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ORIGINAL ARTICLE

Fetal coenzyme Q10 deficiency in intrahepatic cholestasis of pregnancy

Manuela Romina Martinefski[a], Silvina Ema Cocucci[b], María Beatriz Di Carlo[b], Hilda Ruda Vega[c], Silvia Edith Lucangioli[a,d], Beatriz Elizabeth Perazzi[b], Valeria Paula Tripodi[a,d,*]

[a] *Departamento de Tecnología Farmacéutica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 5th Floor, CABA, Buenos Aires, Argentina*
[b] *Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Hospital de Clínicas José de San Martin, Buenos Aires, Argentina*
[c] *Universidad de Buenos Aires, Facultad de Medicina, División de Obstetricia, Hospital de Clínicas José de San Martin, Buenos Aires, Argentina*
[d] *Consejo Nacional de Investigaciones Científicas y Tecnológicas, CONICET, Argentina*

KEYWORDS
Bile acids;
Cord blood;
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Summary
Aim: Intrahepatic cholestasis of pregnancy (ICP) is considered a high-risk condition because it may have serious consequences for the fetus health. ICP is characterized by the accumulation of bile acids in maternal serum which contribute to an imbalance between the production of reactive oxygen species and the antioxidant defenses increasing the oxidative stress experienced by the fetus. Previously, it was reported a significant decrease in plasma coenzyme Q10 (CoQ10) in women with ICP. CoQ10 is a redox substance integrated in the mitochondrial respiratory chain and is recognized as a potent antioxidant playing an intrinsic role against oxidative damage. The objective of the present study was to investigate the levels of CoQ10 in umbilical cord blood during normal pregnancy and in those complicated with ICP, all of them compared to the maternal ones.
Methods: CoQ10 levels and bile acid levels in maternal and umbilical cord blood levels during normal pregnancies ($n = 23$) and in those complicated with ICP ($n = 13$), were investigated.

Abbreviations: ICP, intrahepatic cholestasis of pregnancy; BA, bile acids; ROS, reactive oxygen species; CoQ10, coenzyme Q10; TSBA, total serum bile acids; ALT, alanine-aminotransferase; AST, aspartate-aminotransferase; γ-GT, γ- glutamyltranspeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; Chol, cholesterol; $CoQ10_{Chol}$, CoQ10 levels corrected by cholesterol; UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; CA, cholic acid; LCA, lithocholic acid.

* Corresponding author at: Departamento de Tecnología Farmacéutica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 5th Floor, CABA, 1113 Buenos Aires, Argentina.
E-mail address: vtripodi@ffyb.uba.ar (V.P. Tripodi).

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Results: A significant decrease in neonate CoQ10 levels corrected by cholesterol (0.105 ± 0.010 vs. 0.069 ± 0.011, $P < 0.05$, normal pregnancy vs. ICP, respectively), together with an increase of total serum bile acids (2.10 ± 0.02 vs. 7.60 ± 2.30, $P < 0.05$, normal pregnancy vs. ICP, respectively) was observed.

Conclusions: A fetus from an ICP mother is exposed to a greater risk derived from oxidative damage. The recognition of CoQ10 deficiency is important since it could be the starting point for a new and safe intervention strategy which can establish CoQ10 as a promising candidate to prevent the risk of oxidative stress.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a reversible form of cholestasis that develops in the second or third trimester of pregnancy and disappears spontaneously after birth. ICP is considered a high-risk condition because, despite being usually benign to the mother, it may have serious consequences for the fetus, such as premature deliveries, fetal distress, low weight, intrauterine growth retardation and perinatal mortality [1]. Thus, an early and accurate diagnosis is crucial to find an appropriate medical treatment to improve fetal outcome. This pathology is characterized by the accumulation of bile acids (BA) in maternal serum, which are likely responsible for the development of the disease because of their cytotoxic effects [2]. Increased hydrophobic BA induces oxidative stress and apoptosis leading to hepatic parenchyma injury and, eventually, extrahepatic tissues damage. If this event takes place during gestation, the fetal health may be at risk [3]. Under normal pregnancy conditions, a delicate balance exists between the production of reactive oxygen species (ROS) and the antioxidant defenses, whereas pathological conditions may alter the fetal oxidative burden and antioxidant responses [4].

Previously, it was reported a significant decrease in plasma coenzyme Q10 (CoQ10) in women with ICP [5]. CoQ10 is a redox-active, lipophilic substance integrated in the mitochondrial respiratory chain which acts as an electron carrier to produce cellular energy [6]. In addition, it is recognized as a primary regenerating antioxidant playing an intrinsic role against oxidative damage [7]. It has been hypothesized that CoQ10 interacts earlier than vitamin E in the antioxidant system and its depletion would be an earlier marker of oxidative damage. Moreover, CoQ10 effectively supports the antioxidant activity of vitamin E (considered the most important antioxidant so far) by reducing its oxidized form [8]. Although the importance of CoQ10 in mitochondrial function is well established, the significance of CoQ10 deficiency has recently achieved clinical relevance. In this sense, CoQ10 determination has acquired significance as a biomarker, particularly for metabolic and oxidative stress abnormalities [6].

Considering the increase of hydrophobic BA and the decrease of CoQ10 evidenced in women with ICP and, therefore, the prooxidant environment in which the fetus is exposed to, the objective of the present study was to investigate the levels of CoQ10 in umbilical cord blood during normal pregnancy and in those complicated with ICP, all of them compared to the maternal ones.

Materials and methods

Participants

Women were consecutive, prospective and transversally studied.

Twenty-three healthy pregnant women and 13 ICP patients (both groups in the third trimester of pregnancy) were included in the study. All patients underwent cesarean process with spinal anesthesia and none of the women received oxytocin treatment. ICP diagnosis was based on the presence of pruritus with elevation of total serum bile acids (TSBA) higher than 11 μmol/L and/or at least one of the two aminotransferases [alanine-aminotransferase (ALT) or aspartate-aminotransferase (AST)] higher than the upper normal limit (40 and 31 UI/L, respectively), and/or elevation of γ- glutamyltranspeptidase (γ-GT) above 36 UI/L during the second or third trimester of a pregnancy, where no other condition has been observed, and the absence of infection by hepatitis A, B and C viruses, autoimmune diseases-, alcohol intake, smoking or drug addiction, HIV, skin diseases or biliary obstruction. The patients were not treated with UDCA or other treatment at the time of the study and none of the patients received prenatal supplements containing CoQ10.

Maternal blood and newborn cord blood samples were obtained simultaneously at birth. Samples were centrifuged at 5000 rpm at 4 °C for 10 min and the serum fraction was transferred into another vial and stored at −80 °C until assayed.

Serum Coenzyme Q10

CoQ10 in serum was quantified by an optimized micro-HPLC-UV method as previously detailed [9].

Considering that information about lipid-related concentration of CoQ10 helps to avoid misunderstandings and abolishes its blood content variations [10], CoQ10 levels were normalized by cholesterol levels ($CoQ10_{Chol}$) by straight division.

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Serum Bile Acids

Serum samples (200 μL) were de-proteinized by addition of cold acetonitrile (600 μL), and it was loaded onto a reverse-phase 500 mg SPE cartridge (Strata® C18-E cartridges, Phenomenex, USA) for cleanup. The methanol fraction was collected, evaporated and the residue was dissolved in 50 μL methanol: ammonium acetate 10 mM (80:20) of which 10 μL were injected into the high-performance liquid chromatography- tandem mass spectrometry system (HPLC-MS/MS) following the technique reported by Ye et al. [11].

The separation and quantitation of BA were accomplished by liquid chromatography (UltimateHPLC, Thermo Fisher Scientific, San Jose, CA, USA) coupled to a triple quadrupole (TSQ Quantum Access Max, Thermo Fisher Scientific, San Jose, CA, USA) with an electrospray ionization (ESI) source, operating in the negative ion mode. All data were acquired and processed using Xcalibur software, version 2.1 (Thermo Fisher®). The stationary phase was a BDS HYPERSIL C18 (Thermo Scientific®) (100 mm × 2.1 mm, 2.4 mm) with a C18 guard column Detection limits ranged from 0.001 to 0.012 μmol/L and linearity was confirmed between 0.03 and 60 μmol/L.

Biochemical markers

Biochemical markers were studied as numerical continuous variables. These were measured according to internationally recommended methods in a COBAS Auto-analyzer 6000 C-501 Module-Roche Diagnostics Germany. The following markers were studied: total bilirubin (colorimetric method), total proteins (Biuret-colorimetric method), albumin (colorimetric bromocresol green method), ALT (IFCC-kinetic method), AST (IFCC-kinetic method), alkaline phosphatase (ALP) (DGKC kinetic method), γ-GT (IFCC-kinetic method), lactate dehydrogenase (LDH) (DGKC-kinetic method) and cholesterol (Chol) (CHOD/PAP-enzymatic method).

Statistical analysis

Shapiro—Wilk W-test of normality was performed. Kruskal—Wallis non parametric analysis followed by Mann—Whitney U-test was used. Differences between groups were analyzed by Student's t-test or non parametrical tests, according to the distribution. G power statistic was performed to determine the power of the analysis. Levels of significance were established at $P < 0.05$.

Ethical approval

This study was conducted in women with normal pregnancy and with ICP under gestational control at ''Hospital de Clínicas'' José de San Martin of the Universidad de Buenos Aires, Argentina. This study complied with the Declaration of Helsinki and was approved by the Institutional Review Board and the Bioethical Committee of our Institutions (exp-UBA 48891/16, approved 11/8/16 by Comité de Ética en Investigación Clínica and Comité de Ética del Hospital de Clínicas 7/21/10). Written consent from all participants was obtained.

Results

Thirteen out of twenty-one women diagnosed with ICP fulfilled the inclusion criteria and were included in the study.

Mothers' ages in both groups were comparable at the time of the study. The clinical, obstetrics, perinatal and biochemical markers of patients studied are shown in Table 1. A significant decrease in ICP maternal gestational weeks ($P < 0.01$) and in their newborns weight was observed in comparison to controls ($P < 0.01$).

TSBA and their profiles together with the CoQ10 levels in mothers and their newborns are shown in Table 2. TSBA were significantly higher in mothers with ICP respect to control group ($P < 0.01$), of which two of them had TSBA level higher than 40 μM. Analysis of BA profile shows a significant increase in chenodeoxycholic acid (CDCA) ($P < 0.05$), cholic acid (CA) ($P < 0.01$) and litocholic acid (LCA) ($P < 0.01$) with a three-fold increase in the ratio of primary BA (CA:CDCA) ($P < 0.05$) in ICP mothers than controls (Table 2).

In newborns of mother with ICP, TSBA were higher than controls ($P < 0.05$) showing a significant increase in CA ($P < 0.01$) and LCA ($P < 0.05$) with a six-fold increase in the ratio of primary BA (CA:CDCA) ($P < 0.05$) (Table 2).

The relative contribution of individual BA to TSBA pool, calculated as % BA/TSBA, showed a significant increase in CA in ICP with respect to control pregnancy (57.9 ± 8.3% vs 21.1 ± 0.6%, $P < 0.01$ for mothers and 58.5 ± 10.1% vs. 15.7 ± 1.6%, $P < 0.05$ for newborns). On the other hand, $CoQ10_{Chol}$ levels are reduced in ICP in both, mother (−64%, $P < 0.001$) and neonate (−30%, $P < 0.05$), respect to controls (Table 2). The $CoQ10_{Chol}$/TSBA ratio decreased significantly in mothers with ICP and in their newborns respect to control group ($P < 0.01$, ICP vs controls, in both cases) (Fig. 1).

Discussion

In this study, a decrease in gestational weeks and weight in newborns from ICP mothers was observed, with respect to the control group and this might be associated with the intrauterine low restriction related to the disorder, as it was previously reported [12]. In agreement with Gruccio et al. [12] we have observed an increase in ALP in umbilical cord blood in newborns from ICP mothers, that might be related to cellular damage and inflammatory response associated with the maternal condition in which this enzyme levels are also increased. The decrease in total proteins and albumin observed in the newborns from ICP mothers may be due to the intrauterine low restriction and, particularly, the decrease in ALT suggests a newborn hepatic immaturity reflected on enzyme production [13].

The process of birth is accompanied by an increase in oxidative aggression. The oxidative stress suffered by the neonate is balanced by the maturation of effective antioxidant mechanisms such as the enzymatic systems (superoxide dismutase, catalase, glutathione peroxidase, etc.) [13].

To the end of gestation, there is an increase in oxygen tension causing ROS production and therefore distinct enzymatic antioxidant defenses are modified to reduce

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Table 1 Maternal age and gestational weeks, newborn weight and biochemical parameters in maternal venous blood and umbilical cord blood in control and ICP.

	Mother			Newborn		
	CON	ICP	*P*	CON	ICP	*P*
n	23	13		23	13	
Mother Age (years)	24.7 ± 1.0	29.4 ± 1.5	0.786			
Gestation (weeks)	38.6 ± 0.2	36.8 ± 0.7	< 0.01			
Chol (mg/dL)	233 ± 15	249 ± 13	0.242	60 ± 3	70 ± 6	0.574
Total protein (g/dL)	5.5 ± 0.1	5.7 ± 0.1	0.875	5.5 ± 0.1	5.0 ± 0.1	< 0.01
Albumin (g/dL)	3.0 ± 0.1	3.0 ± 0.1	0.203	4.1 ± 0.1	3.4 ± 0.1	< 0.01
ALT (UI/L)	8 ± 1	84 ± 25	< 0.001	10 ± 1	7 ± 1	< 0.01
AST (UI/L)	23 ± 2	59 ± 14	< 0.01	30 ± 3	30 ± 3	0.868
ALP (UI/L)	203 ± 6	327 ± 47	< 0.05	204 ± 9	289 ± 36	< 0.01
GGT (UI/L)	11 ± 2	32 ± 8	< 0.001	99 ± 13	100 ± 16	0.551
LDH (UI/L)	439 ± 34	470 ± 46	0.551	771 ± 90	706 ± 50	0.637
Total bilirrubin (mg/dL)	0.31 ± 0.03	0.50 ± 0.11	0.305	1.43 ± 0.07	1.37 ± 0.07	0.748
Birthweight (g)				3245 ± 78	2772 ± 157	< 0.01
Apgar score < 8 at 1 min (*n*)				0	3	
Apgar score < 8 at 5 min (*n*)				0	1	

Results are expressed as means ± S.E.M; CON: control, ICP: intrahepatic cholestasis of pregnancy; Chol: cholesterol, ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transpeptidase; LDH: lactate dehydrogenase.

Table 2 Comparison of total serum bile acid levels, serum bile acid profiles and CoQ10 in mothers and their newborns.

	Mother			Newborn		
	CON	ICP	*P*	CON	ICP	*P*
TSBA (μM)	0.734 ± 0.005	20.1 ± 10.5	< 0.01	2.10 ± 0.02	7.60 ± 2.30	< 0.05
UDCA (μM)	0.054 ± 0.005	0.089 ± 0.048	0.523	0.189 ± 0.111	0.846 ± 0.444	0.427
CDCA(μM)	0.254 ± 0.051	4.57 ± 2.65	< 0.05	1.25 ± 0.05	2.12 ± 0.46	0.184
DCA (μM)	0.262 ± 0.049	1.005 ± 0.505	0.414	0.323 ± 0.013	0.240 ± 0.039	0.993
CA (μM)	0.155 ± 0.005	14.30 ± 8.85	< 0.01	0.330 ± 0.031	3.66 ± 1.65	< 0.01
LCA (μM)	0.009 ± 0.002	0.039 ± 0.010	< 0.01	0.009 ± 0.001	0.030 ± 0.006	< 0.05
CA/CDCA	0.628 ± 0.062	1.82 ± 0.30	< 0.05	0.262 ± 0.008	1.356 ± 0.297	< 0.05
$CoQ10_{Chol}$	0.116 ± 0.009	0.033 ± 0.005	< 0.001	0.105 ± 0.010	0.069 ± 0.011	< 0.05

Results are expressed as means ± S.E.M; Bile acids are expressed in their free, glycine and taurine forms; CON: control; ICP: intrahepatic cholestasis of pregnancy; TSBA: total serum bile acids; UDCA: ursodeoxycholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; CA: cholic acid; LCA: lithocholic acid; $CoQ10_{Chol}$: CoQ10 levels corrected by cholesterol expressed as μmol CoQ/mmol cholesterol.

the resultant radicals. Compagnoni et al. [14] suggested that the risk of oxidative stress related to the exposure of neonate to the extrauterine environment, provides the mother with an adequate storage of antioxidant systems as CoQ10.

Frank et al. [15] determined an increase in antioxidant mechanisms at the pulmonary level during the end of gestation in coincidence with the maturation pattern of pulmonary surfactant.

However, when ROS generation exceeds the capacity of the antioxidant defenses, oxidative stress emerges and indiscriminate damage to proteins, lipids and DNA occurs leading to eventual cell death [16]. Since CoQ10 is considered the first line of defense in response to oxidative stress, decreased CoQ10 levels detected in mothers with ICP may pose a risk for the newborn.

Besides, pre-term neonates are at a higher risk for oxidative stress at birth and are very susceptible to oxidative damage by ROS because the extrauterine environment has more oxygen than the intrauterine environment [4]. This problem is aggravated by the low efficiency of natural antioxidant systems in the newborn that could be worsened even more if the antioxidant capacity of the mother is deficient [14].

A previous study in pre-eclampsia [17] showed that in response to an oxidative insult, fetal CoQ10 levels in cord blood are increased compared to normal pregnancies; the authors suggest this could be a compensatory mechanism to protect the newborn from excessive oxidative stress.

In the present study, the levels of CoQ10 in normal pregnant women were similar to those previously reported by other authors [14,18]. Also, as previously described,

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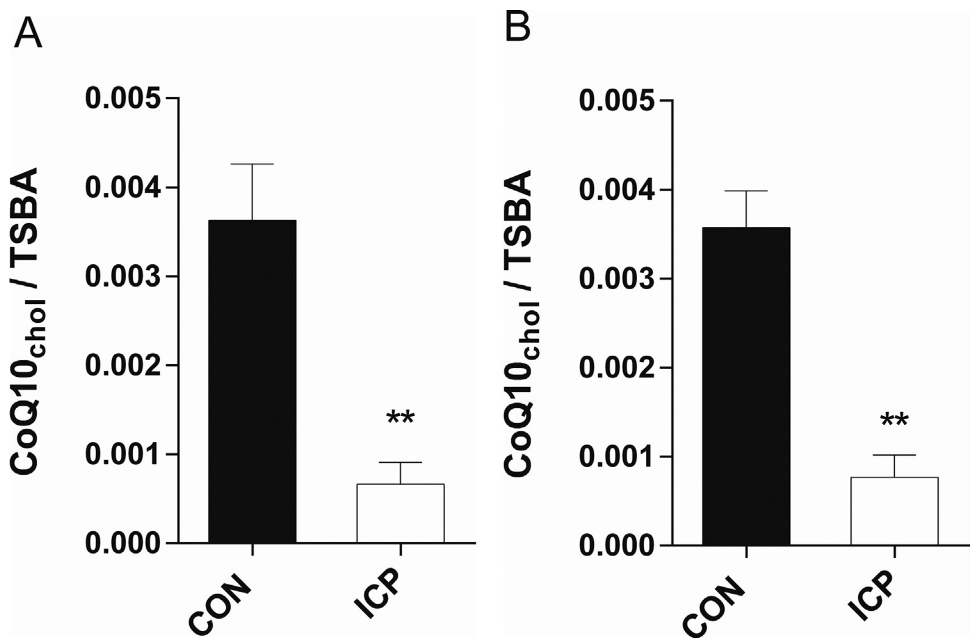

Figure 1 $CoQ10_{Chol}$/TSBA ratio in mothers (A) and newborns (B) from CON and ICP pregnancy, $n = 23$ and $n = 13$, respectively. Results are expressed as means ± S.E.M; $^{**}P < 0.01$, ICP vs CON. $CoQ10_{Chol}$: CoQ10 levels corrected by cholesterol (μmol CoQ10/mmol cholesterol); TSBA: Total serum bile acids (μM); CON: control; ICP: intrahepatic cholestasis of pregnancy.

we detected a decrease of CoQ10 levels together with an increase of TSBA in patients with ICP [5].

Taking into account the results obtained by Teran et al. [17], we have expected an increment of CoQ10 levels in cord blood in ICP as occurs in pre-eclampsia, as a compensatory mechanism to protect the newborn from excessive oxidative stress. Contrary, we have observed that levels of CoQ10 was maintained in ICP cord blood and even was decreased respect to controls when it was normalized to cholesterol. Also, an increase in TSBA in ICP cord blood was observed respect to normal pregnancies. The decreased $CoQ10_{chol}$/TSBA ratio reflects a highly pro-oxidant environment since it combines the increase of BA levels with the decrease of CoQ10 and this fraction is decreased both in the mother and the newborn with ICP respect to normal pregnancies.

As we have shown, the levels of cholesterol did not change during ICP in mother and newborns but TSBA in mothers is increased 30 times while CoQ10 is decreased 3 times. This could be explained taking into account the metabolic pathway. In ICP mothers the synthesis of BA produced from mother's cholesterol, was highly increased. To maintain the concentration of cholesterol it is possible that the mevalonate pathway was accelerated to supply the cholesterol synthesis and even absorbs part of the metabolic flow required to support the CoQ10 synthesis, another branch of the mevalonate pathway [19].

Another possible explanation relays in the decrease of HMG-CoA synthase and HMG-CoA reductase activities mediated by BA (especially CA) as a feedback down-regulation [20]. The increase of TSBA with high concentrations of CA observed in mothers and newborns, could decrease HMG-CoA activities and, hence, decrease mevalonate pathway preserving the cholesterol way but decreasing CoQ10 synthesis. The later could explain why the newborn cannot compensate the oxidative stress present in ICP by increasing CoQ10 levels as it was demonstrated in pre-eclampsia.

On the other hand, one of the proposed final common pathways leading to cholestatic liver injury is the intracellular accumulation of hydrophobic BA. This accumulation associated with cholestasis could increase fetal susceptibility to oxidative stress such as occurs during maternal alcohol ingestion, which could also have a negative impact on fetal development [21,22].

Moreover, hydrophobic BA like LCA, directly stimulate the generation of ROS in hepatocytes and liver mitochondria and its mechanism involves mitochondrial dysfunction with respiratory chain alteration during cholestasis [22,23]. Yerushalmi et al. [23] proposed that ROS are generated at the ubiquinone-complex III interaction of the respiratory chain in hepatic mitochondria upon exposure to BA.

Krähenbül et al. [24] also reported that hydrophobic BA, such as CDCA and LCA, impair the function of enzyme complexes of the mitochondrial electron transport chain in intact and disrupted mitochondria at low concentrations and confirmed the decrease in complex I and III activities. Mitochondrial toxicity induced by lipophilic BA could be relevant in the development of cholestasis liver failure. These events could possibly alter CoQ10 levels by generating large amounts of free radicals that will eventually lead to its consumption.

Additionally, Botla et al. [25] reported that hydrophobic BA initiate the membrane permeability transition in hepatic mitochondria.

Thus, growing evidence propose that accumulated BA impair electron transport in the respiratory chain, promoting electron leak at the ubiquinone-complex III site of interaction, enhancing the formation of superoxide and a

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more toxic oxygen radical species. Indeed, a CoQ10 analogue (idebenone), which could promote a more efficient electron transport in addition to its antioxidant properties, prevents BA stimulation of ROS from hepatic mitochondria and intact hepatocytes [22,23].

It is also important to highlight that many substances, and in particular those derived from nutrients, have an antioxidant effect. The transfer through the placenta of antioxidant substances such as ascorbic acid, alpha-tocopherol, beta-carotenes and CoQ10, is essential for improving defenses against oxidative aggression in normal pregnancies. In premature newborn associated with ICP condition, both the physiological oxidative stress and the diminished antioxidant defenses promote a fetal imbalance. The maternal—fetal nutrient transfer with an antioxidant effect, during the final stage of gestation, is not completed in the premature neonate, and some of the antioxidant enzymatic systems have not maturated [12]. These observations show an oxidant damage of mitochondrial hepatocyte with the consequent functional deterioration promoted by BA along with the immaturity of the fetal antioxidant enzyme systems.

Beyond the hypotheses previously discussed, the fact is that in terms of CoQ10, the newborn does not manage to generate an adaptive response to the oxidative stress observed in cholestasis.

Conclusion

A decrease of CoQ10 levels in ICP cord blood was found. Even though our study dealt with a low number of patients especially due to a difficulty of finding ICP mothers without treatment, our preliminary data seem to suggest that the recognition of CoQ10 deficiency is important in ICP since it could be the starting point for a new and safe intervention strategy based on CoQ10 maternal supplementation to prevent the risk of oxidative stress in ICP even as a general supplement for mothers a the starting point of gestation. More studies are needed to investigate this further.

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Author contributions

All authors contributed significantly to this work. MM and SL: Optimized the analytical methods and extracted the data; MM, SC and MB DiC: collected the samples and conducted experiments, HRV: Selected the patients and was the obstetrician responsible for the patients; BP: analyzed the data and wrote the manuscript and VT: Designed the research, analyzed the data and wrote the manuscript.

All authors read and approved the final manuscript.

Disclosure of interest

The authors declare that they have no competing interest.

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