الأساسية. وخلصت الدراسة إلى أن الرضع والأطفال المصابين بأمراض كبدية مزمنة يرتفع فيهم احتمال حدوث نقص في الأحماض الدهنية الأساسية يترابط مع ارتفاع مُتَرَق في بيليروبين المصل وتدهور متزق في حالة المؤكسدات.

ABSTRACT The relationship between essential fatty acid (EFA) status and degree of hyperbilirubinaemia and oxidant stress in infants and children with chronic liver diseases was evaluated. Thirty patients with chronic cholestasis and 30 with liver cirrhosis were examined; 30 healthy subjects served as controls. Patient groups had significant decreases in EFAs and significant elevation of total bilirubin. Levels of thiobarbituric acid reactive substances were significantly raised and were significantly inversely correlated to decreased EFA levels. There were also significant decreases in retinol, α-tocopherol and α-tocopherol/total lipids ratio, which had significant positive correlations with decreased EFA levels. Infants and children with chronic liver diseases have a high risk of EFA deficiency correlated with progressive elevation of serum bilirubin and progressive deterioration of oxidant status.

Le statut en acides gras essentiels chez les nourrissons et les enfants atteints de maladie hépatique chronique

RESUME On a évalué la relation entre le statut en acides gras essentiels, le degré d'hyperbilirubinémie et le stress oxydant chez les nourrissons et les enfants atteints de maladie hépatique chronique. Trente patients atteints de cholestase chronique et 30 patients atteints de cirrhose du foie ont été examinés ; 30 sujets en bonne santé ont servi de témoins. Les groupes de patients avaient une diminution significative des acides gras essentiels et une élévation significative de la bilirubine totale. Ils avaient une élévation significative des substances réactives acides thiobarbituriques, qui présentait une corrélation inverse significative avec la diminution des acides gras essentiels ; ils avaient en outre une diminution significative du rétinol, de l'α-tocophérol et du ratio lipides totaux/α-tocophérol, qui présentait une corrélation positive significative avec la diminution des taux d'acides gras essentiels. Les nourrissons et les enfants atteints de maladie hépatique chronique ont un risque élevé de carence en acides gras essentiels corrélé à une élévation progressive de la bilirubine sérique et à une détérioration graduelle du statut oxydant.

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Introduction

Essential fatty acids (EFAs) are polyunsaturated fatty acids (PUFAs) that are essential components of structural phospholipids in all tissues and that modulate cell membrane fluidity and functions. The availability of PUFAs (> 18 carbon atoms) such as arachidonic acid and docosahexaenoic acid is important for early human growth and development of membrane-rich tissues such as the brain and retina [1]. Moreover, n-6 and n-3 PUFAs serve as precursors of eicosanoids with important biologic roles as mediators of immune and vascular functions as well as platelet aggregation [2,3]. It has been hypothesized that children with cholestatic liver disease have 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 desaturase and elongase systems that are adversely affected in chronic liver diseases. Furthermore, PUFA depletion of plasma lipid fractions has been 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 EFA status in infants and children with cholestasis and other chronic liver diseases such as autoimmune hepatitis, chronic hepatitis, glyocogen storage diseases and others.

We examined the relationship between EFA status (linoleic, linolenic and arachidonic acids) and serum bilirubin and oxidant status in infants and children with various chronic liver diseases such as chronic hepatitis, autoimmune hepatitis, biliary atresia and others.

Methods

The present study included 90 children and infants (60 with chronic liver diseases and 30 control subjects). All were selected from the Hepatology Clinic, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

The patient groups included: 10 children with autoimmune hepatitis (6 boys and 4 girls aged 10–12 years); 25 children with chronic hepatitis (20 boys and 5 girls aged 3–9 years); 5 children with glycogen storage disease type III (3 boys and 2 girls aged 6–7 years); 6 children with portal vein thrombosis (2 boys and 4 girls aged 7–10 years); 4 children with Wilson disease (3 boys and 1 girl aged 4–5 years); 4 children with corrected extrahepatic biliary atresia (1 boy and 3 girls aged 2–3 years); 3 girls with alpha-1-antitrypsin deficiency (aged 1–2 years) and 3 boys with veno-occlusive disease (aged 11–14 years).

The patient group was subdivided into two groups. The first group included 30 patients with cholestasis, i.e. 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 greater than 20% of total bilirubin, dark urine and pale stools. None of the patients were cirrhotic but they had compensated liver disease. The second group included the other 30 patients. They did not have cholestasis, but they had liver cirrhosis and they had compensated liver disease. Furthermore, 30 healthy children (20 boys and 10 girls) with no history or clinical evidence of liver disease or any other disease were chosen as control subjects. They were aged 1–14 years.

The following 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 gastrointestinal tract endoscopy, liver scanning, 24-hour urinary copper and serum ceruloplasmin; autoantibodies such as antinuclear antibodies, antismooth muscle antibodies and antimitochondrial antibodies; liver function tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum total bilirubin and direct bilirubin [9]; and total lipids [10].

EFA levels (linoleic, linolenic and arachidonic fatty acids) were assayed by gas liquid chromatography (GLC) and 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 solution. Fatty acids were separated from total lipids as saponifiable fraction using 0.5 mmol/L alcoholic KOH according to the method described by Folch et al. [11]. Fatty acids were then recovered by acidification and methyl esterification according to the method decribed by Balint [12]. The extracts were analysed by GLC (Varian) programmed at 160–260 °C, 4 °C/minute, using a glass column (Supelco Inc). 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. Furthermore, plasma retinol and alpha tocopherol levels were assayed by high performance liquid chromatography (HPLC) [13]. Serum levels of thiobarbituric acid reactive substances (TBARS), i.e. malondialdehyde (MDA), were assayed by high pressure liquid chromatography (HPLC) by the method of Steven et al. [14].

Linoleic, linolenic and arachidonic acids, methyl esters, retinol, retinol acetate, α-tocopherol, and α-tocopherol acetate (Sigma Chemical Co., St Louis, Missouri, USA) were used as standards. The chromatographic accuracy (trueness and precision) were confirmed by analytical recovery of known added concentrations of standards. Recovery was 100%. Calibration procedures with pure standards were applied for chromatographic analysis and calculations with computer software (Varian for GLC and LDC/Milton-Roy for HPLC). The technique was optimized by examining its reproducibility on replicated analysis of samples and standards of different concentrations of a wide range of values. The analysis of variance experiment for all the studied parameters was checked. Two replicates per specimen per run and two runs per day for 20 days were estimated. Standard deviations of the analyses for linoleic acid, linolenic acid, arachidonic acid, retinol and α-tocopherol were: 1.28, 0.032, 0.117, 0.303 and 0.049 between days; 1.31, 0.017, 0.118, 0.214 and 0.048 within runs; 1.32, 0.016, 0.119, 0.214 and 0.049 between runs.

The statistical programme package SPSS, version 5.8 (EchoSoft Corp, USA, 1996) was used. Statistical analysis of the data included calculating means, standard deviations and standard errors of mean and Spearman rank correlation to assess the relationship between different studied parameters within a group.

Student t-test for independent samples and probability (P) values were then obtained from the statistical table with $n_1 + n_2 - 2$ degrees of freedom. P-values ≤ 0.05 were considered significant [15].

Results

There were no significant differences in the mean age or sex ratio between patient and control groups. The mean age ± standard deviation were as follows: 6.26 ± 3.9 years (controls); 6.5 ± 3.34 years (cholestasis patients); 6.3 ± 3.79 years (cirrhosis patients). The male/female ratios were 10/5 (controls); 5/7 (cholestasis patients); and 11/2 (cirrhosis patients).

EFAs (linoleic, linolenic and arachidonic acids) were significantly decreased in both patient groups compared with control subjects ($P < 0.01$) (Table 1). Retinol, α-tocopherol and α-tocopherol/total lipids also showed significant decreases in both patient groups compared with control sub-

jects ($P < 0.0001$); these levels in the cholestasis group were significantly lower than in the cirrhosis group ($P < 0.0001$ for retinol, $P < 0.01$ for α-tocopherol and $P < 0.002$ for α-tocopherol/total lipids ratio).

Serum levels of TBARS, AST, ALT, total bilirubin and direct bilirubin were significantly elevated in both cholestatic and cirrhosis groups compared with controls (Table 1). In the cholestasis group, serum total and direct bilirubin levels were significantly elevated compared with the cirrhosis group ($P < 0.0001$) and direct bilirubin was > 20% of total bilirubin.

Statistical comparison between cholestatic and cirrhosis groups for other biochemical parameters revealed no significant differences. In the cholestasis

Table 1 **Comparison of essential fatty acid levels and liver function tests between control and patient groups**

Variable	Controls (n = 30)	Cholestasis patients (n = 30)	P^a	Cirrhosis patients (n = 30)	P^b	P^c
Linoleic acid (μmol/L)	94.43 ± 13.3	81.5 ± 5.6	< 0.01	78.3 ± 3.2	< 0.01	NS
Linolenic acid (μmol/L)	3.93 ± 0.69	2.52 ± 0.27	< 0.01	2.47 ± 0.18	< 0.01	NS
Arachidonic acid (μmol/L)	2.90 ± 0.18	1.87 ± 0.09	< 0.01	1.90 ± 0.06	< 0.01	NS
AST (IU/L)	17.4 ± 3.3	104.1 ± 28.4	< 0.01	96.2 ± 26.9	< 0.01	NS
ALT (IU/L)	12.5 ± 4.9	96.2 ± 18.0	< 0.01	94.8 ± 24.6	< 0.01	NS
Total bilirubin (mg/dL)	0.81 ± 0.08	3.35 ± 0.84	< 0.0001	1.39 ± 0.09	< 0.0001	< 0.0001
Direct bilirubin (mg/dL)	0.10 ± 0.08	0.75 ± 0.10	< 0.0001	0.13 ± 0.01	< 0.01	< 0.0001
TBARS (μmol/L)	0.49 ± 0.05	5.05 + 0.80	< 0.0001	5.41 ± 0.74	< 0.0001	NS
Retinol (μg/dL)	34.4 ± 4.7	17.4 ± 3.3	< 0.0001	26.6 ± 3.7	< 0.0001	< 0.0001
α-tocopherol (mg/dL)	10.14 ± 1.87	2.60 ± 0.96	< 0.0001	3.73 ± 1.05	< 0.0001	< 0.01
α-tocopherol/total lipids	0.65 ± 0.06	0.23 ± 0.06	< 0.0001	0.31 ± 0.04	< 0.0001	< 0.002

[a]Comparing cholestatic and control groups; [b]Comparing cirrhotic and control groups; [c]Comparing cholestatic and cirrhosis groups.
NS = not significant.
n = total number of patients.
AST = aspartate aminotransferase.
ALT = alanine aminotransferase.
TBARS = thiobarbituric acid reactive substances.

group, there was a significant negative correlation between decreased levels of EFAs and elevated levels of total and direct bilirubin (Table 2). In both patient groups, there was a significant negative correlation between decreased EFAs and increased TBARS. There was a significant positive correlation between decreased EFAs and the decreased levels of retinol, α-tocopherol and α-tocopherol/total lipids.

Discussion

EFA deficiency has been reported to occur in advanced liver cirrhosis and other liver diseases such as hepatitis and cholestatic liver diseases [5,16,17]. In our study, plasma levels of linoleic acid (C 18:2 n-6), linolenic acid (C 18: 3 n-3) and arachidonic acid (C 20: 4 n-6) were found to be significantly reduced in both cholestatic and cirrhosis groups compared with controls (P < 0.01). EFA 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 and malnutrition in those patients [16].

Furthermore, plasma levels of α-tocopherol, α-tocopherol/total lipids and retinol were significantly reduced in both cholestasis and cirrhosis patient groups compared with control subjects (P < 0.0001). There was also a significant positive correlation between the reduced EFA levels and the reduced levels of α-tocopherol, α-tocopherol/total lipids and retinol in both patient groups. TBARS levels, however, were significantly elevated in both patient

Table 2 Correlations between essential fatty acid levels and liver function and other tests in patient groups

Variable	Cholestasis patients (n =30)						Cirrhosis patients (n = 30)					
	Linoleic acid		Linolenic acid		Arachidonic acid		Linoleic acid		Linolenic acid		Arachidonic acid	
	r	p	r	p	r	p	r	p	r	p	r	p
Total bilirubin	−0.907	<0.001	−0.936	<0.001	−0.976	<0.001	−0.022	0.1	−0.007	0.1	−0.076	0.1
Direct bilirubin	−0.987	<0.001	−0.976	<0.001	−0.913	<0.001	−0.027	0.1	−0.020	0.1	−0.028	0.1
TBARS	−0.910	<0.001	−0.923	<0.001	−0.945	<0.001	−0.910	<0.001	−0.931	<0.001	−0.879	<0.001
Retinol	0.814	<0.001	0.859	<0.001	0.966	<0.001	0.918	<0.001	0.947	<0.001	0.879	<0.001
α-tocopherol	0.934	<0.001	0.902	<0.001	0.752	<0.001	0.909	<0.001	0.852	<0.001	0.930	<0.001
α-tocopherol/ total lipids	0.974	<0.001	0.963	<0.001	0.865	<0.001	0.957	<0.001	0.914	<0.001	0.961	<0.001

n = total number of patients.
r = Pearson correlation coefficient.
TBARS = thiobarbituric acid reactive substances.

groups, confirming the presence of oxidative stress in those patients. Increased levels of MDA and peroxidation products with thiobarbituric acid connected with hepatocyte injury has been confirmed in both clinical and experimental studies [17–19]. Furthermore, there was also a significant inverse correlation between plasma levels of TBARS and EFA levels in both patients groups ($P < 0.001$). The presence of oxidative stress could explain the reduced levels of EFA by enhancing the susceptibility of EFAs to lipid peroxidation. These findings agreed with those reported by Socha et al. [20,21] who attributed the decrease in PUFA levels in cholestatic liver disease cases to several factors: enhanced lipid peroxidation, reduced dietary intake and 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. The decreased levels could be a factor in enhanced lipid peroxidation as both vitamins A and E are antioxidants.

In both cholestatic and cirrhotic patient groups, serum levels of total and direct bilirubin were significantly elevated compared with control subjects, whereas in the cholestasis group, serum levels of total and direct bilirubin were significantly higher compared with the cirrhosis group and direct bilirubin was > 20% of total bilirubin. Furthermore, our results demonstrated a significant inverse correlation between the decreased EFA levels and the increased serum total and direct bilirubin levels in the cholestasis group. Similar findings were reported by Babin et al. [22] and Dupont et al. [23]. They found depletion of linoleic and arachidonic acid from total plasma fatty acids in children with syndromatic paucity of interlobular bile ducts (Alagille syndrome) and biliary atresia. Yamashiro et al.

[24] confirmed the metabolic benefit of ursodeoxycholic acid treatment on EFA deficiency in patients with biliary atresia.

There was no indication of a direct relationship between hyperbilirubinaemia and the activity of hepatic microsomal desaturase/elongase systems used for LCP fatty acid synthesis [25–27]. 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 TBAR concentrations in our patients as well as in others and in an experimental model [28,29]. Lemonnier et al. [28] also found a significant correlation of plasma TBAR concentration with bilirubin concentration in children with biliary atresia and syndomatic paucity of interlobular bile ducts.

Several investigators have proposed that direct supplementation with long chain PUFAs could provide a unique advantage in the correction of EFA deficiency in patients with chronic liver diseases and end stage liver diseases [27,30,31]. Lapage et al. [32] reported that ursodeoxycholic acid could improve the hepatic metabolism of EFAs and retinol in children with cystic fibrosis associated with liver disease and in cases of chronic hepatitis.

Infants and children with various chronic liver diseases are at high risk of EFA deficiency that may lead to a wide array of both cellular and clinical consequences including poor neurological, visual and psychomotor development. In addition, vitamin E deficiency might be a further factor that contributes to neurological impairment in children with chronic liver disease. Reduced arachidonic acid availability may contribute to disturbed eicosanoid balance and may be one factor in the pathogenesis of altered coagulation, immunologic response and renal functions

[33]. A positive correlation between arachidonic acid status and growth was found in preterm infants and in animal models [34]. It has been reported that poor arachidonic acid status might also contribute to the growth disturbances observed in children with cholestasis [35]. Furthermore, EFA supplementation in these patients needs extensive investigation as regards route, dosage and safety. The use of antioxidants and the use of bile salts should be justified for those subjects.

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Note from the Editor

We would like to inform our readers that the next special issue of EMHJ will be on Tropical and Other Communicable Diseases Research and will be issue No. 4 of Volume 9. The issue will include papers originating from the joint EMRO/DCD/TDR Small Grants Scheme for Operational Research in Tropical and Communicable Diseases for the period 2000–2001.