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Correction of maternal serum bile acid profile during ursodeoxycholic acid therapy in cholestasis of pregnancy

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Background/Aims: **Intrahepatic cholestasis of pregnancy is characterized by pruritus and increased levels of serum bile acids, and is often associated with premature delivery, fetal distress, and perinatal mortality. The aims of the present study were: (i) to better define the serum bile acid profile in intrahepatic cholestasis of pregnancy and its potential usefulness for differential diagnosis; (ii) to investigate the effect of ursodeoxycholic acid treatment on the bile acid pool; and (iii) to investigate possible adverse effects of therapy.**

Methods: **Fifteen patients with intrahepatic cholestasis of pregnancy were enrolled in this study. Ursodeoxycholic acid (14 mg/kg body weight per day) was administered for 13±5 days. Twenty normal pregnant women served as controls. Serum bile acid profile was analyzed by high-performance liquid chromatography.**

Results: **Patients with cholestasis of pregnancy showed significant alterations in the proportion of primary bile acids, with an increase in cholic acid (64.0±3.0% vs. 32.2±1.8%, $p<0.01$), and a decrease in chenodeoxycholic acid (20.8±1.4% vs. 31.9±1.3%, $p<0.01$), as compared to controls, resulting in a marked elevation in the cholic/chenodeoxycholic acid ratio (3.4±0.5 vs. 1.1±0.1, $p<0.01$). The glycine/taurine ratio was reduced in cholestasis of pregnancy (0.8±0.1 vs. 1.4±0.1, $p<0.01$). During ursodeoxycholic acid administration its proportion in serum increased from 1.4±0.6% (0.6±0.2 μmol/l) at baseline to 24.7±2.3% (5.9±1.9 μmol/l) with therapy ($p<0.01$). This increment was accompanied by a significant decrease in the percentage of cholic acid (28.2±2.6%, $p<0.01$) and an elevation in chenodeoxycholic acid proportion (25.0±1.9%, N.S.). Although lithocholic acid concentration in serum was maintained with treatment (1.2±0.2 μmol/l vs. 1.7±0.5 μmol/l), there was a significant increase in lithocholic acid proportion ($p<0.01$) from 3.3±0.5% at baseline to 7.4±1.3% during therapy. The glycine/taurine ratio of serum bile acid pool returned to normal after ursodeoxycholic acid administration (1.7±0.3).**

Conclusions: **These results establish the importance of ursodeoxycholic acid treatment for the correction of maternal serum bile acid profile in cholestasis of pregnancy, indicating that ursodeoxycholic acid may improve fetal prognosis.**

Key words: **Cholestasis of pregnancy; Cholic acid; Serum bile acid profile; Ursodeoxycholic acid therapy.**

INTRAHEPATIC cholestasis of pregnancy (ICP) is a condition of unknown etiology characterized by generalized pruritus, mild jaundice, and abnormal liver function tests that occur predominantly during the third trimester of pregnancy (1,2). Shortly after delivery, biochemical parameters of liver cell function return to normal, serum bile acid levels decrease, and pruritus disappears (3). Although essentially benign, ICP has been considered responsible for urinary tract infection, postpartum hemorrhage, and fat and vitamin K malabsorption (3–5). However, ICP, commonly described in Scandinavia and Chile (3,6), is also associated with increased fetal distress, premature deliveries and perinatal mortality and morbidity (4,7,8). Several treatments failed to improve pruritus, biochemical abnormalities, or fetal prognosis during ICP; cholestyramine, phenobarbital, dexamethasone, S-adenosyl-L-methionine, and epomediol have been

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used without clear evidence of efficacy (9–16). More recently, promising results have been reported by using ursodeoxycholic acid (UDCA) in ICP treatment (16–22).

During recent decades, several clinical studies have established UDCA as a useful drug for the treatment of other cholestatic liver diseases; improvements in clinical and biochemical tests of liver function consistently occur following UDCA administration (23–29), and a trend toward histological improvement was shown in some cases (25,29,30). This highly hydrophilic bile acid reduced pruritus, aminotransferases, and serum levels of total bile acids when administered to patients with ICP (16–22). An important issue raised in some of these studies relates to the safety of UDCA in pregnancy. Despite reports of no adverse effects to the newborns, the number of patients is still too small to answer with certainty the question of whether there are any deleterious effects on the fetus from administration of UDCA. Also, to our knowledge, there have been no data on the influence of UDCA on the maternal serum bile acid profile, which may be directly implicated in the poor fetal prognosis.

We therefore conducted a study in 15 cases of ICP to: (i) better define the typical serum bile acid profile in ICP patients; (ii) establish the effect of UDCA treatment on the serum bile acid pool; and (iii) investigate the effectiveness and possible adverse effects of UDCA administration on both the mother and the fetus. The data obtained confirm the relevance of serum bile acid determination in the early recognition and follow-up of ICP, and establish the importance of UDCA treatment for the correction of maternal serum bile acid profile in this disease. Also, by reducing the circulating bile acid levels in the mother, and consequently its passage to the fetus, UDCA may improve fetal prognosis.

Materials and Methods

Patients

Fifteen patients with ICP were included in this study. Inclusion criteria were defined as: (a) severe pruritus; (b) increase in total serum bile acids and in the percentage of cholic acid, together with an elevation in at least one of the biochemical indices of cholestasis such as aminotransferases or bilirubin; and (c) absence of any other known disease that could cause liver malfunction.

Five of the 15 women had previously suffered from cholestasis of pregnancy, two had a positive family history of ICP, and two had liver disorders due to oral contraceptives. Seven patients had unexplained stillbirths in past pregnancies, and two presented a history of cholecystopathies. Fetal condition was closely monitored by cardiotocography and labor was induced as soon as fetal lung maturity was achieved, or earlier when fetal well-being was compromised. Meconium staining of the amniotic fluid was found in four patients. Other characteristics of the patient population are summarized in Table 1.

Study design

Following the report by Palma et al. (16), UDCA treatment (14 mg/kg body weight per day) was given to 15 patients, between 28 and 36 weeks of gestation, (32±2 weeks) during 13±5 days (1 to 21 days). Treatment was initiated only after biochemical confirmation of the diagnosis and was maintained until delivery, or until 21 days of therapy were completed. Two patients, with earlier onset of the disease, received two periods of treatment, separated by a 14-day interval free of drug (16).

Pruritus assessment and its changes during UDCA treatment were based on complaints from the patients. Liver enzymes and individual bile acids were determined in all patients at the time of admission to the study, which occurred between 13 and 34 weeks of gestation (30±5 weeks), and at the end of the treatment period (36±2 weeks of gestation). The same parameters were also evaluated at 20±12 days after delivery in 14 from the 15 patients with ICP. Fasting serum samples were stored at −20°C until analyzed. As a control group, 20 pregnant women with a gestation time between 20 and 39 weeks (32±6), in an age range comparable to the ICP cases (26 to 37 years, 30±5) were also studied.

Informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Analytical techniques

(i) Liver function tests. Routine laboratory methods were used to determine serum biochemical markers of cholestasis that included serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (GGT), alkaline phosphatase (AP), total bilirubin (TB) and conjugated bilirubin (CB), in patients with ICP.

(ii) Bile acid analysis. Fasting total serum bile acids were measured by an enzymatic-fluorimetric assay (31,32), after solid-phase extraction using Sep-Pak C_{18} cartridges (33). Individual amidated bile acids were determined by high-pressure liquid chromatography (34), and unconjugated bile acids were evaluated using the enzymatic-fluorimetric method, following thin-layer chromatography using silica gel plates (35–37). Unconjugated UDCA was not individually quantified due to

TABLE 1

Clinical characteristics of the patient population

Number of cases	15	
Race		
Caucasian	14	
Indian	1	
Age (years)	29±6	[21–41]
Multiparous	7	
Positive family history	2	
Recurrence	5	
Twin pregnancy	0	
Onset of pruritus (weeks)	27±6	[10–33]
Jaundice[a]		
Incidence (%)	47	
Appearance (weeks)	30±7	[12–33]
Delivery		
Gestational age (weeks)	36±2	[29–39]
Vaginal		
Spontaneous	5	
Induced	8	
Cesarean section	2	
Newborns		
Sex (male:female)	9:6	
Weight (g)	2668±547	[1120–3380]
Apgar score (1 min)	9±1	[7–10]

Values are expressed as mean±S.D. and respective range; [a] total bilirubin over 1 mg/dl.

difficult separation from chenodeoxycholic acid during thin-layer chromatography.

Statistical analysis

A two-tailed Student's *t*-test was used for the statistical analysis of data. Values are expressed as mean±S.E.M. or mean±S.D., as indicated. *p*-values lower than 0.05 were considered statistically significant.

Results

Clinical observations

No major side-effects were reported in the 15 patients studied during UDCA therapy. Compliance was assessed from the increased percentage of UDCA in serum (higher than 16%), and no drop-out was recorded during the treatment period. Itching disappeared or was markedly reduced during UDCA treatment, except in one case where no improvement was observed. Patients still presenting pruritus at labor (n=4) showed a complete remission 10 to 39 days after delivery. Fetal distress was not noticed and no cases of neonatal morbidity or mortality were seen. Newborns of ICP mothers treated with UDCA, with delivery occurring at 35.8±2.2 weeks of gestation, presented birth weight (2668±547 g) and Apgar scores at 1 min (9±1) comparable to those from non-treated ICP mothers (2809±505 g and 8±2, respectively) born at 36.6±1.7 weeks of gestation (data not shown). Other clinical observations are reported in Table 1.

Liver function tests

As reported in Table 2, patients with ICP showed significant increases in serum bilirubin and total bile acids ($p<0.01$), as well as in aminotransferases and GGT levels ($p<0.05$), when compared with controls with normal pregnancies. Some patients, however, showed normal TB and CB concentrations (47% and 7% of the patients, respectively) and normal GGT levels (67% of the patients). Although AP values are influenced by the superimposition of a placental isoenzyme in late pregnancy, a marked elevation was also observed in cholestatic women ($p<0.01$). At 20±12 days after delivery, aminotransferases, bilirubin and total bile acids levels were already close to normal values (Table 2).

UDCA administration significantly improved serum liver enzymes, bilirubin and total bile acid levels (Table 2). As a consequence, AST, ALT, GGT, and AP decreased by 52±10% ($p<0.01$), 51±6% ($p<0.05$), 36±7% ($p<0.01$), and 16±4% ($p<0.05$), respectively. Significant declines in CB (42±10%, $p<0.05$), TB (42±6%, $p<0.01$) and total bile acids (55±8%, $p<0.01$) were also observed.

Serum bile acid composition

Figure 1 shows that percent bile acid composition during ICP is characterized by a marked increase in cholic

TABLE 2

Serum liver enzymes, bilirubin, and total bile acid levels during the third trimester of pregnancy in normal pregnant women, and in patients with intrahepatic cholestasis of pregnancy, before and during ursodeoxycholic acid therapy (14 mg/kg body weight per day), and after delivery

		Intrahepatic cholestasis of pregnancy		
	Normal pregnancies (n=20)	Before UDCA (n=15)	During UDCA (n=15)	After delivery (n=14)
AST (IU/l)	26.1±2.3	151.1±54.6[a]	73.1±31.2	24.6±3.6
ALT (IU/l)	23.6±5.4	186.7±58.7[a]	91.5±41.3	28.5±2.3
GGT (IU/l)	9.8±1.2	18.0±2.4[b]	11.2±2.2	12.9±2.2
AP (IU/l)	124.5±17.1	246.6±18.5[b]	216.0±21.4[c]	190.0±19.6
TB (mg/dl)	0.4±0.1	1.5±0.2[a]	0.8±0.1[c]	0.6±0.1[d]
CB (mg/dl)	0.2±0.1	1.0±0.2[a]	0.4±0.1[c]	0.2±0.1
TBA (μmol/l)	6.8±0.4	68.4±16.1[a]	20.8±5.1	7.0±1.1

Results are expressed as mean±S.E.M.; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: γ-glutamyltranspeptidase; AP: alkaline phosphatase; TB: total bilirubin; CB: conjugated bilirubin; TBA: total bile acids; UDCA: ursodeoxycholic acid; [a] $p<0.05$, cholestasis before UDCA versus all the other groups; [b] $p<0.05$, cholestasis before UDCA versus normal pregnancies and cholestasis during UDCA; [c] $p<0.05$, cholestasis during UDCA versus normal pregnancies; [d] $p<0.05$, postpartum following cholestasis versus normal pregnancies.

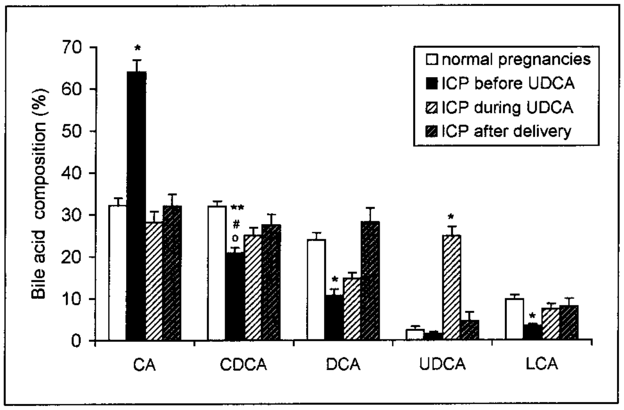

*Fig. 1. Percent bile acid composition (mean±S.E.M.) in serum from normal pregnant women (*n*=20) and from patients with intrahepatic cholestasis of pregnancy (ICP), before and during ursodeoxycholic acid administration (*n*= 15), and after delivery (*n*=14). Analyses were performed as described in Methods. CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; UDCA: ursodeoxycholic acid; LCA: litholic acid; **p*<0.01, ICP before UDCA versus all the others: ***p*<0.01, ICP before UDCA versus normal pregnancies; #*p*<0.05, ICP before UDCA versus ICP after delivery; °*p*<0.10, ICP before versus after UDCA.*

acid and a decrease in chenodeoxycholic, deoxycholic and lithocholic acids ($p<0.01$). In normal pregnant women, cholic acid accounted for 32.2±1.8% (2.2±0.2 μmol/l), whereas during cholestasis this primary bile acid was 64.0±3.0% (45.9±11.4 μmol/l) of the total serum bile acids ($p<0.01$). Accordingly, the cholic/chenodeoxycholic acid ratio was elevated in ICP patients (3.4±0.5 vs. 1.1±0.1, $p<0.01$), as indicated in Table 3. Taurocholic acid was quantitatively the most important conjugated bile acid, attaining 26.5±7.0 μmol/l (35.9±3.4%) versus 0.8±0.1 μmol/l (12.2±1.3%) in normals ($p<0.01$), followed closely by glycocholic acid that increased ($p<0.01$) from 1.0±0.1 μmol/l (14.1±1.0%) to 18.4±4.6 μmol/l (25.6±2.1%) in ICP patients (Fig. 2). As a consequence, the molar ratio glycine/taurine of 0.8±0.1 in patients with ICP was significantly diminished ($p<0.01$) as compared with 1.4±0.1 in normal pregnant women (Table 3).

Bile acids in serum from individual patients, before and during UDCA administration, and pruritus improvement with therapy are depicted in Table 4. Patients with ICP presented a great variability in the concentration of total bile acids, with values ranging from 14.7 to 198.0 μmol/l. A three-fold change was observed during UDCA administration, where total bile acids in serum decreased from 68.2±16.1 μmol/l at baseline to 20.8±5.1 μmol/l with treatment ($p<0.01$). Cases presenting a greater reduction in bile acid levels were simultaneously associated with a marked improvement of pruritus. Patient number 6, who exhibited the highest concentration of total bile acids both before and after UDCA treatment, was, interestingly, the case with the earliest onset (10 weeks of gestation) and the latest remission (39 days after delivery) of the itching symptom.

Individually, the most significant alteration was the reduction ($p<0.01$) in serum cholic acid concentration from 45.9±11.4 μmol/l at baseline to 6.4±1.8 μmol/l with UDCA administration (Table 4), which represents a more than two-fold decrease (64.0±3.0% to 28.2±2.6%, $p<0.01$) in the proportion of this primary bile acid (Fig. 1). Conversely, the percentage of chenodeoxycholic acid was maintained, but there was a significant decrease ($p<0.01$) in its concentration (Table 4). Although the reduction in concentrations of taurocholic and taurochenodeoxycholic acids was marked, there were also reductions in concentrations of glycine conjugated forms (Fig. 2). This change in the composition of the bile acid pool was accompanied by a significant increase in the concentration and proportion

TABLE 3

Bile acid ratios in serum from normal pregnant women and from patients with intrahepatic cholestasis of pregnancy, before and during ursodeoxycholic acid therapy (14 mg/kg body weight per day), and after delivery

		Intrahepatic cholestasis of pregnancy		
	Normal pregnancies (n=20)	Before UDCA (n=15)	During UDCA (n=15)	After delivery (n=14)
Glycine/taurine	1.4±0.1	0.8±0.1[a]	1.7±0.3	1.6±0.2
Primary/secondary	1.9±0.1	7.3±1.0[a]	1.3±0.1[b]	1.8±0.4
Trihydroxylated/dihydroxylated	0.6±0.1	2.3±0.3[a]	0.5±0.1	0.6±0.1
Cholic/chenodeoxycholic	1.1±0.1	3.4±0.5[a]	1.2±0.2	1.4±0.2

Results are expressed as mean±S.E.M.; UDCA: ursodeoxycholic acid; [a]$p<0.01$, cholestasis before therapy versus all the other groups; [b]$p<0.01$, normal pregnancies versus cholestasis during therapy.

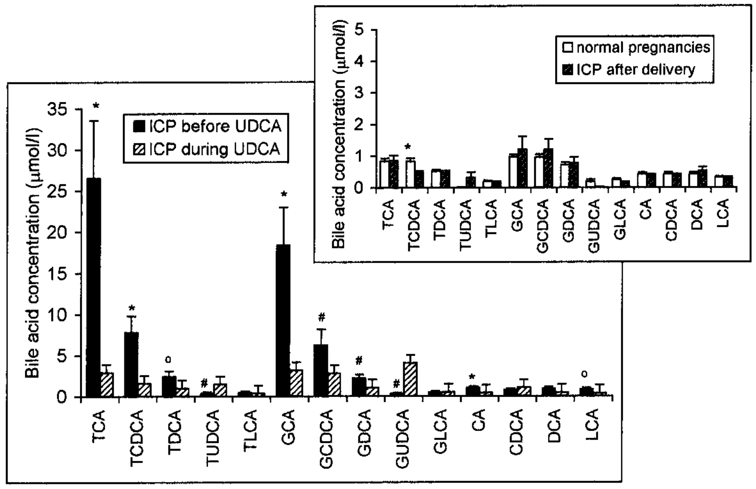

*Fig. 2. Bile acid concentrations (mean± S.E.M.) in serum from normal pregnant women (*n*=20) and from patients with intrahepatic cholestasis of pregnancy (ICP), before and during ursodeoxycholic acid administration (*n*= 15), and after delivery (*n*=14). Analyses were performed as described in Methods. TCA: taurocholic acid; TCDCA: taurochenodeoxycholic acid; TDCA: taurodeoxycholic acid; TUDCA: tauroursodeoxycholic acid; TLCA: taurolithocholic acid; GCA: glycocholic acid; GCDCA: glycochenodeoxycholic acid; GDCA: glycodeoxycholic acid; GUDCA: glycoursodeoxycholic acid; GLCA: glycolithocholic acid; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; **$p<0.01$*; #*$p<0.05$*; °*$p<0.10$*.

of UDCA from 0.6±0.2 μmol/l and 1.4±0.6% to 5.9±1.9 μmol/l and 24.7±2.3%, respectively, at baseline and during treatment (Table 4 and Fig. 1). Within the UDCA fraction, glycoursodeoxycholic acid was quantitatively the most important conjugated species (Fig. 2). Only a small proportion of total bile acids was detected in the unconjugated fraction, even during UDCA administration, despite its increase from 7.2±1.3% at baseline to 12.8±1.6% during treatment.

The percentage of secondary bile acids, such as deoxycholic and lithocholic acids, increased during UDCA administration ($p<0.01$). Nevertheless, lithocholic acid concentration in serum was not significantly altered as a consequence of treatment (1.2±0.2 μmol/l during therapy versus 1.7±0.5 μmol/l at baseline, Table 4).

The glycine/taurine ratio of the serum bile acid pool increased from 0.8±0.1 at baseline to 1.7±0.3 during UDCA therapy (Table 3), reaching a value similar to that found in normal pregnancies (1.4±0.1). Other bile acid ratios and significant changes are shown in Table 3.

TABLE 4

Individual bile acid concentrations (μmol/l) in serum from 15 patients with intrahepatic cholestasis of pregnancy before and during ursodeoxycholic acid administration (14 mg/kg body weight per day), and pruritus improvement with therapy

Patients	Therapy (days)	Pruritus improvement	TBA		CA		CDCA		DCA		UDCA		LCA	
			Before	During	Before	During	Before	During	Before	During	Before	During	Before	During
1	10	+++	27.9	7.3	19.2	2.2	4.7	1.7	1.8	1.5	1.0	1.9	1.2	0.1
2	14	+	28.2	16.9	13.5	5.0	7.5	1.9	5.5	2.7	nd	5.0	1.7	2.3
3	17	+++	151.7	16.7	86.0	6.5	41.5	3.3	15.3	1.4	2.9	3.2	6.0	2.4
4	9	++	14.7	9.0	7.9	1.4	3.0	0.9	2.1	2.1	0.8	3.9	0.9	0.8
5	20[b]	+++	108.7	22.2	71.7	3.1	27.5	7.0	9.5	2.7	nd	7.3	nd	2.1
6	14[b]	+	198.0	80.6	148.3	25.3	38.0	20.2	4.8	4.4	2.2	29.4	4.7	1.3
7	12	+++	12.3	9.1	5.2	2.2	2.6	2.0	3.0	1.6	0.8	2.1	0.7	1.3
8	15	+++	12.4	6.9	6.8	1.6	3.0	1.5	2.0	1.6	nd	1.6	0.6	0.6
9	6	+/−	25.0	15.7	16.4	6.3	5.0	4.9	3.0	2.6	nd	1.3	0.6	0.6
10	9	++	95.5	18.8	54.9	4.9	27.0	5.8	13.3	2.8	nd	5.2	0.3	0.1
11	21	+++	88.4	28.6	68.2	12.7	12.4	5.7	5.6	4.0	nd	5.3	2.2	1.0
12[a]	14	++	34.2	42.7	27.6	16.2	3.3	8.2	2.3	4.8	nd	12.3	0.9	1.2
13	1	+++	19.0	11.9	13.5	3.8	3.5	3.3	1.2	1.3	nd	2.7	0.8	0.8
14	13	+++	149.0	17.2	105.4	3.6	30.1	4.1	9.3	2.3	0.4	5.1	3.8	2.2
15	18	+++	60.6	7.6	43.5	1.1	11.9	1.8	3.1	1.0	1.2	3.1	0.9	0.6
mean	13		68.4	20.8	45.9	6.4	14.7	4.8	5.4	2.4	0.6	5.9	1.7	1.2
S.E.M.	5		16.1	5.1	11.4	1.8	3.7	1.3	1.2	0.3	0.2	1.9	0.5	0.2
p			0.01		0.01		0.01		0.05		0.05		N:S.	

[a] Insulin-dependent, treated with dexamethasone; [b] Second treatment period; +++: marked; ++: moderate; +: poor; +/−: no change; TBA: total bile acids; CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; UDCA: ursodeoxycholic acid; LCA: lithocholic acid; nd: not detected; N.S.: not significant.

Serum bile acid profile in the postpartum period, determined 20±12 days after delivery in 14 of the 15 patients with ICP, was similar to that observed in normals (Fig. 1 and 2), despite the lower levels of taurochenodeoxycholic acid, which probably resulted from UDCA therapy at the end of the gestation period. Additionally, it should be noted that bile acid ratios were not significantly changed after delivery, since their normalization was previously achieved during UDCA administration (Table 3).

Discussion

ICP is a common condition in Scandinavia and in Chile, where prevalence rates of 2% and 14%, respectively, have been described (38). Although in most countries in Europe diagnosis of ICP is made in only one or two patients among every 10 000 deliveries, our experience in Portugal points to a higher incidence, which may reach 1%.

The onset of pruritus is, in general, reported to occur after 28–30 weeks of gestation (3,38). Accordingly, in approximately 87% of the patients included in this study, pruritus started during the last trimester of pregnancy; exceptionally, in one case the onset of pruritus occurred at 10 weeks of gestation, and was probably related to the previous history of ICP. In agreement with the literature (2,3,39), jaundice was clinically noticed in 33% of the cases, 2 weeks after the appearance of pruritus, and biochemically confirmed in 47% of the patients studied, based on a total bilirubin concentration greater than 1 mg/dl. Although serum aminotransferases were also elevated in most patients, from 2 to 10 times the upper normal limits (Table 2), even higher values were reached in one case (AST, 800 UI/l; ALT, 850 UI/l). A similar observation had previously been made by other authors (40,41).

Serum bile acid levels, which may be increased 10 to 100 times over the normal limits (1,42,43), are a more specific sign of cholestasis during pregnancy, and usually precede the onset of pruritus or other laboratory alterations (3,44). Several authors described a poor correlation between routine liver function tests and serum bile acids (42,45), indicating that total bile acid levels give additional information on hepatic dysfunction during ICP. Conflicting data exist on individual bile acid concentrations during ICP and these discrepancies are probably the consequence of differences in sampling and methodology. In normal pregnant women included in this study, serum total bile acid concentrations ranged from 2.6 to 9.9 μmol/l, cholic and chenodeoxycholic acids accounting for 32.2±1.8% and 31.9±1.3% of the total, respectively (Fig. 1). Cholestatic patients showed total bile acids between 12.3 and 198.0 μmol/l, which represents an elevation up to 20-fold when compared with normal pregnancies. Other significant alterations are related to primary bile acid proportions, with increased cholic acid (64.0±3.0%), and decreased chenodeoxycholic acid (20.8±1.4%), when compared with normals ($p<0.01$). The increment in cholic acid concentration during ICP is four times greater than that of chenodeoxycholic acid, indicating that chenodeoxycholic acid is less relevant than cholic acid to the occurrence and prognosis of ICP. Therefore, instead of using primary bile acids for the differential diagnosis of ICP (1,46,47), cholic acid should be considered as a more reliable indicator of the disease. In line with this observation, the ratio of cholic/chenodeoxycholic acid in serum rose from 1.1±0.1 in normal pregnancy to 3.4±0.5 in ICP ($p<0.01$). Our results also confirm earlier findings (48,49) showing cholic acid to be a sensitive indicator of ICP. Interestingly, elevated cholic acid levels in maternal serum have been found to correlate with stronger uterine muscle contractions (50) and placental chorionic vein vasoconstriction (51), which may cause fetal distress. Secondary bile acid percentages were consequently decreased in ICP patients, which is indicative of impaired enterohepatic circulation. Preferential conjugation of bile acids with taurine, expressed by a molar ratio glycine/taurine of 0.8±0.1, seems to be another valuable parameter in characterization of ICP.

Although there is a lack of clear understanding of its mechanism of action, oral UDCA has been increasingly used for the treatment of a large variety of cholestatic liver diseases, and previous reports have demonstrated the usefulness of this drug in ICP (16–22). The present study confirms the rapid relief of pruritus (Table 4) and a significant improvement in laboratory abnormalities (Table 2) during UDCA therapy in all but one patient, in whom treatment benefits were recognized only at a later stage. The beneficial effect of UDCA therapy may be associated with its ability to enrich the bile acid pool, and presumably to increase hydrophilicity (52). In serum, the concentration of total bile acids decreased significantly ($p<0.01$) from 68.4±16.1 μmol/l at baseline to 20.8±5.1 μmol/l during therapy (Table 4), while cholic acid was the bile acid most significantly reduced (45.9±11.4 μmol/l at baseline: 6.4±1.8 μmol/l during therapy, $p<0.01$). The decrease in cholic acid may be clinically relevant since it has been reported to cause fetal distress (50,51), as previously discussed. An improvement in the cholestatic condition during UDCA administration is also reflected in alterations on cholic/chenodeoxycholic acid and glycine/taurine molar ratios (Table 3).

Since UDCA may be converted to lithocholic acid, known by its embryotoxicity in the rat (53), some concern has been expressed regarding UDCA treatment during pregnancy. Our data show that the serum lithocholic acid concentration is maintained during UDCA administration (1.7±0.5 μmol/l at baseline, and 1.2±0.2 μmol/l during therapy), despite an increase in the lithocholic acid proportion (7.4±1.3% vs. 3.3±0.5%, $p<0.01$).

In agreement with reports by others describing the disappearance of biochemical markers of cholestasis within 1 month after delivery, the bile acid profile obtained postpartum showed a trend toward normalization, with a significant decrease in the concentration of the major bile acids. Treatment with UDCA until labor seems, therefore, not to interfere with the normal resolution of the disease after delivery. However, a slight increase in tauroursodeoxycholic acid was observed, counterbalancing the decrease in taurochenodeoxycholic acid, probably still as the result of previous UDCA therapy (Fig. 2).

UDCA therapy was revealed not to cause any detectable deleterious effects during gestation to mother or fetus, and newborns presented no alterations in either birth weight or in Apgar scores at 1 min, when compared with those from normal pregnancies with the same gestation ages. Moreover, no cases of neonatal morbidity or mortality were seen.

In conclusion, these results confirm the relevance of serum bile acid determination in the early recognition and follow-up of ICP, and establish the importance of UDCA treatment for correction of the maternal serum bile acid profile in cholestasis of pregnancy, suggesting an important role for this bile acid in improving fetal prognosis.

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