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BIOMARKERS, MEDICAL TREATMENT AND FETAL INTRAPARTUM SURVEILLANCE IN INTRAHEPATIC CHOLESTASIS OF PREGNANCY

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TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1215 | Medica - Odontologica | Turku 2016

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Front cover Sirpa Erkinharju.

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-6356-0 (PRINT)
ISBN 978-951-29-6357-7 (PDF)
ISSN 0355-9483
Painosalama Oy - Turku, Finland 2016

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ABSTRACT

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Biomarkers, medical treatment and fetal intrapartum surveillance in intrahepatic cholestasis of pregnancy

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Annales Universitatis Turkuensis 2016

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific disorder characterized by maternal pruritus and elevated liver enzymes. It usually begins in the third trimester of pregnancy and resolves spontaneously after delivery. ICP is considered benign for the pregnant woman, but it is associated with an increased risk for unexplained term stillbirth and preterm delivery.

There are no specific laboratory markers to diagnose ICP. The diagnosis is currently based on the presence of maternal pruritus and elevated values of alanine aminotransaminases (ALT) and serum bile acids (BA). Recently, ursodeoxycholic acid (UDCA) has been used for treatment. Mechanisms leading to intrauterine fetal death (IUFD) may be multifactorial and are unknown at present.

For this thesis, 415 pregnant women with ICP were studied. The aim was to evaluate the value of the liver enzyme glutathione S-transferase alpha (GSTA) as a specific marker of ICP and to assess the effect of maternal UDCA therapy on maternal laboratory values and fetal outcome. The specific markers predisposing the fetus to heart arrhythmia were studied by comparing waveform analysis of fetal electrocardiograms (FECG) during labor in pregnancies complicated by ICP with controls.

The levels of maternal GSTA were high and the values correlated with the value of ALT in patients with ICP. UDCA therapy reduced the values of the liver enzymes and alleviated maternal pruritus, but it did not influence maternal hormonal values. Although the newborns experienced an uneventful perinatal outcome, severe ICP was still associated with preterm birth and admission to the neonatal intensive care unit (NICU). There were no significant differences in intrapartum FECG findings between fetuses born to ICP women and controls.

Keywords: intrahepatic cholestasis, pregnancy, glutathione S-transferase alpha, ursodeoxycholic acid, waveform analysis, fetal electrocardiogram

TIIVISTELMÄ

Titta Joutsiniemi

Raskaudenaikaisen maksan toimintahäiriön diagnosointi, hoito ja sikiön synnytyksenaikainen seuranta

Turun Yliopisto, Lääketieteellinen tiedekunta, Naistentaudit ja synnytysoppi, Kliininen kemia, Kliininen tohtoriohjelma, Turku, Suomi
Annales Universitatis Turkuensis 2016

Äidin raskaudenaikainen maksan toimintahäiriö eli hepatogestoosi aiheuttaa ihokutinaa ja maksa-arvojen nousua odotusaikana. Häiriö alkaa tavallisesti loppuraskauden aikana ja korjaantuu itsestään synnytyksen jälkeen. Hepatogestoosi ei ole vaarallinen äidille, mutta siihen liittyy kuitenkin suurentunut sikiökuoleman riski sekä usein ennenaikainen synnytys. Tämän vuoksi hepatogestoosiraskaudet ovat riskiraskauksia ja tarvitsevat polikliinistä seurantaa loppuraskauden aikana sekä synnytyksen suunnittelua.

Hepatogestoosin diagnosointi on perustunut äidin kutinaoireisiin ja kohonneisiin maksa-arvoihin. Mitään erityistä laboratoriokoetta ei ole ollut. Lääkityksenä on yleisesti käytetty ursodeoksikoolihappoa. Sikiön äkkikuoleman syy on edelleen epäselvä, mutta sydämen rytmihäiriö saattaa olla sen takana.

Tähän tutkimukseen kuului yhteensä 415 naista, joilla todettiin raskausaikana hepatogestoosi. Maksan erittämän entsyymin (plasman glutathione S-transferaasi alphan) osuvuutta selvitettiin hepatogestoosin diagnostiikassa. Ursodeoksikoolihapon vaikutuksia tutkittiin äitiin ja syntyvään lapseen. Lisäksi etsittiin sikiön sydänsähkökäyrästä tekijöitä, jotka saattavat altistaa rytmihäiriöille.

Tutkimamme maksaentsyymin todettiin nousevan yleisesti käytetyn maksaentsyymin (ALAT) nousun kanssa ja siten mahdollisesti tarkentavan diagnoosia. Ursodeoksikoolihappo parantaa maksa-arvoja ja vähentää äidin kutinaoireita. Käyttämämme ursodeoksikoolihapon annos oli pieni. Vastasyntyneet lapset olivat hyväkuntoisia. Tutkimuksessani ei löydetty merkittäviä sydänsähkökäyrän muutoksia synnytyksen aikana hepatogestoosiäitien sikiöiltä terveiden äitien sikiöihin verrattuna.

Avainsanat: raskaudenaikainen maksan toimintahäiriö, raskaus, glutathione S-transferaasi alpha, ursodeoksikoolihappo, sikiön sydänsähkökäyrä

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ABBREVIATIONS

ALT, ALAT	Alanine aminotransferase
AST	Aspartate transaminase
APTT	Activated partial thromboplastin time
BA	Bile acid
BMI	Body mass index
CTG	Cardiotocogram
FECG	Fetal electrocardiogram
FXR	Farnesoid X receptor
FIDD	Fibrinogen D-dimers
GA	Gestational age
GW	Gestational week
GSTA	Plasma glutathione S-transferase alpha
ICP	Intrahepatic cholestasis of pregnancy
IUFD	Intrauterine fetal death
HELLP	Hemolysis, elevated liver enzyme and low platelet count
MPR	Multidrug resistance associated protein
MSAF	Meconium staining of amniotic fluid
NICU	Neonatal intensive care unit
RCT	Randomized controlled trial
RDS	Respiratory distress syndrome
SAMe	S-Adenosyl-L-methione
STAN	ST-analyzer
TBA	Total bile acid
UA	Umbilical artery
UDCA	Ursodeoxycholic acid
QT	QT interval
QT_c	Corrected QT interval
VAS	Visual analogue scale

LIST OF ORIGINAL PUBLICATIONS

I Joutsiniemi T, Leino R, Timonen S, Pulkki K, Ekblad U. Hepatocellular enzyme glutathione S-transferase alpha and intrahepatic cholestasis of pregnancy. Acta Obstet Gynecol Scand 2008; 87:1280

II Joutsiniemi T, Timonen S, Leino R, Linden M, Palo P, Ekblad U. Ursodeoxycholic acid in the treatment of intrahepatic cholestasis of pregnancy: A randomised controlled trial. Arch Gynecol Obstet 2014; 289:541

III Joutsiniemi T, Timonen S, Linden M, Suvitie P, Ekblad U. Intrahepatic cholestasis of pregnancy: observational study of the treatment with low-dose ursodeoxycholic acid. BMC Gastroenterology 2015; 15: 92

IV Joutsiniemi T, Ekblad U, Rosén KG, Timonen S. Waveform analysis of the fetal ECG in labor in patients with intrahepatic cholestasis of pregnancy. Submitted.

1. INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disorder characterized by maternal pruritus and elevated serum liver enzymes. It typically arises in the third trimester of pregnancy and disappears spontaneously after delivery. The prevalence of ICP varies worldwide: it is more common in the Scandinavian countries and in South America, especially Chile and Bolivia (Reyes et al. 1978; Lammert et al. 2000; Riely & Bacq 2004). In the Nordic countries the incidence of ICP is 0.5 – 1.9% of pregnancies (Heikkinen J et al. 1982, Berg B et al. 1986, Turunen et al. 2010).

ICP poses a risk for the fetus and is disadvantageous with respect to maternal wellbeing during pregnancy. Increased maternal blood levels of serum bile acids (BAs) are the most sensitive and specific biochemical marker of ICP (Geenes & Williamson 2009) and are widely used as a diagnostic tool, together with serum alanine aminotransferase (ALT). There are, however, no specific diagnostic laboratory parameters for the diagnosis of ICP. Glutathione S-transferase alpha (GSTA) occurs in high concentrations in the human liver and is released into the blood circulation even after minor impairment of hepatocellular integrity, but its role in the diagnosis of ICP has not been evaluated properly. ICP is associated with an increased risk for adverse perinatal outcome, including fetal distress, spontaneous preterm labor and even intrauterine fetal death (IUFD). A proper and timely diagnosis allows for drug treatment of the pregnant woman and close antenatal surveillance of the fetus (Kremer et al. 2014).

There are ongoing controversies concerning treatment and management of ICP. Ursodeoxycholic acid (UDCA) is the most promising medical treatment. A recent meta-analysis concluded that UDCA is effective in reducing maternal pruritus and improving maternal liver function test values and that it also benefits fetal outcome (Bacq et al. 2012). Women with ICP have an increased rate of unexplained IUFD at term, mostly between 37 and 39 gestational weeks (GWs). An increase in the total BA value ($\geq$ 40 μmol/L) is associated with increased fetal risk (Glantz et al. 2005). Active management, including fetal antenatal monitoring and labor induction, is usually recommended. However, this has been contrasted in a recent expert review where individually tailored management of ICP-affected pregnancies is recommended rather than following a routine active management protocol (Henderson et al. 2014).

There are no data available on the value of any antenatal tests as predictors of sudden IUFD in ICP. The mechanisms by which ICP leads to an adverse fetal outcome are unclear. Increased maternal total bile acid (TBA) concentrations may predispose the fetus to heart arrhythmia. Prolongation of the QT-interval may predispose the fetus to ventricular tachycardia and IUFD (Schwartz et al. 1998; Kremer et al. 2014). The

significance of the corrected fetal QT-interval in the evaluation of fetal surveillance has not been studied previously in women with ICP.

The aim of this study was to establish the value of maternal plasma levels of glutathione S-transferase alpha (GSTA) in diagnosing ICP, to evaluate the treatment of ICP with UDCA and its influence on maternal and neonatal outcome and to study the changes in the fetal electrocardiogram intrapartum.

2. REVIEW OF THE LITERATURE

2.1. Intrahepatic cholestasis of pregnancy

2.1.1. Terminology

Intrahepatic cholestasis of pregnancy (ICP), also known as obstetric cholestasis, is a liver disease of pregnancy associated with severe itching, which the patient senses especially in her palms and soles, combined with biochemical evidence of liver dysfunction. It typically arises in the third trimester of pregnancy and disappears spontaneously after delivery. A diagnosis of ICP requires that other pathological liver and skin diseases or conditions are excluded.

As an independent symptom, pruritus is fairly common among pregnant women; it affects as many as 20% of pregnancies. The main differential diagnosis of pruritus is skin diseases and allergic reactions. (Roger et al. 1994; Lammert et al. 2000; Pathak et al. 2010)

ICP was first described in 1883 by Ahlfeld as recurrent jaundice during pregnancy that resolved after delivery (Ahlfeld 1883). As late as in the 1950s severe pruritus with or without jaundice was reported in conjunction with liver disease during pregnancy (Svanborg 1954, Thorling 1955). Also complete resolution following delivery and high recurrence rates in subsequent pregnancies were reported.

2.1.2. Epidemiology

The incidence of ICP varies widely by geographical location and ethnicity (Geenes & Williamson 2009) **(Table 1)**. The estimated prevalence of ICP in the United States is reported to vary from 0.001% to 0.32% and, in contrast, in Chile the number is as high as 6.5% (Wilson & Haverkamp 1979; Lee et al. 2006, Laifer et al. 2001). In some ethnic groups the prevalence is much higher, e.g., in Araucanian, Chile, the prevalence is 27.6% and in Aimaras, Bolivia, 13.8% (Reyes et al. 1979). In Europe, it affects approximately 10 – 150 per 10,000 pregnancies; the incidence is highest in the Nordic countries (Finland 0.9%, Sweden 1.4%) and lowest in France (0.2%) (Gagnaire et al. 1975; Berg et al. 1986, Lammert et al. 2000; Glantz et al. 2004; Turunen et al. 2010). The prevalence of ICP seems to have changed over time, possibly because the diagnostic criteria have become more inclusive in the more recent studies and also because of changes in the environment (Geenes & Williamson 2009). The incidence of ICP has been low and stable for many years in Europe.

Table 1. Prevalence of ICP in some countries and ethnic groups (Modified from Geenes and Williamson 2009)

Country	Prevalence%	Study year	Reference
Australia	0.2	1964–1966	Kater R 1967
	1.5	1968–1970	Steel R 1973
	0.2	1975–1984	Fisk N et al. 1988
Bolivia	9.2	1976	Reyes H et al. 1979
	Aimaras 13.8		
Canada	0.07	1963–1976	Johnston W et al. 1979
Chile	24.0	1974–1975	Reyes H et al. 1978
	Aimaras 11.8		
	Araucanian 27.6		
	Caucasian 15.1		
	6.5	1988–1990	Rioseco A et al. 1994
China	0.32	1981–1983	Jiang Z et al. 1986
Finland	1.1	1971–1972	Laatikainen T 1975
	0.54	1990–1996	Heinonen S et al. 1999
	0.54	1994–1998	Eloranta M et al. 2001
	1.3	1992–1993	Savander M et al. 2003
	0.9	1969–1988	Turunen K et al. 2010
France	0.53	1988–1989	Roger D et al. 1994
India	0.08	2002–2004	Rathi U et al. 2007
Italy	1	1989–1997	Roncaglia N et al. 2002
Poland	1.5	Not available	Wojcicca-Jagodzinska J et al. 1989
Portugal	1	Not available	Brites et al. 1998
Sweden	1.5	1971–1974	Berg B et al. 1986
	1.5	1999–2002	Glantz A et al. 2004
UK	0.7	1995–1997	Abedin P et al. 1999
USA	0.32	1997–1999	Laifer S et al. 2001
	Latino 5.6	1997–1998	Lee R et al. 2006

2.1.3. Etiology and pathogenesis

Certain maternal physiological changes during pregnancy support fetal growth and development. Serum hormonal levels increase and affect metabolic, synthetic and excretory hepatic functions.

Among the risk factors for ICP are environmental factors, nutritional deficiencies, genetic variations and hormonal changes (Reyes et al. 1978).

There is evidence for a primary role of steroid hormones in ICP. The changes caused by genetic factors may increase the subject's sensitivity to normally produced steroid hormones, especially estrogens and progesterones. This is based on following three circumstances: Firstly, ICP usually starts in the last trimester, i.e., the time of the highest maternal estrogen and progesterone concentrations (Reyes 1997). Secondly, the

incidence is higher in twin pregnancies than singletons (Gonzalez et al. 1989; Rioseco et al. 1994; Koivurova et al. 2002) and in pregnancies following in vitro fertilization than spontaneous conception (2.7% vs 0.7%) (Koivurova et al. 2002). Thirdly, ICP resolves promptly after delivery and also recurs in half of the patients during subsequent pregnancies (Heinonen & Kirkinen 1999; Reyes & Sjövall 2000).

Estrogens are cholestastic according to animal studies: they reduce the uptake of BA at the basolateral membrane of hepatocytes. Estrogen can inhibit BA transport from hepatocytes into bile canaliculi by interfering with the bile salt export pump and the multidrug resistance-associated transporter 2 (Chen et al. 2013). Further, progesterone metabolites might play an even more important role in the ICP metabolism than estrogens (Reyes & Sjövall 2000). The levels of sulfated progesterone metabolites in the serum of in women with ICP rise and the pattern of progesterone metabolites differs significantly from that in normal pregnancy (Sjövall & Sjövall 1970). The level of normal metabolism of progesterone in ICP is reduced, resulting in increased formation of metabolites and a larger fraction of sulfates (Meng et al. 1997) (**Figure 1.)** Women with ICP may also have a defect in the secretion of sulfated progesterone metabolites into bile (Reyes and Sjövall 2000), and the normal fetomaternal transfer of BAs across the placenta is impaired. The accumulated BAs are potentially toxic to the fetus (Faran 1999).

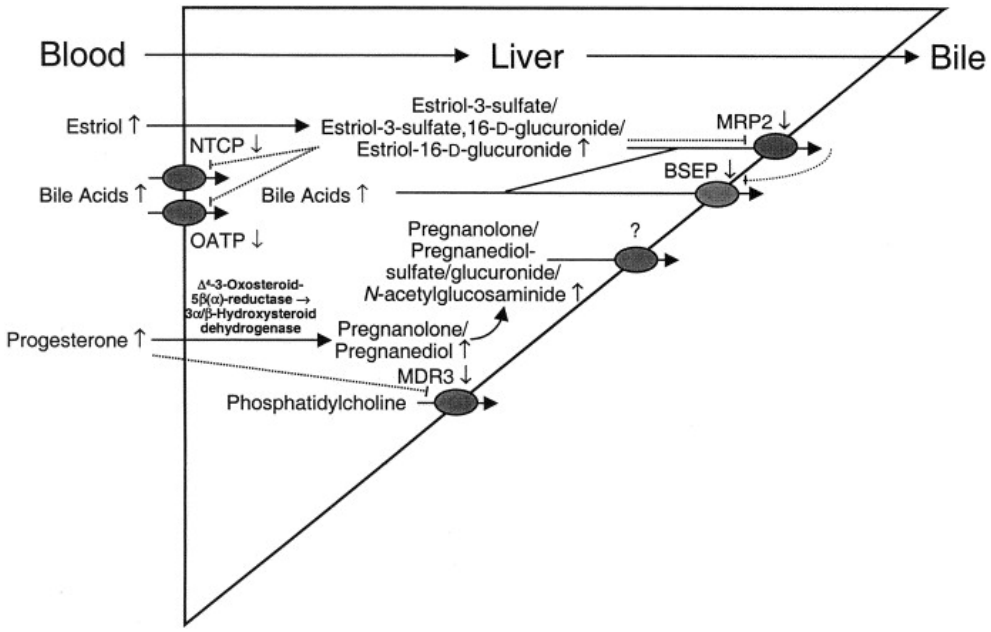


Figure 1. Hepatic metabolism of hormonal factors contributing to ICP. Placental sex hormones affect the functions of membrane transport proteins of the hepatocytes and affect lipid secretion. Hepatic estrogen conjugates inhibit uptake via the cotransporting polypeptides (NTCP, OATP). Estrogen glucuronides are excreted via MPR2 (multidrug resistance associated protein 2) into bile, and the bile salt export pump is inhibited. Progesterone metabolites are conjugated. The existence of an export pump for sulfated metabolites has not been verified. Progesterone modulates the activity of MDR3 (phosphatidylcholine translocase). Reprinted with permission.

The genetic etiology of ICP is largely unknown, but several investigators have studied potential genes associated with ICP (Eloranta et al. 2003; Müllenbach et al. 2003; Pauli-Magnus et al. 2004; Painter et al. 2005; Jacquemin 2012). Genetic predisposition may lead to changes in the membrane composition of the bile ducts and hepatocytes, and to dysfunctional transporters in the biliary canaliculi (Savander et al. 2003). Considerable evidence for a genetic predisposition to ICP comes from familiar clustering and an increased risk of ICP among first-degree relatives to affected individuals (Turunen et al. 2013).

Currently, the primary genes of interest are genes that encode biliary transport proteins (ABCB4, ATP8B1, and ABCB11). Mutations in these genes cause recessively inherited progressive familial intrahepatic cholestasis, which is a rare, early-onset condition associated with intrahepatic cholestasis in infancy or early childhood and resulting in death. Affected individuals benefit from transplantation. ABCB4 is a gene encoding for multidrug resistant protein 3 (MDR3) P-glycoprotein, and mutations in this gene may affect BA trafficking and lead to a rise in BA concentrations (Lammert et al. 2000; Wasmuth et al. 2007). ABCB4 gene variants have particularly been linked to a severe form of ICP (Wasmuth et al. 2007). Various single nucleotide polymorphisms (SNP) in ABCB11 and ABCB4 proteins are associated with ICP (Dixon et al. 2014). ATP8B1 is also a candidate gene for ICP, but not for ICP in Finnish ICP patients (Savander et al. 2003; Painter et al. 2005).

Seasonal variations affect the prevalence of ICP. ICP is most common in the winter months in Finland, Sweden, Chile and Portugal (Berg et al. 1986). Other reported risk factors for ICP are a family history of biliary disease, hepatitis C, prior ICP, multifetal gestation and maternal age greater than 35 years (Gonzalez et al. 1989; Heinonen & Kirkinen 1999). ICP patients have also often low concentrations of selenium in their serum (Kauppila et al. 1987; Reyes et al. 2000). Selenium deficiency may lead to defective bile formation or secretion because of selenium is a cofactor for several oxidative hepatic enzymes (Kauppila et al. 1987; Reyes et al. 2000).

2.1.4. Symptoms

Clinical symptoms of ICP arise when the capacity of malfunctioning BA canalicular transporters are overwhelmed by the high steroid hormone levels produced in pregnancy (Saleh & Abdo 2007).

The most common symptom of ICP is pruritus which typically appears in the third trimester and starts in the palms and soles. It often becomes generalized. The pathophysiology of the pruritus is still unknown. Bile salts are thought to be deposited on nerve endings of the skin causing itching. The pruritus is typically most severe at night and can cause insomnia and considerable discomfort for the patients. Other causes of itching must be excluded (atopic eczema, allergic reactions, urticarial or gestational pemphigoid and virus infections). Clinical examination of the skin is

normal except for evidence of scratching. Usually 80% of patients have symptoms after 30 GWs and the condition presents in the late second and third trimester of pregnancy, although ICP has been reported as early as at 6 – 10 weeks of gestation (Brites et al. 1998; Kenyon et al. 2002; Saleh & Abdo 2007).

The old term icterus gravidarum describes the old aggravated course of ICP. In rare cases ICP can cause steatorrhea with decreased absorption of fat-soluble vitamins and weight loss. Theoretically steatorrhea can increase the risk for postpartum hemorrhage as a result of malabsorption of vitamin K, although such cases have been reported only rarely (Reid et al. 1976). ICP resolves spontaneously in a few days following delivery.

2.1.5. Diagnosis

The diagnosis of ICP based on the clinical presentation, laboratory results and exclusion of other causes for the clinical and biochemical findings. The differential diagnosis includes other forms of hepatic disease. In patients with very high levels of serum alanine aminotransferase (ALT) and/or aspartate transaminase (AST), hepatitis A, B, C, severe pre-eclampsia, the HELLP syndrome, acute fatty liver of pregnancy, and drug toxicities should be excluded.

2.1.5.1. Biochemical features

Liver function does not change during normal pregnancy, although it has been recommended that adjusted upper limits of the reference range be used during pregnancy (Geenes & Williamson 2009). The upper limit of the reference range for ALT and AST should be reduced by 20% for healthy pregnant subjects (Girling et al. 1997). The level of gamma-glutamyl transpeptidase (GGT) is reduced similarly in later pregnancy (Bacq et al. 1996). The alkaline phosphatase level is usually elevated in pregnancy, particularly during the third trimester, and is thus not a reliable test for cholestasis.

The liver tests should be performed in every pregnant woman who complains of pruritus. ALT and TBA concentrations have been widely used to diagnose ICP. Minor elevations of liver enzymes are observed in up to 60% of ICP patients (Tan 2003).

Serum BA levels are sensitive indicators of hepatobiliary disease. Elevated serum BA levels have been used to screen for other cholestatic disorders than ICP, e.g., biliary atresia and bile acid synthesis defects (Mushtaq et al. 1999; Haas et al. 2012). A BA level ≥10 μmol/L during pregnancy was the diagnostic criteria for ICP in the study of Glantz et al. (Glantz et al. 2004). In Finnish studies the diagnostic laboratory criteria for ICP were BA ≥ 6 μmol/L or ALT > 40 U/L or AST > 35 U/L (Turunen el. al. 2010). Earlier studies reported that 20 – 60% of women with pruritus during pregnancy and elevated serum BA levels have 2 – 10-fold levels of transaminases (Lunzer et al. 1986; Rioseco et al. 1994). Elevated serum BA levels are the most sensitive indicator

of ICP (Lammert et al. 2000), but serum ALT levels usually also rise in ICP, while other biochemical features remain normal (Lammert et al. 2000; Dann et al. 2004). The levels of both AST and ALT in the serum can rise in patients with ICP, but ALT seems to be the more sensitive indicator of the two (Bacq 1999). An earlier Finnish study reported no statistically significant correlation between TBA and ALT (Laatikainen 1975).

The levels of serum bilirubin are elevated in the most severe form of ICP, and the level of total bilirubin is associated with preterm delivery (Oztas et al. 2015). The activity of GGT is increased in about 10 – 15% of the women with ICP.

As the aminotransferases and GGT are located within the periportal hepatocytes, they are relatively poor markers of damage to the centrilobular hepatocytes (Beckett & Hayes 1993).

The glutathione S-transferase group of enzymes occurs in abundance in the liver. Cytosolic glutathione S-transferases are dimeric proteins which are divided into four classes: α, μ, π and θ (Mannervik et al. 1992). These enzymes are released in detectable amounts after only minor impairment of hepatocellular integrity (Beckett & Hayes 1993). GSTA occurs in high concentrations in the liver and the levels in the plasma are known to rise exclusively in hepatic diseases (Beckett & Hayes 1993). Measurement of the activity of this enzyme in plasma samples may provide a fast and specific marker of acute hepatocellular damage (Mannervik et al. 1992; Beckett & Hayes 1993). Previously, elevated maternal concentrations of GSTA have been associated with obstetric complications, e.g., severe pre-eclampsia and the HELLP syndrome (Steegers et al. 1995; Knapen et al. 1998; Kumtepe et al. 2002). Dann et al. showed that high serum GSTA levels could be a useful indicator of liver dysfunction also in ICP and might help to distinguish ICP from pruritus gravidarum (Dann et al. 2004).

In a recent study by Kremer et al., high serum autotaxin activity correlated with the onset of ICP-related pruritus (Kremer et al. 2015). Elevated autotaxin levels might be a highly sensitive and a specific diagnostic marker for ICP, not influenced by the circadian rhythm or food intake.

Also some routine laboratory parameters can predict an adverse perinatal outcome in ICP. An increased mean platelet volume is associated with preterm delivery in ICP patients (Oztas et al. 2015). The neutrophil-to-lymphocyte ratio is elevated in pregnancies complicated with ICP and may predict the severity of ICP (Kirbas et al. 2014).

2.1.5.2. Ultrasonography

Ultrasonography of the maternal liver and upper abdomen is an important examination for ICP patients with puzzling laboratory test results, largely as a means to exclude

some important liver disorders and to provide support to a working diagnosis of ICP. In ICP, it usually reveals no dilatation of the intrahepatic nor extrahepatic bile ducts (Krueger et al. 1990), although the fasting and ejection volumes of the gallbladder seem to be higher than in healthy pregnancy (Krueger et al. 1990; Bacq 1999). ICP is associated with a predisposition for cholesterol gallstones and the incidence of ICP is higher among patients with gallstones (Samsioe et al. 1975; Leevy et al. 1997). Several liver and biliary diseases are significantly more common among patients with ICP than during healthy pregnancy (Ropponen et al. 2006). Some patients with ICP are at risk of liver cirrhosis or other severe chronic liver diseases (Ropponen et al. 2006).

2.1.5.3. Pathology

A liver biopsy is rarely needed in the diagnosis of ICP. If a biopsy is taken, pure cholestasis and sometimes bile plugs are seen in hepatocytes and canaliculi (**Figure 2**). Usually inflammation and necrosis are not present and the portal tracts are not affected (Rolfes & Ishak 1986; Bacq 1999).

Untreated ICP patients present histopathologically with chorangiosis (increased number of capillaries in terminal villi), an increased surface volume of capillaries and terminal villi, and an increased number of syncytial knots (Wikström Shemer et al. 2012). In a recent histopathological case-control review of placentas from ICP patients and controls, there were no differences in placental histopathology between the groups (Patel et al. 2014). Placental histology shows usually nonspecific hypoxic changes, but it cannot be established whether these changes are primary or secondary (Costoya et al. 1980). Treatment with ursodeoxycholic acid (UDCA) may alleviate the histological findings of villitis of unknown etiology (Müllenbach et al. 2005).

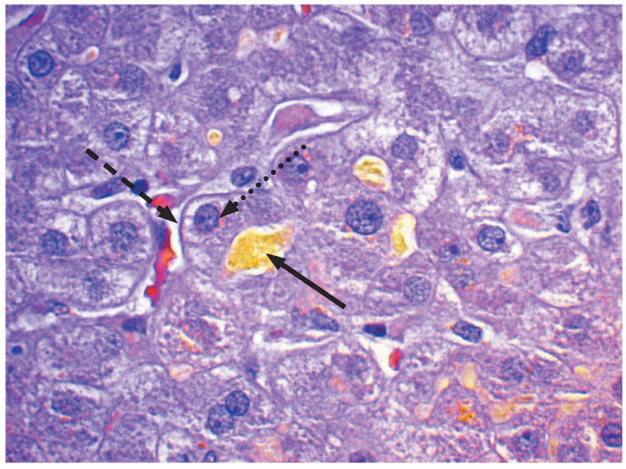


Figure 2. Photomicrograph of liver biopsy of an icteric patient with obstructive cholestasis: bile canaliculus filled with bile pigment (solid arrow), hepatocyte (arrow with dotted shaft), sinusoid (arrow dashed shaft). Picture Harry Kujari, pathologist.

2.1.6. Impact on fetus

Although severe maternal complications are rare in ICP, the fetus may be affected by preterm labor and birth, meconium aspiration and even perinatal death **(Table 2)** (Reid et al. 1976; Fisk & Storey 1988; Alsulyman et al. 1996). The etiology of the fetal complications is poorly understood, but it is generally believed that the fetal complications are related to the elevated maternal BA concentrations. IUFD is thought to occur suddenly, as there is no evidence of preceding intrauterine growth restriction or uteroplacental insufficiency and the fetal autopsy is usually normal (Fisk & Storey 1988; Davies et al. 1995; Williamson et al. 2004).

High maternal serum BA levels are a significant predictor of preterm delivery, spontaneous preterm delivery, stillbirth and MSAF (Geenes et al. 2014). The doubling the level of maternal serum BA increases the risk for preterm delivery by 68%, spontaneous preterm delivery by 66%, MSAF by 55% and IUFD by 200%. Maternal serum ALT and preterm delivery are also significantly, albeit more weakly, associated. In the study of Geenes et al. (2014), none of the other biochemical markers were significantly associated with any perinatal complications (Geenes et al. 2014). Brouwers and associates showed that in severe ICP (BA levels > 100μmol/L) sudden and unpredicted IUFD was even more common. A more aggressive approach to elective delivery may be justified when maternal BA levels are >100 μmol/L (Brouwers et al. 2015). In that study, the levels of BAs correlated between mother and fetus and this implies a causal relationship between the level of fetal exposure to BA and fetal complications and, ultimately, an adverse outcome (Brouwers et al. 2015).

It is estimated that the probability of fetal complications, such as spontaneous preterm deliveries, asphyxial events and MSAF, increase by 1 – 2% per additional μmol/L of serum BA (Glantz et al. 2004). When the BA levels are < 40 μmol/L, the fetal risk does not seem to be increased (Glantz et al. 2004).

The normal feto-maternal transfer of BA across the placenta is impaired in ICP patients. The fetus has no ability to excrete cholic acid, which remains elevated in the meconium in ICP, even after UDCA treatment (Rodrigues et al. 1999). Elevated BA may cause fetal arrhythmias. It is hypothesized that IUFD is caused by impaired fetal cardiomyocyte function, resulting in fetal cardiac arrest caused by raised fetal serum taurocholate concentrations (Williamson et al. 2001). Taurocholate also impairs the conduction in the fetal heart and ectopic electrical activity may arise (Miragoli et al. 2006). The fetal PR interval is significantly increased in the ECG in ICP (Strehlow et al. 2010). Fetal atrial flutter and supraventricular tachycardia occur in pregnancy complicated by ICP (Al Inizi et al. 2006; Shand et al. 2008) and BAs can cause vasoconstriction in isolated human placental chorionic veins (Sepúlveda et al. 1991).

Corticosteroid secretion is a part of the fetal stress response and in severe ICP it is suppressed. Elevated maternal TBA levels are associated with reduced fetal concentrations

of cortisol, dehydroepiandrosterone sulfate and corticotropin-releasing hormone (Wang et al. 2011; Zhou et al. 2013). The impaired fetal stress control might contribute to the risk for IUFD. Corticotropin-releasing hormone is one of the most potent vasodilatory factors in the human fetoplacental circulation (Clifton et al. 1994). Downregulation of maternal serum and placental corticotropin-releasing hormone expression might result in poorer feto-placental vascular perfusion and adverse fetal outcomes (Zhou et al. 2013).

The cause of the spontaneous onset of preterm delivery in ICP is unknown. Increased levels of BA may affect the contractility of the myometrium (Rioseco et al. 1994). Also, myometrial contractility may be increased as a consequence of increased levels of cholic acid (Mullally & Hansen 2002); this activates the oxytocin receptor pathway (Germain et al. 2003) following a cholic acid-mediated increase in oxytocin-receptor expression (Germain et al. 2003).

There is an increased risk for meconium passage in ICP, but there is not sufficient evidence to suggest its role in fetal death (Kafkasli et al. 1997). The incidence of MSAF varies between 10% and 44% in ICP (Bergasa 1995; Glantz et al. 2004; Lee et al. 2006) and correlates positively with the severity of ICP (Glantz et al. 2004; Lee et al. 2008, Kawakita et al 2015). The risk for meconium expulsion increases near term, similarly as in healthy pregnancies. BAs stimulate the motility of the large intestine of the fetus, which may be the reason for meconium expulsion (Kirwan et al. 1975), although Jain and associates did not establish an association between meconium passage and the severity of ICP (Jain et al. 2013).

Table 2. Studies on the incidence and risks of fetal complications of ICP (modified from Šimják P et al. 2014)

	Preterm delivery	MSAF	RDS	Intrauterine fetal death
Brouwers 2015	13%	20.5%	-	0.9%
Geenes 2014	25%, OR 4.68 in severe ICP	16% in severe ICP	-	1.5% OR 3.05 in severe ICP
Oztekin 2009	11.7%	-	-	-
Zecca 2006	-	-	28.6%	-
Lee 2006	-	10.5%-12.5%	-	-
Glantz 2004	2.2% in mild ICP 16.7% in severe ICP	44% in severe ICP	-	-
Bacq 1997	60.0%	-	-	-

MSAF meconium-stained amniotic fluid
RDS respiratory distress syndrome
OR odds ratio
mild ICP TBA 10 – 39 μmol/L, severe ICP TBA ≥ 40 μmol/L

Maternal ICP is also significantly associated with the occurrence of the respiratory distress syndrome (RDS) in the newborn (Zecca et al. 2006; Zecca et al. 2008). Zecca et al.

concluded that the incidence of RDS in infants from mothers with ICP was almost double that of infants born after healthy pregnancies. They hypothesized that raised level of TBA in the feto-maternal unit could decrease fetal surfactant production. Another pathophysiological mechanism may consist of a direct toxic effect of BAs on type II pneumocytes (Oelberg et al. 1990). BAs are thought to be the direct causal factor for BA-induced pneumonia during early postnatal period (Oelberg et al. 1990; Zecca et al. 2004).

Sudden IUFD occurs in 0.4% – 1.5% of pregnancies complicated with ICP (Glantz et al. 2004; Geenes et al. 2013). The cause of the IUFD seems to be multifactorial and is still unknown. BAs are thought to play a key role in its pathogenesis, although IUFD can occur at virtually physiological TBA levels (Sentilhes et al. 2006). There is currently no effective way of identifying the fetuses at risk for IUFD.

2.1.7. Management

2.1.7.1. Treatment of the mother

The treatment of ICP has mainly been symptomatic. Several medications during pregnancy, e.g., phenobarbital, cholestyramine, S-Adenosyl-L-methionine (SAMe), dexamethasone and ursodeoxycholic acid (UDCA), have been evaluated in the treatment of ICP (Heikkinen et al. 1982; Ribalta et al. 1991; Roncaglia et al. 2004; Glantz et al. 2005). These therapies aim at altering the enterohepatic circulation of BA and in this way at reducing the concentration of BA in the maternal serum (Pathak et al. 2010). The goal of these treatments is to improve perinatal outcome with continued pregnancy (if safe) and relief of maternal symptoms. The published randomized controlled trials on the treatment of ICP are summarized in **Table 3**.

Currently, UDCA is the most promising treatment for ICP. It is a naturally occurring hydrophilic BA that replaces more cytotoxic BAs and constitutes less than 3% of the physiological BA pool in humans. It is clinically used for the treatment of various cholestatic disorders, e.g., primary biliary cirrhosis (Lazaridis et al. 2001). The main modes of action of UDCA are protection of cholangiocytes against the cytotoxicity of hydrophobic BAs, stimulation of hepatobiliary secretion and protection of hepatocytes against BA induced apoptosis (Paumgartner & Beuers 2002). It might also improve BA transport and detoxification (Marschall et al. 2005). UDCA lowers the levels of cholestatic estrogen metabolites in the serum. Overall, however, the mechanisms of these beneficial effects are not fully understood.

UDCA has also a beneficial effect on the BA transport mechanisms in human placentas (Serrano et al. 1998) by the following mechanism: ICP induces impairment of the placental antioxidant system, which causes oxidative damage. These alterations are accompanied by enhanced activation of the mitochondrial pathway of apoptosis (Perez et al. 2006). Treatment with UDCA prevented partly these placental changes in a rat model (Perez et al. 2006).

Treatment with UDCA reduces serum BA in the maternal compartment and reduces the transplacental gradient between fetus and placenta. There are no major differences between the level of BA in the umbilical cord artery and vein serum samples, which implies that there is no significant fetal metabolism of BA of maternal origin in ICP (Geenes et al. 2014). UDCA treatment decreases urinary excretion of disulfated progesterone metabolites. This suggests that the amelioration of the pruritus of ICP is connected to enhanced hepatobiliary excretion of progesterone disulfates (Glantz et al. 2008).

Maternal serum and placental corticotropin-releasing hormone expression in ICP patients are up-regulated during UDCA treatment, which may be important for our understanding of the therapeutic mechanisms of UDCA treatment. UDCA may reduce fetal distress through the up-regulation of placental and maternal serum corticotropin-releasing hormone levels (Zhou et al. 2014).

Several studies have confirmed the positive effects of UDCA in ICP at a daily dosage ranging from 450 mg to 2 g (Diaferia et al. 1996; Floreani et al. 1996; Palma et al. 1997; Mazzella et al. 2001; Roncaglia et al. 2004; Glantz et al. 2005; Zapata et al. 2005; Binder et al. 2006; Glantz et al. 2008; Gurung et al. 2013). UDCA is well tolerated even at high doses (1.5 – 2 g/day) by pregnant women, and thus far no adverse effects in newborns have been recorded (Mazzella et al. 2001; Zapata et al. 2005).

The use of UDCA was first reported as a treatment for ICP in 1992 by Palma et al. (1992). Later they reported a significant reduction in pruritus and a decrease in serum bilirubin, AST and ALT concentrations after 3 weeks treatment at a dose of UDCA of 1 g daily (Palma et al. 1997). Also Diaferia et al. reported that pruritus abated and biochemical parameters improved in ICP when the dose of UDCA was 600 mg/day (Diaferia et al. 1996).

Later Glantz et al. reported that 3 weeks of UDCA treatment improved the values of some of the biochemical markers of ICP irrespective of disease severity, whereas significant relief from pruritus and a marked reduction of serum BA took only place in patients with severe ICP and BA levels $\geq$ 40 μmol/L (Glantz et al. 2005). More recently, a meta-analysis showed that UDCA therapy is effective for reducing pruritus and improving liver test values in women with ICP (Bacq et al. 2012). Chappell et al. found that UDCA reduces pruritus, but that the benefit may be modest (Chappell et al. 2012). According to a meta-analysis including both non-randomized and randomized controlled trials, UDCA-treatment reduces pruritus and improves the biochemical features of patients with ICP (Grand'Maison et al. 2014).

UDCA therapy during ICP benefits also fetal outcomes. Bacq et al. (2012) reported that this treatment reduces the occurrence of neonatal RDS and that fewer neonates need NICU treatment for RDS. There were fewer cases of fetal distress or asphyxia events in the groups on UDCA compared to placebo, but the difference was not statistically significant (Chappell et al. 2012). There were also significantly fewer total preterm births

among the patients treated with UDCA (Chappell et al. 2012). UDCA treatment did not increase the rate of Cesarean sections, but it was associated with less prematurity, a reduced need for treatment at the NICU and there was also a trend favoring increased birth weight and decreased meconium staining (Grand'Maison et al. 2014). Zapata et al. reported that UDCA treatment during pregnancy had no adverse effects on 26 infants followed up for a mean of 6 years after delivery (Zapata et al. 2005).

On the basis of a meta-analysis, which included both non-randomized and randomized controlled trials, UDCA was recommended for women with ICP to reduce adverse maternal and fetal outcomes (Grand'Maison et al. 2014).

Dexamethasone therapy reduces circulating estriol levels which are thought to be increased in ICP. Dexamethasone relieves pruritus and normalizes serum levels of ALT and BA but suppresses fetoplacental estrogen production (Hirvioja et al. 1992). Also clinical and biochemical improvement in ICP patients has been reported in another small study and no maternal or fetal adverse effects were recorded (Diac et al. 2006). In an larger randomized study on the effects of dexamethasone, UDCA and placebo were compared with dexamethasone in the treatment of ICP (Glantz et al. 2005). Dexamethasone provided no alleviation of pruritus or reduction of ALT levels and was less effective than UDCA at reducing BA and bilirubin (Glantz et al. 2005). Worsening of ICP following treatment with dexamethasone has been documented (Kretowicz & McIntyre 1994). In conclusion, dexamethasone is not an optimal treatment of ICP and is no longer used (Pathak et al. 2010).

S-Adenosyl-L-methionine (SAMe) alters hepatic surface membrane function in a way that improves impaired bile flow due to increased levels of ethinyl estradiol (Boelsterli et al. 1983). According to a randomized study, a high daily dose of SAMe (800 mg intravenously daily) is associated with significantly decreased levels of serum transaminases, conjugated bilirubin and TBA (Frezza et al. 1984). Pruritus was also decreased (Frezza et al. 1984). In contrast, Ribalta and associates showed no similar advantages, not even at a higher dosage of SAMe (900 mg intravenously daily) compared to placebo (Ribalta et al. 1991). Roncaglia et al. compared UDCA with SAMe and reported that UDCA was more effective than SAMe in improving maternal liver tests, although both therapies were equally effective in reducing maternal pruritus (Roncaglia et al. 2004). Floreani et al. showed that UDCA is more effective in controlling pruritus and reducing TBA-levels than SAMe (Floreani et al. 1996). Combination studies of UDCA and SAMe imply a synergistic effect (Binder et al. 2006; Zhou et al. 2014). SAMe is usually given intravenously or intramuscularly and the therapeutic effect is not predictable and thus SAMe has not become primary medication in the treatment of ICP.

Two Finnish study groups studied phenobarbital as a treatment for ICP years back (Laatikainen 1978; Heikkinen et al. 1982). These studies showed inconsistent reduction

in maternal itching and only a negligible effect or no effect at all on serum biomarkers (Laatikainen 1978; Heikkinen et al. 1982).

Geenes et al. reported recently on the effect of rifampicin in the treatment of ICP (Geenes et al. 2015). Rifampicin has been used in primary biliary cirrhosis and it reduces bilirubin, enhances hepatic efflux of organic anions including BA and reduces pruritus. Combined treatment with UDCA and rifampicin was effective in treating women with severe ICP who had not responded to treatment with UDCA monotherapy (Geenes et al. 2015).

Cholestyramine is an anion exchange resin that binds to BA and decreases their absorption in the ileum. In women with ICP cholestyramine did not show adequate benefits. Itching was not well or consistently controlled and serum BAs did not decrease consistently (Laatikainen 1978; Heikkinen et al. 1982). UDCA was more effective than cholestyramine and did not cause any adverse effects in a larger randomized study comparing those two preparations (Kondrackiene et al. 2005). Cholestyramine reduces the absorption of fat-soluble vitamin K and may thus increase the risk for hemorrhage for the mother as well as for the fetus (Sadler et al. 1995). Given the poor performance of cholestyramine in ameliorating maternal pruritus, improving maternal liver tests or newborn outcomes, its use as a treatment in ICP has not gained acceptance (Pathak et al. 2010).

Activated charcoal absorbs BA and decreases the levels of TBA in the serum, but has no influence on liver enzyme activities nor on pruritus symptoms (Kaaja et al. 1994). Guar gum is a dietary fiber binding to BA and provides some relief of pruritus (Gylling et al. 1998; Riikonen et al. 2000). However, neither activated charcoal or guar gum has proven to be clinically effective for ICP.

A recent study by Wu et al. demonstrated a regulatory effect of the farnesoid X receptor (FXR) agonist. A highly selective and potent FXR agonist protected against placental oxidative stress in an ICP mouse model (Wu et al. 2015). The data demonstrated that the FXR agonist is a promising group of specific drugs for treating ICP (Wu et al. 2015).

To relief pruritus, antihistamines are widely used. Hydroxyzine (25 – 50 mg/d) may alleviate the discomfort of pruritus.

Generally, UDCA is the most promising maternal treatment for ICP. According to a recent Cochrane review, there is insufficient evidence to introduce other medications (for example SAMe, guar gum, activated charcoal, dexamethasone, cholestyramine alone or in combination) to the treatment of obstetric cholestasis (Gurung et al. 2013).

The randomized controlled trials on treating ICP are summarized in **Table 4.**

Table 3. Randomized controlled treatment trials of ICP.

Reference	Study protocol	Dose/day	N	Result
	UDCA vs placebo			
Chappell 2012		1000 mg	125	reduces pruritus
Diaferia 1996		600 mg	8	improves clinical and biochemical results
Glantz 2005		1000 mg	94	improves some biochemical markers
Liu 2006		900 mg	68	itching scores, serum ALT and TBA significantly reduce
Meng 1997		14 mg/kg	11	decreases serum BAs and several sulfated progesterone metabolites
Nicastri 1998		600 mg	16	reduces itching and improves biochemical results
Palma 1997		1000 mg	15	reduces pruritus and improves biochemical results
	SAMe vs placebo			
Frezza 1984		200 – 800 mg	18	reduces pruritus and transaminases, BA, bilirubin at higher dose
Frezza 1990		800 mg	30	reduces pruritus, improves liver function tests
Nicastri 1998		800 mg	16	no side effects, reduces itching and biochemical abnormalities
Ribalta 1991		900 mg	18	no improvement
	Guar cum vs placebo			
Riikonen 2000		5 – 15 g	48	relieves pruritus, prevents rise in TBA
	Activated charcoal vs no treatment			
Kaaja 1994		150 g	20	decreases TBA
	Dexamethason vs placebo			
Glantz 2005		12 mg	80	no alleviation of pruritus, no reduction of ALT

Reference	Study protocol	Dose/day	N	Result
	UDCA vs SAMe			
Binder 2006		750 mg vs 1000 mg	78	synergistic effect on biochemical parameter
Floreani 1996		450 mg vs 1000mg	20	UDCA more effective than SAMe
Roncaglia 2004		600 mg vs 1000 mg	46	UDCA more effective than SAMe
	UDCA vs dexamethason			
Glantz 2005		1000 mg vs 12 mg	83	UDCA more effective than dexamethasone
	UDCA vs cholestyramine			
Kondrackiene 2005		8-10 mg/kg vs 8 g	84	UDCA more effective than cholestyramine
	UDCA + SAMe vs placebo			
Nicastri 1998		600 mg + SAMe 800 mg	24	combination therapy effective
	UDCA + SAMe vs SAMe			
Binder 2006		750 mg + 1g vs 1g	52	combined therapy and the monotherapy with UDCA improves the concentrations of BA and transaminases
Nicastri 1998		600 mg + 800 mg vs 800 mg	16	combination therapy more effective than SAMe alone
	UDCA + SAMe vs UDCA			
Binder 2006		750 mg + 1g vs 750 mg	53	combination therapy and monotherapy with UDCA improves concentrations of BA and transaminases. Combined therapy reduces a faster serum BA and transaminases compared with UDCA monotherapy
Nicastri 1998		600 mg + 800 mg vs 600 mg	16	combination therapy more effective than UDCA alone

Table 4. Characteristics of randomized controlled trials of UDCA treatment (modified from Bacq et al. 2012)

Reference	Double blinded	Controls	No of patients in each control group	No of patients in UDCA group	UDCA dose (mg/d)
Diaferia 1996	yes	placebo	8	8	600
Nicastri 1998	no	SAMe SAMe+UDCA vitamin (placebo)	8 8 8	8	600
Palma 1997	yes	placebo	7	8	1000
Floreani 1996	no	SAMe	10	10	450
Roncaglia 2004	no	SAMe	22	24	600
Glantz 2005	yes	placebo dexamethasone	47 36	47	1000
Kondrackiene 2005	no	cholestyramine	42	42	750
Binder 2006	no	SAMe SAMe+UDCA	25 27	26	750
Liu 2006	no	low fat +vitamin C	34	34	900
Chappell 2012	no	placebo	55	56	1000

2.1.7.2. Prenatal surveillance

There is no ideal method of fetal surveillance in ICP and antenatal testing has thus far had only limited predictability with regard to fetal outcome. Monitoring of fetal wellbeing during pregnancy is mandatory for all women with ICP and it is pleasing to learn that no less than 95% of maternity units in Europe have some policy for antenatal routine monitoring of women with ICP (Saleh & Abdo 2007).

Fetal surveillance has included various kinds of follow-up protocols, but usually daily maternal recording of fetal movements and regular (at intervals of 1 – 2 weeks) nonstress CTG-test as of 34 GW until delivery are performed (Rioseco et al. 1994). However, earlier studies have not clearly established the value of weekly non-stress CTG-testing in the antepartum management of ICP (Rioseco et al. 1994). Also non-stress testing just before the onset of delivery did not predict fetal asphyxia in a group of ICP patients (Oztecin et al. 2009). Usually, then, antenatal CTG has been reported in ICP (Rioseco et al. 1994, Glantz et al. 2004).

Most fetal deaths in ICP occur towards the end of pregnancy. Roncaglia et al. used a protocol including search for meconium with amnioscopy, amniocentesis, semi-weekly non-stress CTG-testing and amnionic fluid volume determinations. Labor was induced at 37 weeks or earlier if there was meconium, nonreassuring fetal testing or severe maternal symptoms. They suggested that their protocol might significantly reduce the stillbirth rate without increasing the cesarean delivery rate (Roncaglia et al. 2002). Doppler UA velocimetry is only of little value to evaluate the risk for fetal distress in ICP (Zimmermann et al. 1991, Oztecin et al. 2009). However, Doppler ultrasonography might have some value in recognition of the risk for fetal compromise in ICP (Suri et al. 2012). Suri and associates reported that UA Doppler velocity waveform indices in pregnancies complicated by ICP usually exceeded the reference range (Suri et al. 2012). However, abnormal UA Doppler results were not associated with the severity of ICP or abnormal fetal outcome (Suri et al. 2012).

Various strategies have been tested to predict the fetal outcome and to improve the obstetric outcome in ICP. Thus, many studies have evaluated specific active management protocols, e.g., various GW limits (37 – 38 GWs) when elective delivery should be induced. The hypothesis is that active management improves fetal outcome (Fisk & Storey 1988). In contrast to this, Kenyon and associates showed that a policy of active management in ICP may result in increased intervention and labor-associated complications. This must be balanced against the gain of a possible reduction in perinatal mortality. Kenyon et al. therefore concluded that appropriate consideration and advice should accompany active management of ICP, not forgetting the iatrogenic risks of labor associated complications (Kenyon et al. 2002).

Ataalla et al. reported recently on a study of FECG and a tissue Doppler imaging. They observed significant differences in myocardial tissue velocities of both the mitral and

the tricuspid valve between a study group of fetuses to mothers with ICP and TBA levels of < 40 µmol/L and a control group, versus a study group of fetuses to mothers with ICP and TBA levels > 40 µmol/L (Ataalla W et al. 2015). There was a positive correlation between maternal TBA levels and fetal myocardial tissue velocities at the mitral and tricuspid annuli and, on the other hand, between maternal TBA and fetal diastolic myocardial tissue Doppler velocities. They analyzed the motion velocities at both the mitral and the tricuspid annuli during systole and early and late diastole (Ataalla W et al. 2015).

2.1.7.3. Timing of delivery

Because most unexplained fetal deaths in ICP occurs after 37 GW, guidelines generally recommend delivery in ICP at 37 – 38 GW (Geenes & Williamson 2009). There is a general agreement from previous years that all women with ICP should deliver no later than 37 – 38 GW (Rioseco et al. 1994; Heinonen & Kirkinen 1999; Mullally & Hansen 2002; Roncaglia et al. 2002; Tan 2003). Delivering at 37 GW is associated with a low risk for an adverse perinatal outcome due to ICP among ICP patients with a total serum BA concentration ≥ 40 µmol/L (Lee et al. 2008).

A more aggressive approach to elective delivery may be justified in severe ICP (BA > 100 µmol/L) (Brouwers et al. 2015). In a recent, prospective population-based case-control study there was a significantly increased risk for adverse perinatal outcomes including IUFD among patients with severe ICP (Geenes et al. 2014). The risks for preterm delivery, neonatal unit admission and stillbirth were higher in ICP than controls. The authors suggests close antenatal monitoring of pregnancies affected by severe ICP (Geenes et al. 2014).

Henderson et al. analyzed the evidence in support of ICP as a medical indication for early term delivery. They reviewed sixteen studies with respect to IUFD that had occurred at the end of a term pregnancy (Henderson et al. 2014). They claim that empiric, active management of ICP is inappropriate. Rather, they recommend individual management of ICP-affected pregnancies in favor of routine active management. The studies concerning active and expectant management of ICP and unexplained stillbirth rate are summarized in **Tables 5** and **6**.

Table 5. Articles concerning expectant management of ICP and unexplained stillbirth (modified from Henderson C et al. 2014)

	STUDY		CONTROLS		
	N	Unexplained Stillbirth at >37 GW N	N	Unexplained Stillbirth at >37 GW N	P-value
Friedlaender 1967	103	3	0		NS
Roszkowski 1968	49	3	0	N/A	N/A
Jonston 1979	42	1	42	0	NS
Laatikainen 1975	116	0	0	N/A	N/A
Reid 1976	56	1	0		N/A
Qiu 1983	22	1	0	N/A	N/A
Laatikainen 1984	117	1	0	N/A	
Fisk 1988	83	0	0	N/A	
Berg 1986	100	1	100	0	

N/A, not applicable; NS, not significant

Table 6. Articles concerning active management of ICP and unexplained stillbirth (modified from Henderson C et al. 2014)

	STUDY		CONTROLS		
	N	Unexplained Stillbirth at >37 GW N	N	Unexplained Stillbirth at >37 GW N	P value
Alsulyman 1996	79	0	79	0	NS
Rioseco 1994	320	4	319	3	NS
Roncaglia 2002	206	0	N/A	N/A	
Glantz 2004	690	3	N/A	N/A	NS
Turunen 2012	687	8	1374	20	
Rook 2012	101	0	N/A	0	

N/A not applicable, NS not significant

In a recent large retrospective cohort study on fetal, neonatal and infant mortality in ICP, mortality was minimized by delivery at 36 GWs if ICP had been diagnosed at 36 GWs or earlier (Puljic et al. 2015). Immediate delivery minimized perinatal mortality at 36 GWs, after which mortality increased (**Figure 3).** Timing of delivery must take into account both the reduction in risk for stillbirth and the morbidities associated with preterm delivery.

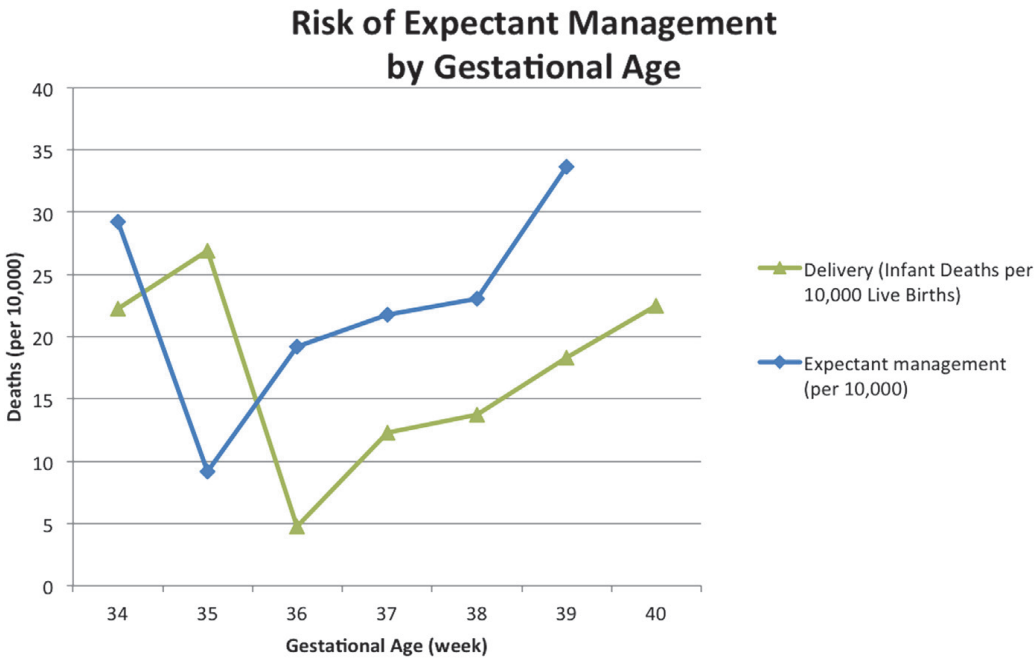


Figure 3. Risk for perinatal mortality associated with delivery vs expectant management stratified by GA in women with ICP. Reprinted with permission.

ICP patients delivering after 38 GWs had a higher incidence of MSAF, abnormal cardiotocography and need for NICU treatment compared to those delivering between 35 – 38 GWs, regardless of maternal laboratory values (Simják et al. 2014). The rate of cesarean sections was not increased by early induction of labor (Roncaglia et al. 2002; Chappell et al. 2012) and active management was associated with a low incidence of maternal and neonatal complications (Lee et al. 2008). Also a large recent cohort of twin pregnancies showed that there is a significantly increased risk for adverse perinatal outcomes, including stillbirth and preterm birth, in twin pregnancies with ICP (Liu et al. 2015). ICP and stillbirth also occurred at an earlier GA in twin pregnancies, suggesting that the policy of delivery at 37 GW in singletons may not be optimal for twin gestations (Liu et al. 2015).

The obstetrical decision to proceed with early delivery is influenced by several factors. The first is the duration of GWs, since the risk for fetal death is increased near term. The second is the severity of cholestasis. There is a relationship between maternal serum BA levels and signs of the fetal distress. Thirdly, the signs of fetal distress are an indication for delivery. **Figure 4** proposes a management algorithm for ICP.

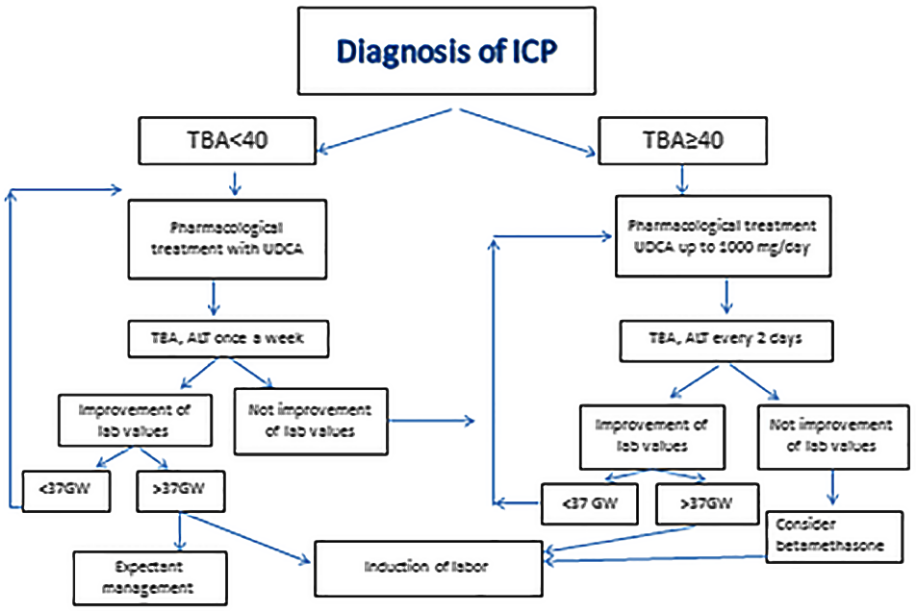


Figure 4. Proposed management algorithm of ICP (modified from Simják et al. 2014)

2.2. Fetal intrapartum surveillance

Traditional cardiotocographic (CTG) recordings use the R–R intervals to calculate the fetal heart rate. Complete fetal electrocardiography (FECG) is available when an electrode is affixed to the scalp of the presenting fetus. Automatic ST interval analysis (STAN) of FECG has been developed as an adjunct to CTG as a part of intrapartum fetal surveillance of term pregnancies (>36 full GW). This system has been designed to determine the point at which obstetric intervention is warranted for women in labor with a fetus at high risk for metabolic acidosis.

The first fetal electrocardiographic (ECG) examination was made by Cremer in 1906 (Cremer 1906). The next leap was taken the early 1950s when a fetal signal large enough was obtained using a silver wire electrode passed into the amniotic sac (Smyth 1953). Fetal scalp electrodes were introduced in the 1960s. A study of FECG during labor and its relation to the maternal and fetal acid-base status was reported in 1971 (Symonds 1971). The first studies were controversial. Rosen et al. reported from experimental studies that there are progressive changes in the ST-interval as an early sign of hypoxia (Rosén & Kjellmer 1975). Later they reported a correlation between the T/QRS-ratio and lactate values (Lilja et al. 1985). Intrauterine hypoxia was reflected in the FECG, a T-wave increase was paralleled by lactacidemia and a stable T-wave indicated acid-base homeostasis (Rosén et al. 1984). Later Reed et al. (1996) studied the potential impact of PR-interval analysis of the FECG on intrapartum fetal monitoring and found that a number of fetal scalp blood samples and newborns with acidosis were reduced (Reed et al. 1996).

Previously, six randomized controlled trials (RCTs) have been published on the use of FECG for intrapartum fetal monitoring; results have been conflicting (Westgate et al. 1993, Amer-Wåhlin et al. 2001, Ojala et al. 2006, Vayssière et al. 2007, Westerhuis et al. 2010, Amer-Wåhlin et al. 2011, Westerhuis et al. 2011, Belfort et al. 2015). These studies have recently been re-evaluated and the conclusion is that CTG with ST-monitoring may reduce significantly the need for fetal blood sampling, the rate of operative delivery and the occurrence of metabolic acidosis (Olofsson et al. 2014). The most recent study in this field comes from the US and could not confirm these results. Importantly, however the study settings differed from those of previous RCTs (Belfort et al. 2015).

Because of the conflicting RCT-results, these studies have undergone several meta-analyses (Becker et al. 2012, Neilson 2012, Salmelin et al. 2013 Olofsson et al. 2014, Schuit et al. 2013). In a meta-analyses of Olofsson et al. (2014) the rates of fetal scalp blood sampling, operative deliveries and metabolic acidosis were reduced (Olofsson et al. 2014). According to the meta-analysis of Becker et al. the use of ST-analysis for intrapartum monitoring reduces the incidence of operative vaginal deliveries and the need for fetal blood sampling but not the incidence of metabolic acidosis at birth (Becker et al. 2012).

RCTs may not reflect everyday practice, and therefore several observational studies on the effect of FECG method have been made. During the years after the implementation a decrease in metabolic acidosis and operative deliveries took place, according to follow-up studies (Norén & Carlsson 2010; Doret et al. 2011; Timonen S 2012; Chandraharan E et al. 2013; Kessler et al. 2013). There is a clear relationship between changes in the ST-segment and fetal stress and distress. The observational studies prove that FECG combined with intensive training of its use improves obstetric outcome (Visser & Kessler 2014).

The morphology of the fetal ECG-complex, e.g., the ST-segment and T-wave configuration, provides information on fetal intrapartum wellbeing. Hypoxia leads to metabolic acidosis, and this reduces significantly the fetal QT-time and corrected QT-time (QT_c), irrespective of changes in the heart rate (Oudijk et al. 2004). The QT-shortening is not related to the general stress of labor, since fetuses with no metabolic acidosis do not have QT-shortening. A QT-shortening seems to provide similar information as noted an increase in the T-wave amplitude and the T/QRS-ratio. It is assumed that QT-shortening is related to the ability of the fetal myocardium to respond to catecholamine surges and β-receptor activation which elicit a rise in T-wave amplitude (Oudijk et al. 2004). A prolonged QT-interval predisposes to ventricular tachycardia and even sudden fetal death (Schwartz et al. 1998).

The ST-segment and T-wave of the ECG relate to the repolarization of myocardial cells in preparation for the next cycle of contraction. This repolarization process is energy consuming. An increase in T-wave height occurs when the energy balance within the myocardial cells is threatened. During hypoxia this balance becomes

negative and the cells produce energy by β-adrenoceptor mediated anaerobic breakdown of glycogen reserves. This process not only produces lactic acid but also potassium ions which affect the myocardial cell membrane potential and cause a rise in the ST-waveform (Rosén et al. 2004).

ST-segment depression and an inverted T-wave (biphasic ST) indicate an imbalance between the endo- and epicardium: the perfusion pressure of the endocardium is always the lower at the same time as the mechanical strain is always the larger causing a delay in the repolarization (recovery) phase. This means that, unless the myocardium is generally activated (β-receptor activation and an increased Frank-Starling relationship, i.e., the myocardium is able to respond to volume load), a decrease in myocardial performance for whatever reason may generate a biphasic ST. Thus, not only hypoxia per se may cause biphasic ST as a sign of maladaptation, but the same is also true for factors substantially altering the balance and performance characteristics within the myocardial wall. So far a number of clinical situations have been associated with ST-depression/negative T-waves, e.g., prematurity, infections, maternal fever, myocardial dystrophy and cardiac malformations (Rosén et al. 2004). Yli and colleagues have documented an above-normal occurrence of ST-depression in fetuses of mothers with diabetes mellitus, a disorder associated with fetal myocardial dystrophy (Yli et al. 2008).

Raised maternal BA levels are clearly associated with a risk for fetal distress (Laatikainen & Ikonen 1977; Laatikainen & Tulenheimo 1984). BA taurocholate impairs rat cardiomyocyte function (Williamson et al. 2001). Taurocholate also affects calcium dynamics and leads to a loss of synchronous pulsation (Williamson et al. 2001). Cholic acid decreases the pulse rate of cardiac myocytes in a dose-dependent manner, increases the concentration of intracellular free calcium and could inhibit the activity of cardiac myocytes leading to the sudden fetal loss in ICP (Gao et al. 2014). Fetal death in ICP patients is acute and sudden, and a possible mechanism is fetal cardiac arrhythmia (Al Inizi et al. 2006). The fetal cardiac conduction system is altered in ICP. Significant differences in the PR-interval between the fetuses of women with ICP and fetuses of normal controls have been reported (Strehlow et al. 2010).

Currently, no antenatal testing method is available to predict sudden fetal death in ICP. A recent study on fetal antenatal testing and delivery outcomes in severe and mild ICP evaluated fetal heart rate decelerations as a predictor of a disadvantageous outcome, but there were no differences between these two groups in antenatal testing (Sheibani et al. 2014). The QT-interval in FECGs provides information on electrophysiological changes in the fetal myocardium. A significant shortening of the fetal QT-interval (QT) and the corrected QT-interval (QT_c) is present when intrapartum hypoxia results in metabolic acidosis; the QT-changes emerge irrespective of changes in the heart rate (Oudijk et al. 2004). Metabolic acidosis is also associated with an elevation of the ST-segment and T-wave, and the ST-rise as well as the QTc-shortening are apparently due to activation of membrane pumping resulting from the surge of catecholamines and the activation of β-

adrenoreceptors which occur by stimulation of beta-receptor and subsequent myocardial glycogenolysis. Furthermore, the QT-interval may be prolonged as a part of a genetic disorder (prolonged QT-syndrome) (Schwartz 2006), and this condition predisposes individuals to tachyarrhythmia in situations of physical stress, perhaps like labor-induced fetal stress. Maybe this offers an explanation for sudden IUFD in connection with ICP?

2.3. Health risks for offspring and mother

Although the symptoms of ICP resolve spontaneously after delivery, there are potential risks for both the mother and the newborn later in life. The intrauterine exposure to environmental stimuli including maternal disease can predispose to alterations in gene expressions (Barnes & Ozanne 2011). Papacleovoulou et al. have studied adolescent offspring of mothers who have had ICP during pregnancy in Northern Finland. They found that males had a higher BMI than the average population and females had an increased waist and hip girth compared with the offspring of uncomplicated pregnancies (Papacleovoulou et al. 2013). Women with ICP are at an increased risk for hepatobiliary disease, hepatitis C, cirrhosis and gallstones (Ropponen et al. 2006; Marschall et al. 2013). ICP also increases the risk for perinatal complications, like gestational diabetes and pre-eclampsia (Wikström Shemer et al. 2013; Martineau et al. 2014). A recent population-based study reported that women with ICP have an increased risk for hepatobiliary cancer, immunomediated disease (specially diabetes mellitus and thyroid disease) and cardiovascular disease later in life (Wikström Shemer et al. 2015). The study gave rise to the suggestion that women with ICP should have a follow-up of biochemical evidence of liver dysfunction 6 − 12 weeks after delivery and if the liver enzymes are elevated, further evaluation by a hepatologist is recommended (Wikström Shemer et al. 2015).

Summary

The etiology of ICP is unclear, although it seems to result from a combination of genetic and hormonal factors. ICP poses a risk for fetal wellbeing. The disorder is associated with an increased risk for preterm birth, meconium passage and even fetal death. The risk for spontaneous preterm delivery, fetal asphyxia and MSAF is the greater in pregnancies with the higher the maternal BA concentration. There is no specific laboratory test for ICP. The diagnosis of ICP is important and fetal wellbeing must be followed-up regularly after maternal diagnosis. The most effective pharmacological treatment is UDCA which reduces maternal pruritus, the concentrations of serum BA and the levels of liver enzymes. UDCA improves fetal outcome. BAs can provoke abnormal contractility of the fetal myocardium. Delivery is a stressful event for the fetus and demands cardiovascular and metabolic adaptation. The fetal QT-interval can be analyzed during labor which may provide more information on the condition of the fetus. Maybe the future will witness gene tests for identifying high-risk patients before ICP becomes evident.

3. AIMS OF THE STUDY

1. To evaluate the value of maternal plasma levels of glutathione S-transferase alpha (GSTA) in diagnosing ICP

2. To evaluate the safety and efficacy of ursodeoxycholic acid as a treatment of ICP patients

3. To evaluate oral low-dose ursodeoxycholic acid as a treatment strategy in ICP patients.

4. To evaluate intrapartum fetal ECG, and especially QT-interval changes, in patients with ICP

4. PATIENTS, SUBJECTS, MATERIALS AND METHODS

This study was carried out during the years 2008-2015 at the Department of Obstetrics and Gynecology, Turku University Hospital, Finland. The materials of studies I and II were collected earlier (in 1997-1998). The total study population consisted of 525 pregnant women, of whom 415 had ICP and 110 were controls.

4.1. Patient characteristics and study design

4.1.1. Study I

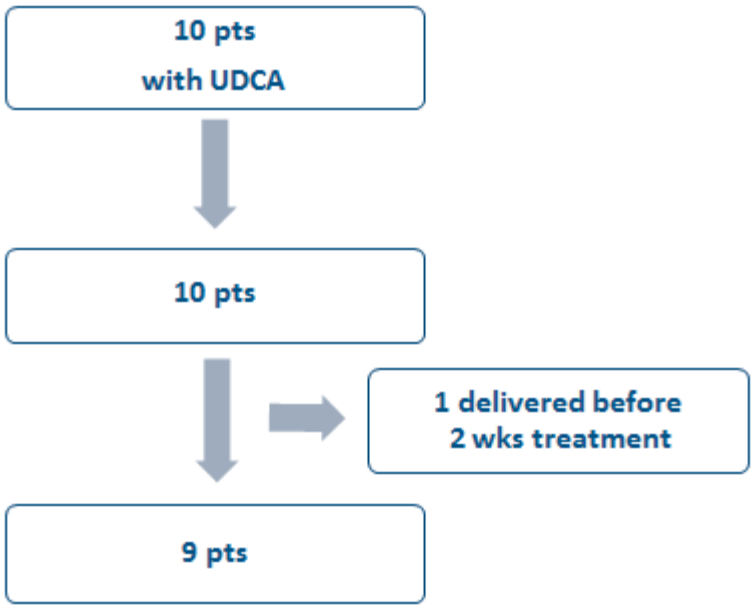
Study I was carried out in the Maternity Unit and Department of Clinical Chemistry of the Turku University Hospital. Maternal plasma glutathione S-transferase α (GSTA) concentrations were analyzed in the third trimester of pregnancy of women with ICP (N=27) and healthy pregnant women (N=49). The diagnosis of ICP was based on the presence of maternal pruritus and abnormal biochemical features. Other medical conditions possible associated with pruritus were excluded, e.g., gallstone disease was excluded by ultrasonography. Most women had singleton pregnancies, but there were three twin and one triplet gestation in the ICP group and one twin pregnancy in the control group. The control group consisted of healthy pregnant women with no complications of pregnancy, who were visiting the antenatal clinic for evaluation of the mode of delivery, or who were hospitalized for elective cesarean delivery because of suspected fetopelvic disproportion. Also women with an abnormal glucose tolerance test but with a normal daily blood glucose profile later during follow-up were included in the control group.

Fasting blood plasma samples for analyses were taken of 76 women during the third trimester after the diagnosis of ICP in the study group and at the time of study recruitment in the control group. The prenatal and neonatal data were collected from the hospital computerized delivery room logbook, where all necessary information on pregnancy outcome, deliveries and neonatal outcome is routinely recorded. Serum ALT and BA concentrations were measured by standard laboratory techniques. The upper reference limit of ALT was set at 45 U/L and of BA at 6 μmol/L. These reference values had been determined using serum samples from non-pregnant women. GSTA was measured with an enzyme-linked immunoassay (HEPKIT^{TM}-Alpha, Biotrin, Sinsheim-Reihen, Germany). The minimum detection limit is 0.25 μg/L. According to the data of manufacturer, the intra-assay variation of the GSTA assay ranges from 3 to 7% and the interassay variation varies from 7 to 10%. The assay is highly specific for the detection of GSTA and there is no significant cross-reactivity with the π or μ isoforms of glutathione S-transferase.

4.1.2. Study II

Study II was a randomized, double-blind, placebo-controlled trial (N = 20) which evaluated the safety of UDCA in pregnancies complicated with ICP. The trial was registered in Clinicaltrials.gov (NCT01576458). Twenty previously healthy pregnant women with a diagnosis of ICP participated in the study. The diagnostic criteria for ICP were maternal pruritus and elevation of biochemical markers, including ALT and/or total BAs. Written informed consent was obtained from each participant. Clinical and further laboratory evaluation were performed to diagnose and follow-up ICP and the pregnancy until delivery. At enrolment, a detailed obstetric and medical history and clinical examination was done for all participating women. All patients complained of a recent onset of pruritus. Other causes of itching were excluded. Hepatitis B carrier status was negative in all patients. The markers of hepatitis A and C were assessed in two patients to exclude viral hepatitis because of high values of ALT. On admission, 17 out of the 20 patients presented with elevation of both serum ALT (>45 U/L) and TBA (>6 μmol/L). Two patients with normal serum ALT values had a clearly elevated serum TBA concentration. One mother with a normal serum TBA concentration had a high serum ALT level. Ten of the patients were multiparous and six of them had had ICP in a previous pregnancy. Three of the primiparous mothers had a history of elevated levels of liver enzymes while taking oral contraceptives. Two patients in the group on placebo had to be excluded at the time of analysis because the mothers had received dexamethasone for fetal lung maturation. Nine patients remained in the study group after treatment of two weeks, as one patient delivered before the end of the treatment period. In the group on placebo, five mothers had delivered before end of treatment (**Figure 5**).

Patients treated with UDCA



Patients on placebo

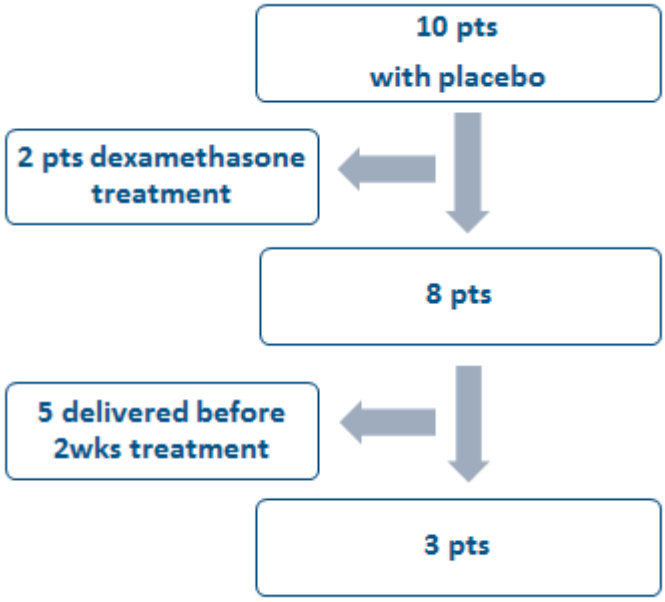


Figure 5. Study II protocol

After entering the study, the patients were randomized in a double-blind fashion to receive either UDCA 450 mg/day (Adursal®, Leiras, Turku, Finland) or placebo (tablets prepared by the hospital pharmacy) for two weeks. No other drugs were used.

In all cases the treatment started after 24 GWs. Clinicians and midwives approached the women attending the study. A member of the research team confirmed participant eligibility, and randomization followed. Serum ALT and TBA were assessed before treatment and once weekly thereafter at least three times or until delivery. Simultaneously serum levels of estradiol, progesterone, prolactin, cholesterol, HDL-cholesterol and triglycerides were assessed. The platelet count, the activated partial thromboplastin time (APTT) and fibrinogen D-dimers (FIDD) were also measured. Blood samples were taken in the morning after overnight fasting. Standard laboratory techniques were used for the analyses. The severity of pruritus was assessed before treatment and then every other day using a visual analogy scale (VAS): 0–10; 0 = no pruritus and 10 = continuous intensive pruritus which disturbs sleep. Obstetrical surveillance of the patients and fetal monitoring were performed according to the protocol adopted by our hospital for the care of patients with ICP. This included regular CTG and weekly ultrasound examination including Doppler measurements of the fetal UA flow. If signs of fetal distress were detected, active management was carried out by the obstetrician.

4.1.3. Study III

The third study was a retrospective observational study performed at the Department of Obstetrics and Gynecology in the Turku University Hospital comprising 307 pregnant women with ICP in the maternal unit during the years between 2000 and 2005. Thirty-four women had two pregnancies during the study period and both were affected with ICP, so the number of individual patients was 273. The diagnosis was made by the presence of maternal pruritus and elevated fasting total BA (>6 μmol/L) and/or ALT (>45 U/L). UDCA was used to treat 208 patients; 99 patients did not receive UDCA (non-UDCA group). There were fourteen twins, altogether 321 newborns. (UDCA group 13 twins, altogether 221 newborns and in the non-UDCA group one twin and altogether 100 newborns). Patients were started on UDCA medication according to our standard protocol, usually 450 mg/day. All participating patients fulfilled the diagnostic criteria, but those with only mild symptoms or only slightly elevated biochemical features were not started on UDCA. Those whose laboratory values were high and labor induction was started immediately did not use the medication.

Questionnaire data on maternal age, gravidity, parity and complications of earlier pregnancies, BMI, use of drugs, medical history, smoking and heredity of ICP were collected retrospectively. All follow-up visits in the antenatal clinic and the dose and side-effects of UDCA were recorded. There was no formal scale to show changes in pruritus. The follow-up visit data included GA, blood pressure, urinalysis and blood sample testing for ALT and TBA, among others. The fetal ultrasound findings and CTG recordings were also registered. The obstetric outcome was evaluated. GA at delivery, induction and duration of labor and obstetrical managements were recorded. Data of the neonatal outcome (Apgar scores, birth weight, pH and base excess values)

were collected. Maternal and neonatal data were analyzed and data of the patients who used UDCA during pregnancy was analyzed separately.

4.1.4. Study IV

Study IV was a retrospective case-control study and involved the population from the delivery unit of the Turku University Hospital during the years 2008 – 2013. We assessed the results of fetal ECG ST-waveform analyses in labor in patients with ICP. The intrapartum QT-interval was measured in 61 fetuses born to mothers with ICP and in a control group of similar size. The diagnostic criteria for ICP were maternal pruritus and elevation of liver enzymes (serum alanine aminotransferases, ALT>45 U/L) and/or TBA (TBA>6 μmol/L). The ALT and TBA values at the diagnosis and before delivery were used for analysis. Mothers with multiple pregnancies, other perinatal complications except ICP, gestational diabetes or pre-eclampsia were excluded to eliminate confounding factors. Obstetric surveillance and fetal monitoring before delivery followed the routines at our hospital for the care of patients with ICP: regular CTG-assessments and weekly ultrasound examinations, including fetal UA flow. If signs of fetal distress were detected, the attending obstetrician carried out active management.

The study data included women with ICP and ST-analysis available during labor. Each mother had a control patient that was the next healthy mother who was giving a birth with the same GWs (+/- one week). Data on maternal demographics (maternal age, BMI, parity, smoking), obstetric and medical history, biochemical values (TBA, ALT), medical treatments (UDCA) and pregnancy outcome (gestational age at delivery, induction and duration of labor and obstetrical managements, birth weight, Apgar scores, UA pH-values, treatment in neonate intensive care unit etc.) were retrieved from the hospital data unit.

When a maternal or a fetal risk factor for fetal hypoxia is identified, ST-analysis is added to standard CTG at 36 completed GWs. For this, a scalp electrode is applied to the presenting fetus. The fetal ECG and QT-interval were reviewed retrospectively from stored data with a special software application (STAN Viewer, Neoventa Medical, Sweden). The fetal ECG data were stored digitally as averages of 30 accepted ECG complexes. The STAN Viewer displayed the fetal ECG on the computer screen. The T/QRS ratio and the appearance of biphasic ST segments were automatically displayed. Elevation of the ST segment was displayed on the screen as baseline ST events or episodic ST events. Biphasic ST events displayed on the screen corresponded to ST segment depression. A specialized software tool was used for the assessment of fetal ECG time intervals, in which markers were placed by visual assessment on the corresponding ECG components of the onset of the QRS-complex and the end of the T-wave (**Figure 6**). The computer calculated the intervals in milliseconds (ms). As expected, these intervals may change profoundly in ICP pregnancies and

measurements were obtained at the beginning of the STAN recording and before delivery. The QT-interval corrected for heart rate (QTc) was calculated using Bazett's formula: QT/√RR. The measurements were obtained from four different time points during labor. Data recorded at the beginning and end of the ST-analysis were used for statistical analysis.

Figure 6. The measurement of the QT-interval. The markers (vertical lines) are placed manually by visual assessment at the onset of the Q-wave and end of the T-wave. The computer automatically calculates the interval in milliseconds, 262 ms in this case. (Adapted from Joutsiniemi et al. 2015, submitted)

4.2. Statistics

Statistical analyses were performed using the SAS Software Package Release 6.12. GLM Procedure and PAWS Statistics 19 Software (SPSS Inc., IBM, Chigaco, USA).

In the study I, Student's t-test was used to compare the groups regarding age, BMI and duration of pregnancy. The χ^2-test or Fisher's exact test were used to test whether tobacco smoking, BMI, duration of pregnancy, medication or the number of fetuses affected GSTA values. Results were expressed as mean group values ±SEM or mean group values ±SD. Spearman's correlation coefficients were used to test the correlation between ALT, BA, pregnancy duration and the response variable (GSTA).

In study II, changes in the intensity of the maternal pruritus and in the laboratory values were evaluated by analysis of variance of repeated measures. The t-test was used to compare pregnancy outcomes between the two groups. Results were expressed as mean group values ± SEM or SD.

In study III, the categorical baseline characteristics were compared by using the χ^2-test between UDCA users and non-users. Numerical characteristics were compared between groups with Fisher's T-test, if the variable was normally distributed and with Mann-Whitney's U-test, if the variable was not normally distributed.

In study IV, statistical analyses were performed using Fisher's exact test and the χ^2-test and Mann-Whitney's U-test.

Probability values <0.05 were considered statistically significant.

4.3. Ethics

The Ethics Committee of the University and the Hospital approved the study protocols. Study II was a randomized controlled trial and was registered in ClinicalTrials.gov (Identifier: NCT01576458).

5. RESULTS

5.1. Study I

Seventy-six patients were enrolled into study I, 27 with ICP and 49 healthy pregnant women.

There was no significant correlation between maternal smoking, medication, BMI or gestational age at sampling and plasma GSTA concentrations, neither in the study group nor in the control group. The mean GSTA concentration in the study group was 51.0 μg/L (range: 2.1 – 183.5) compared with the control group 1.62 μg/L (range: 0.25 – 8.3) (**Figure 7**, **Table 7**). The upper reference value for GSTA was 5.7 μg/L (mean + 2SD), which was estimated among samples of healthy pregnant women based on the measurement of the concentration of GSTA in 49 women with uncomplicated pregnancy.

There was a significant correlation between the number of fetuses and the GSTA concentration (p=0.032). The results of the liver test are presented in **Table 7**. There was a significant correlation between maternal serum ALT and plasma GSTA concentration in the ICP group (r=0.694, p=0.0001), but no significant correlation between maternal serum BA and plasma GSTA concentration in the study group. There were five very mild cases of ICP in the study group. In these patients, the diagnosis was based mainly on clinical symptoms or on only slightly elevated serum BA levels, while the ALT value was within the reference range. Except for two patients, the GSTA concentration was elevated in these patients, as well.

The average birth weight was significantly lower in the study group, which was in concordance with the higher GA in the control group (36.1 vs 39.1 weeks). Sixty-six percent of the deliveries in the ICP group were preterm (<37 weeks of pregnancy), compared to only 22% of the deliveries in the control group.

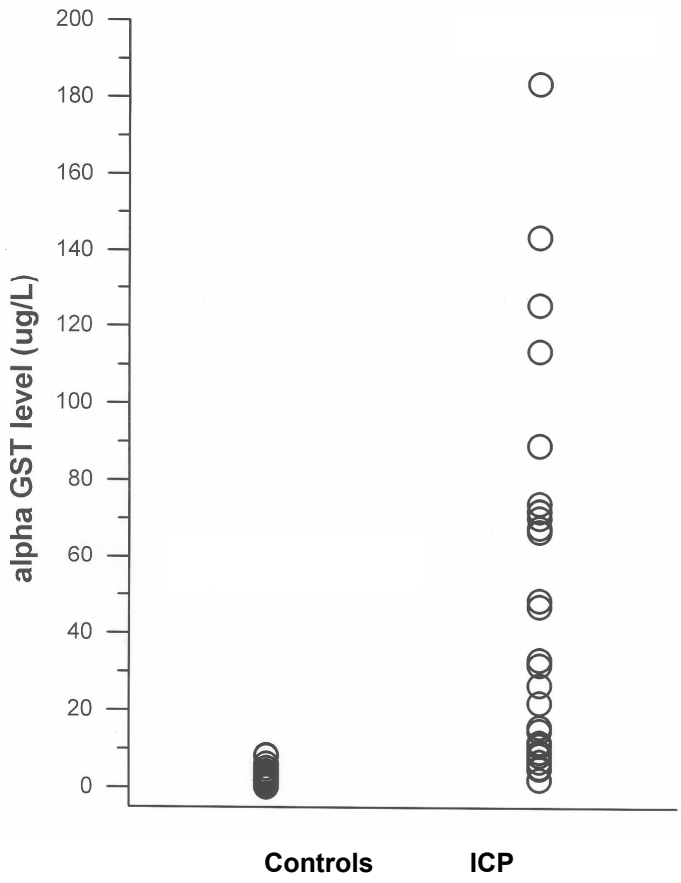


Figure 7. Individual values of GSTA among ICP patients and healthy controls (study I).

Table 7. Liver values (means ± S.E.M., number (%), * =laboratory reference values for non-pregnant women)

	ICP (n=27)	**Controls (n=49)**	**P-value**
GSTA (µg/L)	51±9.2	1.6±2.0	< 0.001
GSTA > 5.7 (µg/L)	24 (89%)		
ALT (U/L)	145.7±20.8	<45*	
TBA (µmol/L)	19.2±3.2	<6*	

5.2. Study II

There was a statistically significant improvement in pruritus scores in the group who were using UDCA (**Figure 8**).

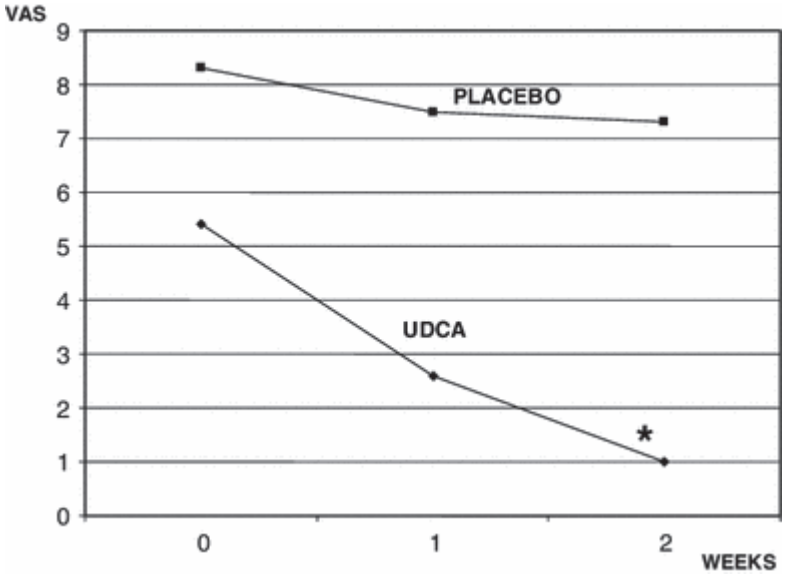


Figure 8. Maternal itching scores in the study group (UDCA) and in the placebo group. VAS (visual analogue scale) 0–10 was used. 0 = at the diagnosis of ICP, 1 = 1 week after starting the treatment, 2 = 2 weeks after starting the treatment. *Asterisk* improvement in pruritus scores was statistically significant in the group using UDCA (p = 0.007). Itching scores at mean, median (range) in UDCA group 5.4, 5.0 (1-10), 2.6, 3.0 (0-9), 1.0, 3.0 (0-4), in placebo group 8.3, 8.0 (6-10), 7.5, 7.0 (3-10), 7.3, 7.0 (7-8). Adapted from Joutsiniemi et al. 2014.

Serum levels of ALT and TBA at the beginning and at the end of the study are presented in **Table 8**. The differences in the changes of the serum ALT (F = 2.26, df = 1, p = 0.15) and TBA (F = 0.02, df = 1, p = 0.88) concentrations before and after UDCA treatment were not statistically significant (**Figure 9 and 10**). One patient had very high level of ALT (860 U/L) before the randomization and this influenced on the study group prerandomization values.

Table 8. Serum levels of ALT (U/L) and TBA (µmol/L) at the beginning of the study and after two weeks treatment. The values are given as a mean (range).

	STUDY GROUP		**GROUP ON PLACEBO**	
Days of treatment	0 [n=10]	14 [n=9]	0 [n=8]	14 [n=3]
ALT (U/L)	307 (23–860)	64 (8–137)	156 (29–406)	70 (49–83)
TBA (µmol/L)	26 (3–59)	6.6 (2–10)	23 (6–53)	11.3 (6–15)

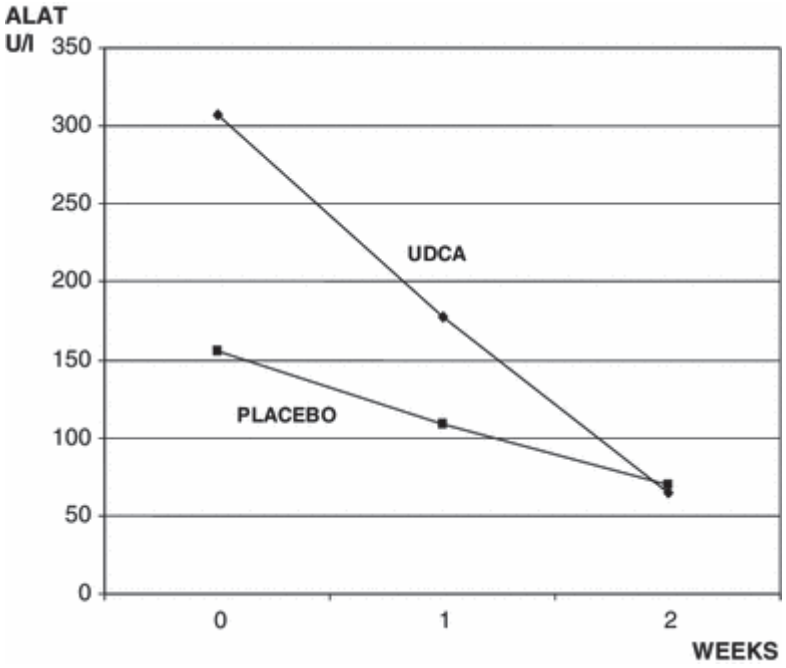


Figure 9. Changes in serum ALT values in the study group (*filled diamond*) and in the group on placebo (*filled square*). 0 = at the time of diagnosis of ICP, 1 = 1 week after starting treatment, 2 = 2 weeks after starting treatment. ALT at mean, median (range) in UDCA group 307, 230 (23-860), 177, 73 (12–440), 64, 67 (8–137), in group on placebo 155, 165 (29–406), 108, 110 (47–212), 70, 77 (49–83). Adapted from Joutsiniemi et al. 2014.

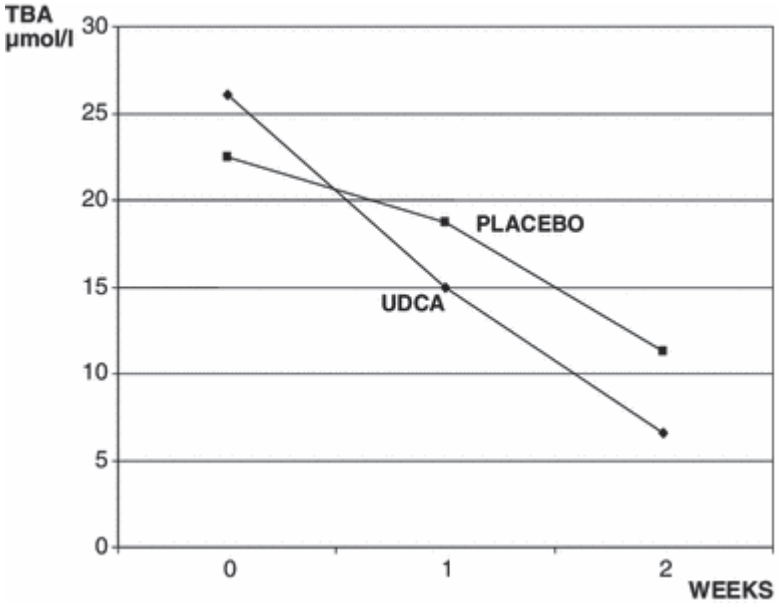


Figure 10. Changes in serum TBA concentrations in the study group (*filled diamond*) and in the group on placebo (*filled square*). 0 = at the time of diagnosis of ICP, 1 = 1 week after starting treatment, 2 = 2 weeks after starting treatment. TBA at mean (range) in UDCA group 26, 22 (3-59), 15, 12 (5−44), 7, 6 (2-10), in group on placebo 22, 16 (6−53), 18, 10 (5−74), 11, 13 (6−15). Adapted from Joutsiniemi et al. 2014.

There were no significant changes in the maternal serum values of cholesterol, HDL-cholesterol, triglycerides, estradiol, progesterone or activated partial tromboplastin time, platelet count or FIDD values between samples obtained before and after UDCA therapy ($p>0.05$) (**Table 9)**.

Table 9. Laboratory values before and after the treatment, study group and group on placebo. Values are as means [SEM]. Modified from Joutsiniemi et al. 2014.

	STUDY GROUP		**GROUP ON PLACEBO**	
Days of treatment	0 [n=10]	14 [n=9]	0 [n=8]	14 [n=3]
Cholesterol mmol/L	6.98 [0.43]	6.92 [0.54]	7.50 [0.39]	9.36 [1.07]
HDL-cholesterol mmol/L	1.15 [0.12]	1.27 [0.11]	1.11 [0.08]	1.0 [0.2]
Triglycerides mmol/L	2.84 [0.34]	2.86 [0.4]	3.31 [0.22]	3.33 [0.41]
Estradiol pmol/L	77660 [8863]	98750 [15233]	105428 [16168]	72333 [20610]
Progesterone nmol/L	497 [79]	520 [71]	488 [72]	576 [99]
Prolactin µg/l	124 [11]	172 [32]	131 [9]	130 [6]
FIDD mg/l	1.90 [0.42]	1.97 [0.33]	1.64 [0.27]	1.95 [0.3]
APTT s	25.4 [0.9]	24.5 [1.0]	25.4 [0.7]	24.8 [0.6]
Platelet count E9/l	229 [21]	212 [24]	210 [28]	171 [9]

FIDD= fibrinogen D-dimers, APTT= activated partial thromboplastin time.

5.3. Study III

The patient characteristics in study III are presented in Table 10. Patients who started UDCA treatment (N=208) had lower GA at symptoms start ($p<0.05$) and they had ICP diagnosed approximately 5 weeks earlier than women without UDCA medication ($p<0.05$). In most women without UDCA, the onset of ICP took place during the last weeks of pregnancy and treatment was not required, either because of mild symptoms or labor induction.

Table 10. Patient characteristics in study III. Values are given as numbers (%) or means [SD]. Modified from Joutsiniemi et al. 2014.

	ALL n=307	**No medication n=99**	**UDCA n=208**	**P**
Maternal age (years)	29.6 [5.2]	28.8 [4.6]	29.9 [5.4]	NS
Smoking	26 (8%)	12 (12%)	14 (7%)	NS
BMI kg/m^2	23.5 [4.1]	22.9 [3.7]	23.8 [4.2]	NS
Former deliveries (range)	1.0 (0-8)	1.0 (0-8)	0.8 (0-5)	NS
ICP in earlier pregnancy	92 (30%)	27 (27%)	65 (31%)	NS
Heredity	7 (2%)	3 (3%)	4 (2%)	NS
Beginning of itching (GWs)	33.6 [3.9]	36.8 [2.5]	32.2 [3.6]	P<0.05
ICP diagnosed (GWs)	34.8 [3.5]	38.0 [1.5]	33.3 [3.1]	P<0.05
Bile acid at diagnosis (µmol/L)	19.2 [24.9]	16.7 [26.7]	20.5 [24.0]	NS
ALT at diagnosis (U/L)	152.8 [166.1]	96.4 [106.6]	179.2 [181.9]	P<0.05
Abdominal ultrasound done	35 (11%)	4 (4%)	31 (15%)	P<0.05
Bile stones	9 (3%)	1 (1%)	8 (4%)	NS

The mean UDCA dose was 450 mg/day (range 150 – 900). The mean GA at the time of diagnosis was 33.3 GWs and at the first control visit 34.7 GWs. Most women started UCDA medication at their first visit to the maternal care unit (N=196) and 12 patients started medication later because ICP was mild at diagnosis. During the follow-up the symptoms got worse, the liver enzyme values rose and medication was started. Only 2.4% of the patients had side effects from the medication, usually gastrointestinal symptoms. At diagnosis, the levels of BA and ALT were higher in the group on UDCA compared to the group without UDCA ($p<0.05$). Both these laboratory parameters began to decrease right after medication start (**Figure 11** and **Figure 12**). Mothers on UDCA delivered earlier than mothers without UDCA ($p<0.05$). There were more preterm deliveries (<37 GWs) in the group on UDCA due to severity of ICP. Most of the deliveries were induced. The cesarean section rate in our hospital during the study

period varied between 13.9 – 17.4% and in the study cohort the rate was 15%. The vacuum extraction rate was 5.8 – 7.3% in the hospital and in this study 5.5%.

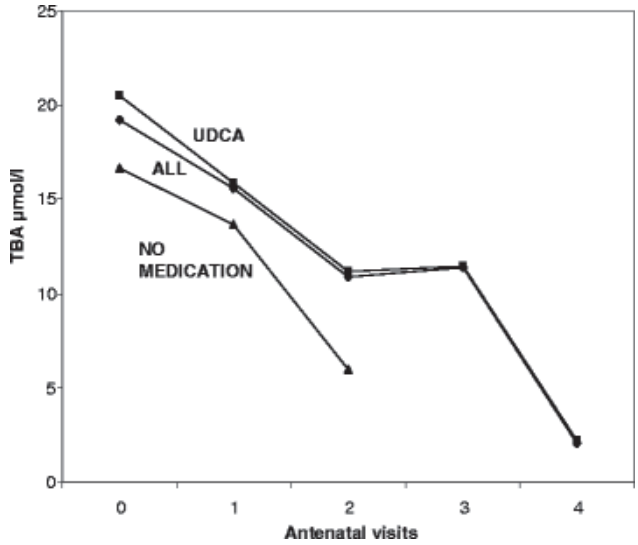


Figure 11. TBA concentrations (µmol/L) among patients on UDCA (■), all patients (♦) and patients without UDCA (▲) at diagnosis (0) and at visits (1–4) to the antenatal clinic. UDCA group: Decrease in TBA levels was statistically significant between time of diagnosis and first antenatal visit and between first and second antenatal visit ($p < 0.05$) Adapted from Joutsiniemi et al. 2014.

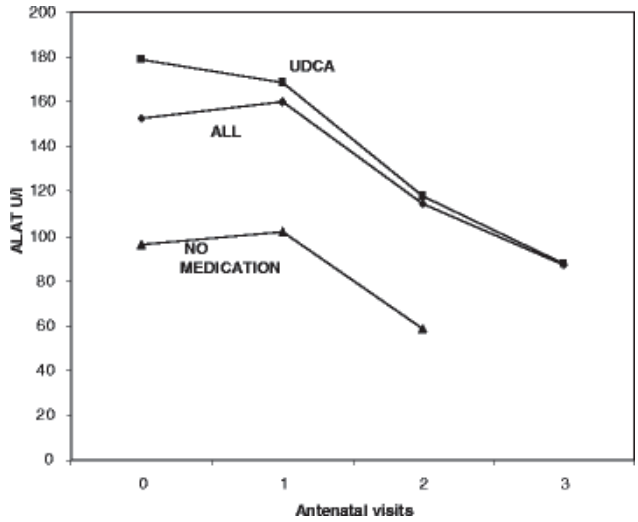


Figure 12. ALT concentrations (U/L) among patients with UDCA (■), all patients (♦) and patients without UDCA (▲) at diagnosis (0) and at visits (1–4) to the antenatal clinic. The statistical analysis was calculated during two weeks. All patients and UDCA group: Decrease in ALT levels was statistically significant between time of diagnosis and second antenatal visit and also between first and second antenatal visit ($p < 0.05$). Adapted from Joutsiniemi et al. 2014.

The perinatal outcome was good and there were no perinatal deaths at all. UA pH values were similar in both groups (p>0.05). The rate of admission to the neonatal unit was higher for children in the UDCA group (30% vs 11%) mostly due to preterm birth (p<0.05). Women with preterm delivery had significantly earlier onset pruritus, and ICP was diagnosed at early GW. Laboratory parameters (ALT and TBA) were also higher at the time of diagnosis and at first control. The Apgar-scores of the neonates were lower (p<0.05), but UA pH values were similar (**Table 11** and **Table 12**).

Thirty women had severe ICP (TBA≥40 μmol/L): 24 on UDCA and six patients without UDCA. Most of the deliveries were induced (22/30). Preterm labor was significantly more common among women with severe ICP compared to women with less severe forms (p<0.05). Almost half of the neonates (14/30) born to mothers with severe ICP needed treatment in the NICU (**Table 13).**

Table 11. Obstetric outcome. Values are given as numbers (%) or mean [SD].

Characteristics	**No treatment (*N* = 99)**	**UDCA (*N*= 208)**	**All (N=307)**	**P**
GA at labor (wks)	38,9 [1,2]	37,4 [1,5]	37,9 [1,6]	p<0.05
Preterm delivery (< 37 GWs)	4 (4%)	53 (25%)	57 (19%)	p<0.05
Induction of labor	79 (80%)	143 (69%)	222 (72%)	p<0.05
Normal vaginal delivery	84 (85%)	161 (77%)	245 (80%)	NS

Table 12. Neonatal outcome. Values are given as numbers (%) or mean [SD].

Newborns	**No maternal UDCA (*n* = 100)**	**With maternal UDCA (*n* = 221)**	**All (*n* = 321)**	**P-value**
Females	46 (46%)	98 (44%)	144 (45%)	NS
Males	54 (54%)	123 (56%)	177 (55%)	NS
Birthweight (g)	3525 [428]	3262 [553]	3344 [531]	p<0.005
Apgar score (5 min)	9.0 [0.9]	8.7 [1.0]	8.8 [1.0]	p<0.05
UA pH	7.28 [0.10]	7.28 [0.09]	7,28 [0,09]	NS
UA pH <7,05	-	2 (1%)	2 (1%)	NS
UA BE (mmol/L)	-4,1 [3,4]	-3,6 [3,7]	-3,7 [3,6]	NS
UA BE <-12 (mmol/L)	1 (1%)	3 (1%)	4 (1%)	NS
NICU treatment	11 (11%)	67 (30%)	78 (24%)	p<0.05
Duration of NICU treatment (days)	7,2 [9,0]	7,8 [6,1]	7,8 [6,1]	NS

Table 13. Obstetric and neonatal outcome in women with severe ICP (TBA > 40µmol/L)

TBA > 40µmol/L	**No treatment (*N* =6)**	**UDCA (*N*= 24)**
Preterm delivery, N	1	14
Induction of labor, N	5	17
Vaginal delivery, N	5	15
UA pH < 7.05	0	0
Need for NICU (newborns)	4	10
Duration of treatment in NICU (days)	7	8.3

5.4. Study IV

Baseline characteristics of the study population are shown in **Table 14**. There were no differences between the groups as regards maternal age, parity, BMI, GA and smoking. Deliveries in women with ICP were significantly more often induced (82%) compared to controls (16.2%) ($p<0.001$). Otherwise the perinatal outcome was similar between the groups. ICP was diagnosed at a mean of 34.6 GWs. Fifty-eight patients (95%) in the ICP group were treated with UDCA. Three patients did not use medication, because labor was induced immediately due to high liver enzyme levels and TBAs and intensive skin pruritus at the time of diagnosis. In the ICP group one infant had an acidotic UA pH-value (pH 7.01) after birth and the one-minute Apgar score was 1, but the newborn recovered well and at 5 minutes the score was 8. One infant in the ICP group had low Apgar scores but no acidosis in UA (pH 7.28).

Table 14. Baseline characteristics of the study population in study IV. Values are given as mean [SD], N (%)

	ICP (n=61)	**Controls (n=61)**	
Maternal age, years	28.7 [4.6]	28.7 [4.7]	NS
Parity	0.6 [0.96]	0.54 [0.9]	NS
BMI kg/m^2	24.6 [4.5]	23.6 [4.7]	NS
Smoking N	5 (8.3%)	7 (11.7%)	NS
Gestational age	38.7 [1.2]	39.2 [1.3]	NS
BA at diagnosis (µmol/L)	17.7 [18.4]		
ALT at diagnosis (U/L)	118.1 [114.0]		
BA before labor (µmol/L)	15.9 [16.0]		
ALT Before labor (U/L)	83.6 [103.3]		
Labor ind.	50 (82.0%)	10 (16.4%)	p<0.0001
Vacuum extraction	9 (14.8%)	12 (19.7%)	NS
Cesarean section	5 (8.2%)	6 (9.8%)	NS
Newborn weight, g	3343.3 [452.4]	3456.1 [434.7]	NS
Apgar scores (5min)	8.9 [0.1]	8.9 [0.8]	NS
UA pH	7.28 [0.072]	7.26 [0.076]	NS

The fetal QTc-intervals of each patient were analyzed separately. The mean of the QTc at the beginning and the end of recording were calculated (**Table 15**). The mean QTc at the beginning was 157 ms [SD 29.6] in ICP patients and 158 ms [SD 25.2] in healthy controls, and at the end of the registration 174 ms [SD 32.0] in ICP patients and 171 ms [SD 30.1] in healthy controls. There were no significant differences in these data between the ICP patients and healthy controls (p=0.467, [95% CI 0.993, 1.015]). In addition, the ICP patients who still had increased levels of ALT or/ and TBA regardless of treatment with UDCA before labor (n=44) were analyzed separately and compared with healthy controls (n=61), but there were no statistically significant differences in the QTc-values compared to healthy controls (p=0.992, [95% CI 0.986, 1.014]). The patients without UDCA medication (n=3) and the patients whose infants had low Apgar scores or acidosis at birth (n=2) were analyzed separately and compared with healthy controls. The number of these patients was small (n=5) but there were no differences between the QTc-values (p=0.906 and p=0.232). In the whole study material there were seven patients with biphasic ST-depression. None of these had ICP. Three had a normal vaginal delivery, one had a vacuum extraction and two patients had cesarean section due to an abnormal CTG and ST-events. All of these newborns had normal UA pH-values (pH >7.05) and normal Apgar scores.

Table 15. Corrected QT-time (QT_c) of patients with ICP and healthy controls at the beginning and end of delivery. The difference between the end and the beginning of delivery was not statistically significant.

	N	Variable	Label	Mean	Std Dev
Patients with ICP	61	QT_c	mean QTc at the beginning	157ms	29.6
			mean QTc at the end	174ms	32.0
Healthy controls	61	QT_c	mean QTc at the beginning	158ms	25.2
			mean QTc at the end	171ms	30.1

Table 16. Summary of studies I – IV. The values are given as means (range). Study II UCDA group/placebo, Study III all, UDCA group/ group without UDCA.

	Study I	Study II	Study III	Study IV
Number of ICP women	27	20	307	61
GSTA µg/l	51.0 (2.1-183.5)			
ALT U/L at first sampling	145.7 (6-393)	307.2 (23-860) / 155.5 (29-406)	152.8 179.2 (9-1021)/ 96.4 (8-520)	118.1 (6-485)
TBA µmol/L at first sampling	19.2 (3-52)	26.1 (3-59) / 22.5 (6-53)	19.2 20.5 (3-162) / 16.7 (1-235)	17.7 (7-127)
ALT U/L after*/ **after starting the treatment / ***before the labor		*64.4 (8-137) / 70 (49-83)	**160.0 **168.7 (5-1492)/ 129.0 (8-334)	***83.6 (7-538)
TBA µmol/L after*/ **after starting the treatment / ***before the labor		*6.6 (2-10) / 11.3 (6-14)	**15.6 **15.9 (3-146)/ 12.7 (3-118)	***15.9 (3-70)
UDCA		450g/day / -	450 g/day , 68%	450g/day, 95%
GWs at dg	35.5	32.6/33.8	34.8	34.9
GWs at delivery		37.1/36.1	37.9 37.4 / 38.9	38.7
UA pH	7.25	7.30/7.30	7.28 7.28/7.28	7.28

6. DISCUSSION

ICP is the most common liver disease during pregnancy. It is characterized by unexplained pruritus usually in the late second and third trimester of pregnancy and elevated BA and/or transaminases. It is a relatively nonthreatening condition to the mother, but it is associated with several fetal complications: higher risk for preterm delivery, MSAF, fetal distress and even IUFD (Brouwers et al. 2015). It is also known to recur in subsequent pregnancies. According to reviews ICP is related to an increased risk for hepatobiliary diseases among women, even hepatobiliary cancer, immune-mediated and cardiovascular disease in later life (Ropponen et al. 2006; Marschall et al. 2013).

6.1. Toward a more accurate diagnosis of ICP

The diagnosis of ICP is based strongly on the clinical symptom of pruritus mainly in the palms and soles and on elevated serum ALT values and/or BA concentrations. There may be several pathogenic entities behind these clinical and laboratory markers. Pruritus during pregnancy is quite common and should be distinguishable from ICP. Therefore; better specific and sensitive markers to diagnose true ICP are needed. However, it is often difficult to establish an accurate diagnosis by performing only routine laboratory tests because they are also affected by some other conditions during pregnancy.

According to our results, GSTA may be a new, promising diagnostic tool for ICP. We found that the mean GSTA concentration in the study group was significantly higher than in the group of women with a normal pregnancy. There was also a significant correlation between serum ALT and plasma GSTA levels, but not between the serum BA concentration and the plasma GSTA concentration in the study group. This may obviously be due to the large interindividual variation in TBA concentrations during pregnancy among subjects with ICP. UDCA is a bile acid itself and when serum TBA is analyzed, also the BAs administered in connection with UDCA treatment will be included. This may explain the increase in TBA during the first days after starting UDCA treatment (Glantz et al. 2005). This has to be taken into consideration especially for patients who have low TBA concentrations at diagnosis and start UDCA treatment (Lindstedt et al. 2010). Dann et al. reported that concentration of GSTA in the serum increased with gestation in patients with ICP (Dann et al. 2004), but in the present studies, there was no such association.

Concentrations of GSTA are higher in patients with ICP compared to patients with pruritus gravidarum and healthy pregnant controls (Dann et al. 2004). Abnormal high values of GSTA occur also in pre-eclampsia and the HELLP syndrome (Steegers et al.

1995). The rise of GSTA precedes that of ALT by several hours and it is an earlier and more sensitive indicator of hepatocellular damage in pre-eclamptic patients than other liver function tests (Steegers et al. 1995). Thus, GSTA might be an earlier and more sensitive laboratory test also for ICP patients, which would make it easier to identify those patients who need closer antepartum surveillance. However, samples for GSTA measurements are recommended to take after overnight fasting as well as TBA samples.

Other new diagnostic markers of ICP have been evaluated recently. The neutrophil-to-lymphocyte ratio was elevated in ICP patients and high levels predicted the severity of cholestasis (Kirbas et al. 2014). Also, an increased level of serum autotaxin activity (lysophospholipase D) is a highly sensitive, specific and robust diagnostic marker of ICP patients to distinguish ICP from pruritus gravidarum and other pregnancy-related liver diseases (Kremer et al. 2014). Serum autotaxin has no circadian rhythm and is not influenced by food intake (Kremer et al. 2015), which is an advantage for clinical acceptance. Different ratios calculated from individual bile acid determinations have been studied. An elevated litocholic acid and ursodeoxycholic acid / litocholic acid ratio may provide a more accurate diagnosis of ICP than TBA alone (Martinefski et al. 2012). Metabolites of progesterone sulfates in the serum are increased before the clinical signs of ICP emerge and may be used to distinguish ICP from benign pruritus (Abu-Hayyeh et al. 2015).

6.2. UDCA as treatment for ICP

Currently, the best treatment for ICP is UDCA. A recent meta-analysis reported that UDCA is effective in normalizing maternal serum ALT levels compared to controls and placebo (27.8% vs 9.4% and vs 14.3%) and at reducing ALT levels (65.9% vs 25.4%, and vs 20.0%) (Bacq et al. 2012). According to the same meta-analysis, serum TBA concentrations are reduced better by UDCA (in 54.3% of patients) than by no drugs (24.4%) and placebo (18.6%) (Bacq et al. 2012). Also the severity of pruritus was reduced statistically significantly better with UDCA than with placebo or no drugs (Bacq et al. 2012).

In our randomized study, the effect of UDCA on liver function was evaluated with repeated and extended laboratory testing. Alkaline phosphatase may be elevated in ICP, but it does not have a diagnostic value since alkaline phosphatase activity is enhanced due to placental and bone production during uncomplicated pregnancies. Triglyceride and total cholesterol levels are often increased in ICP (Zhang et al. 2014), as are FIDD levels (Kebapcilar et al. 2010). In our study serum levels of cholesterol, HDL-cholesterol and triglycerides, APTT, FIDD and estradiol, progesterone, prolactin and platelet count were not modified by UDCA administration. In a study by Dann et al. (2006) ICP was associated with an abnormal lipid profile but UDCA did not affect

plasma lipid concentrations (Dann et al. 2006). Since the levels of estradiol, progesterone and prolactin were not modified in our study by UDCA therapy, it seems obvious that UDCA has no suppressive effect on the placental hormonal function in contrast to dexamethasone, which is a clear benefit in favor of UDCA.

Corelik and associates showed in vitro study in rats that dexamethasone and UDCA protect against the arrhythmogenic effect of taurocholate (Gorelik et al. 2003). Most patients in our study with ICP had UDCA therapy and this may have decreased the fetal risk for arrhythmia by stabilizing the QTc-interval which was close to normal. Several experimental models have shown that UDCA may have a direct protective effect on the fetal compartment (Geenes et al. 2011). Treatment with UDCA reduces the levels of BA in the maternal and fetal compartments (Geenes et al. 2014) and there is no significant fetal metabolism of the increased exposure of BA of maternal origin in obstetric cholestasis (Geenes et al. 2014).

The dose of UDCA has varied between different randomized controlled trials. In most trials, the dose of UDCA has been between 600 and 900 mg/d (Diaferia et al. 1996; Nicastri et al. 1998; Roncaglia et al. 2004; Kondrackiene et al. 2005; Binder et al. 2006; Liu et al. 2006). In the studies of Palma el al. (1997) and Glantz et al. (2005) the UDCA dose was quite high, 1000 mg/d (Palma et al. 1997; Glantz et al. 2005). Floreani (1996) and associates used the same dosing as we did, 450 mg/d (Floreani et al. 1996). According to our results low-dose UDCA treatment was effective in ICP patients. The perinatal outcome was good, liver enzyme levels decreased during treatment and maternal side-effects were minimal. Also Bacq et al. concluded in their meta-analysis that UDCA therapy might benefit fetal outcomes (Bacq et al. 2012). It might reduce fetal distress, and the need for NICU treatment might decrease.

6.3. Risk for the fetus

In a Swedish prospective cohort study, the relationship between an adverse fetal outcome and the level of maternal BA was examined; the risk for spontaneous preterm delivery, asphyxial events and MSAF rose by 1 – 2% for each 1 μmol/L increase in maternal serum BA (Glantz et al. 2004). This became statistically significant when fasting maternal TBA exceeded 40 μmol/L. In a recent prospective, population-based case-control study there was a significantly increased risk for an adverse perinatal outcome, also fetal death, if ICP was severe (Geenes et al. 2014). Also in the present observational study, preterm birth and admission to NICU were related to severe ICP.

A prolonged QT-interval may predispose to ventricular arrhythmias and even sudden neonatal death (Schwartz et al. 1998). Changes in the QT-interval seem to be related to fetal wellbeing. In intrapartum hypoxia there is a significant shortening of the fetal QT-interval and the corrected QT-interval (Oudijk et al. 2004). The former study also showed that the QT-shortening was neither dependent on the fetal heart rate nor related

to the general stress of labor. Our study demonstrated that the corrected QT-intervals of fetuses to women with maternal ICP are similar to the intervals of healthy controls. We had no adverse neonatal outcomes and no fetal asphyxia or IUFD. The mean corrected QT-time at the beginning and the end of delivery showed no significant differences between ICP patients and healthy controls. Nor were there any differences in QT-times between women with ICP and persistently elevated liver enzymes before labor compared to healthy controls. This is a finding that has not been reported previously.

For now, there is no means to predict IUFD in ICP patients by antenatal testing. A recent study focused on fetal antenatal testing of fetal heart rate decelerations and delivery outcomes in severe and mild ICP. There were no differences in antenatal testing results between these two groups in comparison to healthy pregnant women (Sheibani et al. 2014). Nor could we demonstrate in our study any differences in corrected QT-intervals which would have implied a risk for fetal arrhythmia in ICP pregnancies during labor. Maybe the administration of UDCA to women with ICP resulted in a reduced risk and protection of the fetus.

Although recent studies have given a better understanding of the underlying pathophysiology of obstetric cholestasis, the pathogenesis and prognosis of ICP pregnancy are still obscure. Several study reports have concluded that IUFD in ICP may not be predictable by traditional antepartum monitoring and, unfortunately, we did not gain any new, clinically valuable information about the value e of FECG during labor in ICP women and healthy controls.

6.4. Timing of delivery

Nowadays, most deliveries to ICP patients are induced preterm with the aim of reducing the risk for an adverse fetal outcome. However, preterm babies have an increased risk for RDS and the risk is even higher with elective cesarean section than induced vaginal delivery (Curet et al. 1988). In addition, maternal cholestasis may predispose the newborn to unexpected RDS (Zecca et al. 2006). Zecca et al. reported that the incidence of RDS in newborns born to women with ICP is twice as high as in the reference population. They hypothesized that BA can cause surfactant depletion in the alveoli (Zecca et al. 2006).

The risks and benefits of preterm delivery must be considered individually. We evaluated our own hospital treatment strategy. We showed that there were more preterm deliveries (<37 weeks) in the group using UDCA due to an earlier onset and severity of ICP. The ICP diagnosis was also made five weeks earlier in UDCA group than in the group of pregnant women without medication. The rate of admissions to the neonatal unit was higher for children in the UDCA group (30% vs 11%), mostly due to preterm labor. However, Apgar scores at 5 minutes and fetal UA pH values were similar in both groups.

In the present study, most deliveries for patients with ICP were induced. The rates of vacuum extraction and cesarean deliveries were not different in ICP patients compared to the general population in our hospital during the study period. Our study results show that routine induction of labor in mothers with ICP does not increase the need for instrumental deliveries. It is suggested that in ICP, elective delivery at 37 GW, in addition to standard monitoring of fetal well-being, may significantly reduce IUFD rates without increasing the cesarean section rate (Roncaglia et al. 2002). In a semifactorial randomized clinical trial, early term delivery in ICP was not associated with an increased incidence of cesarean section (Chappell et al. 2012).

In our department we practice active management and close antenatal monitoring of pregnancies affected by ICP. Most patients are treated with UDCA and most deliveries of ICP patients are induced. In severe ICP there is a significant risk for an adverse perinatal outcome, including IUFD, and close antenatal monitoring of ICP pregnancies is recommended (Geenes et al. 2014).

The management of obstetric cholestasis consists of weighing the risk for premature delivery against the risk for sudden IUFD. According to a recent systematic review, which had identified only 14 published cases of unexplained term IUFD associated with ICP-affected pregnancies during a period of no less than 53 years, there is not enough scientific power to accept the wide practice of active management of ICP patients (Henderson et al. 2014). In 2006, the Royal College of Obstetricians and Gynecologists (RCOG) concluded that there are no evident findings to support or to refute the practice of active management of pregnancies complicated by obstetric cholestasis (RCOG 2006). Henderson et al. arrived at a recommendation of individual management of ICP-affected pregnancies rather than of active management routinely (Henderson et al. 2014). A recent comment on the management of ICP maintains that early elective delivery should be performed on an individualized basis (Marschall 2015). Maternal liver enzymes should be followed-up for normalization 6 – 12 weeks after delivery (Marschall 2015).

However, the number of IUFD due to ICP is small. Page and associates calculated the number needed to prevent one IUFD in the ICP cohort in a mean scenario based on the mean point of estimation: the number of induced deliveries needed to prevent one stillbirth no less than 2,127 (Page et al. 2015).

6.5. Future research

A critical arena for future research is to explore the crucial factors related to the expression of maternal ICP. Treatment with UCDA reduces the levels of BA in women with ICP. Can that optimize the intrauterine milieu for the fetus and prevent IUFD or preterm birth? Can it prevent the long-term metabolic consequences for the neonate? Although ICP is not usually symptomatic until the second or third trimester, is it

present earlier? At what point in time should UCDA be administered to provide maximum benefit? Papacleovoulou and associates suggested that exposure to BA in utero can lead to metabolic reprogramming in the developing child through epigenetic alterations (Papacleovoulou et al. 2013). They also showed in a mouse model and in vitro with a human model that the fetoplacental fenotype can be altered by maternal hypercholanemia (Papacleovoulou et al. 2013).

6.6. Study limitations and strengths

The number of patients was limited in the GSTA measurement and UDCA treatment studies (studies I and II). Unfortunately, in the randomized, controlled study (study II), many women on placebo delivered before the end of the follow-up, which reduced the statistical power of the study. However, it is possible that high TBA values and receiving placebo instead of UDCA were circumstances associated with this phenomenon. The observational study (study III) was retrospective and a subgroup of patients with severe ICP was induced at the time of diagnosis without any medication. In the study on fetal ECG (study IV) there were no power analyses and the sample size might have been too small to detect fetal arrhythmias, since such events are probably rare and the risk might be larger in genetically predisposed fetuses.

GSTA as a diagnostic marker of ICP has been studied only in one trial previously (Dann et al. 2004), and we confirmed earlier results of its value as an effective marker of ICP. UDCA was studied in a randomized trial and its safety was confirmed with an extensive evaluation of laboratory tests. In the retrospective study we evaluated all patients with ICP over a 5-year period in our hospital. The efficacy of a small dose of UDCA was proven. Our study was the first one in which FECG was evaluated as a potential, novel parameter for detecting fetal cardiac arrhythmias related to maternal ICP. Future large-scale studies are needed to clarify the perinatal influence on the neonatal outcome in the long term, the impact of UDCA on maternal and fetal compartments and early elective delivery on fetal outcome.

7. CONCLUSIONS

1. Plasma GSTA may provide a new diagnostic tool for the diagnosis of ICP in addition to ALT and TBA.

2. UCDA reduces maternal pruritus and improves liver function laboratory values. The side effects for the mother are minimal. UCDA treatment does not affect fetoplacental estrogen production. Maternal cholesterol, APTT and FIDD values are not affected by the treatment.

3. The obstetric outcome is good also with low-dose UDCA treatment. Patients with severe ICP (TBA $\geq$ 40μmol/L) have an increased risk for preterm delivery. In this group, ICP is diagnosed earlier during pregnancy and also ALT levels are significantly higher than in the group with mild ICP.

4. There are no differences between ICP patients and controls regarding fetal ECG waveforms, corrected QT-intervals and ST-waveforms.

ACKNOWLEDGEMENTS

This study was carried out in the Department of Obstetrics and Gynecology, Turku University Hospital, University of Turku, in collaboration with the Department of Clinical Chemistry during the years 1997-1998 and 2008-2015. I express my warmest gratitude to the following persons.

Head of Department, Professor Seija Grénman for her positive attitude toward the scientific work carried out at the clinic. You have inspired me with your energy and kindness. You have not only encouraged me to finalize my theses but also to become a part of the gynecological oncology team.

Professor Juha Mäkinen for crucial advice and support during these years. Your tremendous experience and knowledge were priceless especially near the completion of this work.

My supervisor, Susanna Timonen, MD, PhD, for guidance and a positive and supportive attitude toward me and my work throughout these years. You have always had time for me and encouraged me through all these years. You have shown a deep understanding for my other duties during these years, have carefully listened to my worries and pushed me forward.

My supervisor, Ulla Ekblad, Adjunct Professor, for guidance and support. Your experience as a scientist and a clinician has inspired me profoundly. Both of my supervisors have also introduced me to the art of obstetrical practice and guided me along the learning curve. I truly admire your knowledge as a clinicians and scientists.

All co-authors of the original articles: Riitta Leino, MD, who collected the clinical data for the two first studies, Professor Kari Pulkki for the analyses of GSTA, Pertti Palo, MD, PhD, for scientific contributions and a kind and constructive attitude, Maria Lindén, MD, for help with data collection, Professor Karl G. Rosen for sharing his expertise in fetal electrocardiographic waveforms and for revising the last manuscript, and Pia Suvitie, MD, for statistical advice and enormous help whenever needed.

The official reviewers of these theses, Leea Keski-Nisula, Adjunct Professor, and Kalevi Laitinen, Adjunct Professor, for constructive criticism and valuable comments during the review process.

The steering group members of this dissertation project: Mika Nuutila, Adjunct Professor, and Kirsi Rinne, MD, PhD, for valuable advice and a proactive and supportive attitude toward my work.

Robert Paul, Adjunct Professor, Certified translator, for revision of the English language of the thesis.

Anne Kaljonen and Teemu Kemppainen for the statistical analysis of the studies.

Sirpa Erkinharju for permission to use her portrayal as a cover.

My boss and senior, Sakari Hietanen, Adjunct Professor, for a warm, generous attitude toward my scientific endeavors. You have also created an excellent gynecological oncology team in which I am honored to work. You have taught and inspired me and led me to a deep appreciation of gynecological oncology. I am also thankful for your positive attitude toward everyday life.

Annika Auranen, Adjunct Professor, for guidance and for being my mentor for several years.

My wonderful colleagues in the gynecological oncology team: Marjut Rintala, MD, PhD, Johanna Hynninen, MD, PhD, Sara Narva, MD. Your support and our time together have been invaluable to me. Johanna has also been my mentor and solved my chronic technical computer problems. I also thank you for our friendship. Minna Mäenpää, MD, for support and friendship.

All my other lovely colleagues and all the nurses at the Department of Obstetrics and Gynecology. The time we have spent together, so full of life and zest, is something I shall cherish throughout my life. My colleagues in science on the 12th floor: Thank you for our time together with our respective projects, working side by side. Thank you Johanna, Pia S, Pia H and others.

And, of course, all participants in this study, without whom this work would never have been brought about.

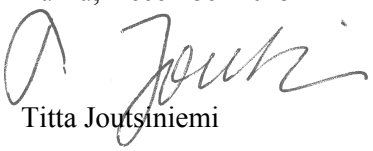
All my friends for kindness and support. My neighbors, Cami and Ekki for your enormous help with my daily duties with the dogs and the chores in the house. You helped me out whenever I needed. Thank you for the lovely evenings and our trips together.

My parents, Tuula and Kari Manninen, for never ending love and support through all the days of my life. You have always had faith in me and encouraged me. I am so grateful to my mother for her wholehearted and overwhelming care of my children, pets and house – and even me, when needed. My sister Paula and her family for trust and friendship. Elmo and Silja, you are so cute.

Finally I express my deepest gratitude to my precious children, Siiri and Alvar. You are my everything. I love you so much.

This study was financially supported by the EVO Foundation of the Turku University Hospital, the Turku University Foundation, the Turku University Medical Faculty Foundations and the Finnish Medical Society Duodecim.

Turku, December 2015



Titta Joutsiniemi

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