ORIGINAL ARTICLE

Case Control Study

Liver stiffness in pregnant women with intrahepatic cholestasis of pregnancy: A case control study

Juliane Nees, Franziska J Ammon, Johannes Mueller, Herbert Fluhr, Sebastian Mueller

Juliane Nees, Franziska J Ammon, Department of Gynecology and Obstetrics, University Hospital Heidelberg, Heidelberg 69120, Germany

Johannes Mueller, Sebastian Mueller, Center for Alcohol Research, University Hospital Heidelberg, Heidelberg 69120, Germany

Herbert Fluhr, Division of Obstetrics, Department of Obstetrics and Gynecology, Medical University of Graz, Graz 8036, Austria

Corresponding author: Sebastian Mueller, PhD, Professor, Center for Alcohol Research, University Hospital Heidelberg, Im Neuenheimer Feld 350, Heidelberg 69120, Germany. sebastian.mueller@urz.uni-heidelberg.de

Abstract

BACKGROUND

Intrahepatic cholestasis of pregnancy (ICP) is a rare but severe complication for both the mother and the unborn child. The diagnosis is primarily based on elevated serum levels of bile acids. In a large ICP cohort, we here study in detail liver stiffness (LS) using transient elastography (TE), now widely used to non-invasively screen for liver cirrhosis within minutes.

AIM

To specifically explore LS in a large cohort of women with ICP compared to a control group with uncomplicated pregnancy.

METHODS

LS and hepatic steatosis marker controlled attenuation parameter (CAP) were measured in 100 pregnant women with ICP using TE (Fibroscan, Echosens, Paris, France) between 2010 and 2020. In 17 cases, LS could be measured postpartum. 450 women before and 38 women after delivery with uncomplicated pregnancy served as control group. Routine laboratory, levels of bile acids and apoptosis marker caspase-cleaved cytokeratin 18 fragment (M30) were also measured.

RESULTS

Women with ICP had significantly elevated transaminases but normal gamma-glutamyl transferase (GGT). Mean LS was significantly increased at 7.3 ± 3.0 kPa compared to the control group at 6.2 ± 2.3 kPa ($P < 0.0001$). Postpartum LS decreased significantly in both groups but was still higher in ICP (5.8 ± 1.7 kPa *vs*

4.2 ± 0.9 kPa, *P* < 0.0001), respectively. In ICP, LS was highly significantly correlated with levels of bile acids and M30 but not transaminases. No correlation was seen with GGT that even increased significantly after delivery in the ICP group. Bile acids were mostly correlated with the liver apoptosis marker M30, LS and levels of alanine aminotransferase, aspartate aminotransferase, and bilirubin. In multivariate analysis, LS remained the sole parameter that was independently associated with elevated bile acids.

CONCLUSION
In conclusion, LS is significantly elevated in ICP which is most likely due to toxic bile acid accumulation and hepatocyte apoptosis. In association with conventional laboratory markers, LS provides additional non-invasive information to rapidly identify women at risk for ICP.

Key Words: Intrahepatic cholestasis of pregnancy; Transient elastography; Bile acids; Liver stiffness; High risk pregnancy

Core Tip: Intrahepatic cholestasis of pregnancy (ICP) is a rare but severe complication in both mothers and unborn children. In a large ICP cohort, we studied liver stiffness (LS) in detail using transient elastography, which is now widely used for non-invasive screening of liver cirrhosis within minutes. LS is significantly elevated in pregnancies with ICP, most likely owing to toxic bile acid accumulation and hepatocyte apoptosis. Interestingly, no correlation was observed with γ-glutamyl transferase. In association with conventional laboratory markers, LS provides a novel non-invasive tool to rapidly identify women at risk for pregnancy complications.

INTRODUCTION

Approximately 3% of pregnant women have liver disorders that can cause severe problems for the mother and unborn child, *e.g.*, liver failure, preterm labor, and stillbirth[1-3]. Despite intensive research on pregnancy-related liver complications in recent decades, treatment options are still insufficient, and no effective screening tests for early assessment have been established[4-6]. Intrahepatic cholestasis of pregnancy (ICP) with elevated serum bile acid levels higher than 20 µmol/L is the most common pregnancy-specific liver disease. Its etiology is complex and consists of genetic, endocrine (circulating estrogen and progesterone), and environmental factors (reduced vitamin D and selenium levels in winter). Severe forms with bile acids levels higher than > 40 µmol/L are associated with abnormal fetal echocardiography, meconium-stained amniotic fluid, spontaneous preterm labor and fetal asphyxia. Moreover, women with a total serum bile acid level of 100 µmol/L have an increased risk of stillbirth. The incidence varied between 0.05% and 27.6% for all pregnancies[7-12].

ICP typically presents in the third trimester as nocturnal pruritus of the soles and palms[13]. ICP also increases the risk of gestational diabetes and pre-eclampsia[14]. Long-term consequences of ICP include a higher risk of cancer of the liver and biliary tree, diabetes mellitus, thyroid disease, autoimmune diseases (psoriasis, inflammatory polyarthropathies, and Crohn's disease), and cardiovascular disease[15]. Treatment with ursodeoxycholic acid has been proven to significantly improve itching, blood levels, and fetal outcomes in numerous studies; however, pregnancy termination remains the only causal therapy[2,7]. A rapid diagnosis of ICP is essential to protect mothers and children from (long-term) complications [16]. The diagnosis is normally based on elevated serum levels of bile acids. Unfortunately, these blood tests usually take several hours, even at maximum care facilities and are not available at any time. To date, no screening tests are available for liver disease during pregnancy, except serological testing for viral hepatitis in the third trimester.

Despite the scarcity of ICP data, enormous progress has been made in the molecular understanding of cholestatic liver disease in recent decades[10]. Hepatocytes and cholangiocytes cooperatively produce bile, which is a mixture of organic and inorganic compounds[17]. Cholestasis usually describes the impairment of bile flow caused by defects in hepatocytes, which form and secrete bile, and/or defects in the secretory machinery of cholangiocytes[17]. The detergent properties of bile render it highly toxic to cells and tissues[17]. In addition to drugs, inflammation, liver disease, and hormones, several gene mutations have been discovered that can cause cholestasis and ICP[8-10,18-21].

Measurement of liver stiffness (LS) using elastographic techniques has become the gold standard for the noninvasive diagnosis of liver fibrosis and cirrhosis and it often avoids invasive liver biopsies[22]. Transient elastography (TE) (FibroScan, Echosens, Paris, France), the first elastographic technique, is an ultrasound-based technique that uses a transducer probe to create an elastic shear wave[23]. Pulse-echo ultrasound is used to measure shear wave velocity, which is directly associated with LS expressed in kilopascals (kPa). TE requires only a few minutes, is highly accurate, and has a lower sampling error than biopsy, thus allowing for repetitive measurements[24]. LS values below 6 kPa are

considered normal, while the generally accepted cutoff values for liver fibrosis (F3) and cirrhosis (F4) are 8 and 12.5 kPa [25]. However, LS is not only elevated by the fibrosis stage but also by other important confounding factors, including physiological conditions such as food and alcohol intake, or pathological confounders such as inflammation or pressure elevation[22]. Of note, all these confounders and artifacts will always increase LS but never decrease it, which is the most important reason, while normal LS has a very high negative predictive value in excluding liver pathologies[24].

Therefore, liver elastography is an ideal diagnostic tool to address hepatic complications during pregnancy. In the first elastography study of > 500 pregnant women without liver disease, we recently demonstrated that LS increased significantly in the third trimester and was an independent predictive factor for pre-eclampsia[26]. These findings were independently confirmed in a smaller study in Denmark[27]. Moreover, in pregnant women with cirrhosis, LS predicts hepatic decompensation after delivery[28]. The aim of the present study was to specifically explore LS in a large cohort of women with ICP compared with a control group with uncomplicated pregnancies.

MATERIALS AND METHODS

Study design and patient cohort

The study protocol (435/2006 and S201/2015) of this observational, prospective, case-control study was approved by the Ethics Committee of the University of Heidelberg and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. The study design is shown in Figure 1. Briefly, between February 2010 and March 2020, 652 women were recruited at the Department of Gynecology at the University of Heidelberg or Salem Medical Center in Heidelberg during prenatal ultrasound or who presented to the prenatal outpatient department with prenatal complications or in the ward. Postpartum examinations occurred 24 h after delivery. Inclusion criteria were age ≥ 18 years and an intact pregnancy at weeks 9 to 42 or status postpartum. The healthy control cohort was obtained from our previous study[26]. ICP was diagnosed based on typical clinical symptoms, such as pruritus, and laboratory markers, such as elevated transaminase and serum bile acid levels > 20 µmol/L. The exclusion criteria were as follows: No signed informed consent; other pregnancy complications, such as preeclampsia or HELLP syndrome; and no valid LS measurements.

Laboratory parameters

Routine blood parameters were measured at the General Laboratory of University Hospital Heidelberg and Limbach Laboratory in Heidelberg. In 100 patients, serologically detected caspase-cleaved (M30) and total (M65) cytokeratin 18 levels as markers of liver apoptosis was measured as described previously using ELISA (Peviva, Bromma, Sweden)[29]. We also measured total bile acids not only in women suspected of having ICP but also in 60 women in the control group for comparative purposes.

LS and controlled attenuation parameter

LS and controlled attenuation parameter (CAP) were measured using TE (FibroScan, Echosens, Paris, France). M or XL probes were used according to the manufacturer's specifications and placed in the right lobe of the liver at the intercostal position, as described previously[30]. LS and CAP values were calculated as the medians of at least 10 consecutive measurements. In parallel to liver elastography in the control group, routine abdominal ultrasound was performed to exclude liver pathologies, such as liver cirrhosis, liver congestion, or liver tumors. In addition, the degree of liver steatosis was graded (0–3) and the spleen size was determined. Cutoff values from a recent meta-analysis were used[31]. Valid LS measurements were obtained for all the women.

Statistical methods

Statistical analyses were performed using the SPSS Statistics [version 23.0 (IBM, New York, United States), Excel 2016 (Microsoft, Redmond, United States), and GraphPad Prism 6 (GraphPad Software, San Diego, United States)]. For group comparisons, means and standard deviations were calculated, and an independent samples t-test was used. The Spearman rank-order correlation coefficient was calculated to conduct correlation analysis. Univariate and multivariate binary logistic regression analyses were used to identify the independent predictors of pregnancy complications, and receiver operating characteristic (ROC) analysis was performed.

RESULTS

Patient characteristics

For a better comparison, Table 1 presents only the characteristics of women (control and ICP) in the third trimester and after delivery. Almost all women with ICP (98 of 100, 98%) were in their third trimester, which is consistent with the literature[32]. In the control cohort, 228 of 450 patients (50.7%) were in the third trimester. A smaller number of women were followed up 1 d after delivery for both controls and ICP (*n* = 38 and *n* = 17). Supplementary Table 1 shows patient characteristics of the entire cohort in all trimesters. Accordingly, differences between the controls and patients with ICP remain. Women with ICP were significantly younger, and by definition, bile acids were significantly increased by a factor of approximately 6 ($P < 0.0001$). Women with ICP also had significantly elevated transaminase [predominantly alanine aminotransferase (ALT)] and bilirubin levels, but not elevated levels of alkaline phosphatase (AP) and gamma-glutamyl transferase (GGT). The levels of caspase 3-cleaved CK18, liver apoptosis marker M30, and uncleaved CK18 (M65), repres-

Table 1 Patient characteristics for the third trimester and after delivery

Parameters	Normal range[1]	ICP		Control	
		3. trimester, *n* = 98	Postpartal, *n* = 17	3. trimester, *n* = 228	Postpartal, *n* = 38
Age (years)		31.0 ± 4.2[a]	28.3 ± 3.5[c]	32.2 ± 5.2	32.9 ± 4.3
Bile acids (μmol/L)	2-5	39.0 ± 29.4[d]	23.7 ± 11.6[a]	6.8 ± 6.9	2.3 ± 1.2
AST (U/L)	< 35	116 ± 106[d]	88.2 ± 80[a]	26 ± 27	28 ± 6
ALT (U/L)	< 35	211 ± 202[d]	127 ± 106[b]	18 ± 26	15 ± 8
GGT (U/L)	< 40	23 ± 22	48 ± 40[a]	30 ± 55	18 ± 18
AP (U/L)	35-105	178 ± 68	182 ± 73	150 ± 95	283 ± 344
Bilirubin total (mg/dL)	< 1.3	0.65 ± 0.27[d]	0.52 ± 0.56	0.47 ± 0.26	0.65 ± 0.35
M30 (U/L)	< 200	432 ± 220[a]	293 ± 75[a]	339 ± 207	296 ± 50
M65 (U/L)	< 400	1180 ± 571[d]	777 ± 426[d]	680 ± 343	486 ± 89
Creatinine (mg/dL)	< 1.3	0.69 ± 0.38[a]	0.88 ± 0.39[a]	0.57 ± 0.12	0.61 ± 0.12
Urea (mg/dL)	< 50	19.8 ± 6.8[a]	25.5 ± 6.4[b]	16.9 ± 4.5	19.4 ± 5.3
Uric acid (mg/dL)	3.5-7.2	5.6 ± 2.4[d]	6.3 ± 2.8	3.9 ± 0.9	4.7 ± 1.0
Total protein (g/L)	66-83	67.6 ± 4.2[b]	65.2 ± 6.8	64.7 ± 4.4	66.3 ± 9.5
Albumin (g/L)	38-59	35.6 ± 2.7	36.3 ± 12.7	35.7 ± 2.7	
Leukocytes (1/nL)	3.7-10.0	8.8 ± 2.4[d]	12.0 ± 3.4[a]	10.8 ± 3.0	15.5 ± 4.8
Erythrocytes (1/pL)	4.1-5.1	3.9 ± 0.4	3.6 ± 0.6	4.0 ± 0.4	3.7 ± 0.5
Hemoglobin (g/dL)	12-16	11.4 ± 1.1	10.3 ± 1.5	11.7 ± 1.3	10.8 ± 1.5
Hematocrit (%)	36-43	33 ± 3	30 ± 6		
MCV (/pL)	80-96	86.1 ± 4.4	88.0 ± 3.5		
Platelets (1/nL)	150-360	228 ± 72	241 ± 61	227 ± 68	237 ± 61
Haptoglobin (g/L)	0.3-2.0	0.8 ± 0.4	1.0 ± 0.8		
Quick (%)	70-120	123.3 ± 5.1	119.4 ± 21.1	123.0 ± 13.0	124.5 ± 4.4
INR	< 1.1	0.91 ± 0.04	0.90 ± 0.03	0.90 ± 0.03	0.90 ± 0.03
Grade of steatosis in United States (0-3)	0	0.42 ± 0.50[a]	0.14 ± 0.38	0.18 ± 0.42	0.00 ± 0.00
Spleen size (cm)	< 11	11.4 ± 1.9	11.3 ± 1.1	11.1 ± 1.5	11.1 ± 1.7
Liver stiffness (kPa)	< 6	7.3 ± 3.0[c]	5.8 ± 1.7[d]	6.2 ± 2.3	4.2 ± 0.9
CAP (dB/m)	< 290 (S3)	206 ± 49[c]	213 ± 43	228 ± 39	224 ± 46

[1]Normal range for women.

[a]*P* < 0.05 *vs* controls in the corresponding group (3. trimester or postpartal).

[b]*P* < 0.01 *vs* controls in the corresponding group (3. trimester or postpartal).

[c]*P* < 0.001 *vs* controls in the corresponding group (3. trimester or postpartal).

[d]*P* < 0.0001 *vs* controls in the corresponding group (3. trimester or postpartal).

ICP: Intrahepatic cholestasis of pregnancy; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; AP: Alkaline phosphatase; MCV: Mean corpuscular volume; INR: International normalized ratio; CAP: Controlled attenuation parameter; M65: Soluble cytokeratin 18; M30: Caspase-cleaved cytokeratin 18 fragment.

entative of liver necrosis, were significantly elevated. However, M65 showed a twofold increase in women with ICP, whereas M30 was only slightly higher in this group. Interestingly, although all liver-related parameters decreased after delivery, GGT was the only marker with postpartum elevation compared with controls.

LS is significantly increased in women with ICP

As shown in Figure 2A and Table 1, LS was significantly higher in women with ICP, both before and after delivery. Moreover, in both groups, we observed an increase in LS as pregnancy progressed. In the ICP group, mean LS was significantly increased at 7.3 ± 3.0 kPa compared with the control group at 6.2 ± 2.3 kPa (*P* < 0.0001). An increase in LS to > 6 kPa was observed in 24.9% of healthy pregnant women and 44.2% of the ICP group, whereas an elevated LS of > 8
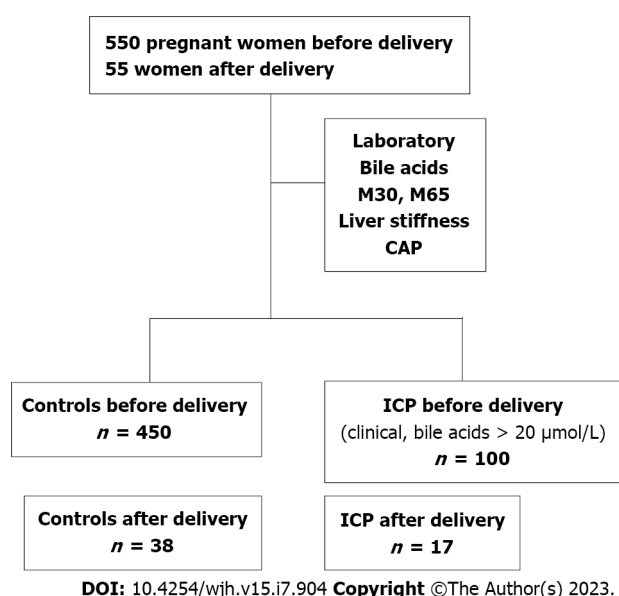
Figure 1 Study design. ICP: Intrahepatic cholestasis of pregnancy.
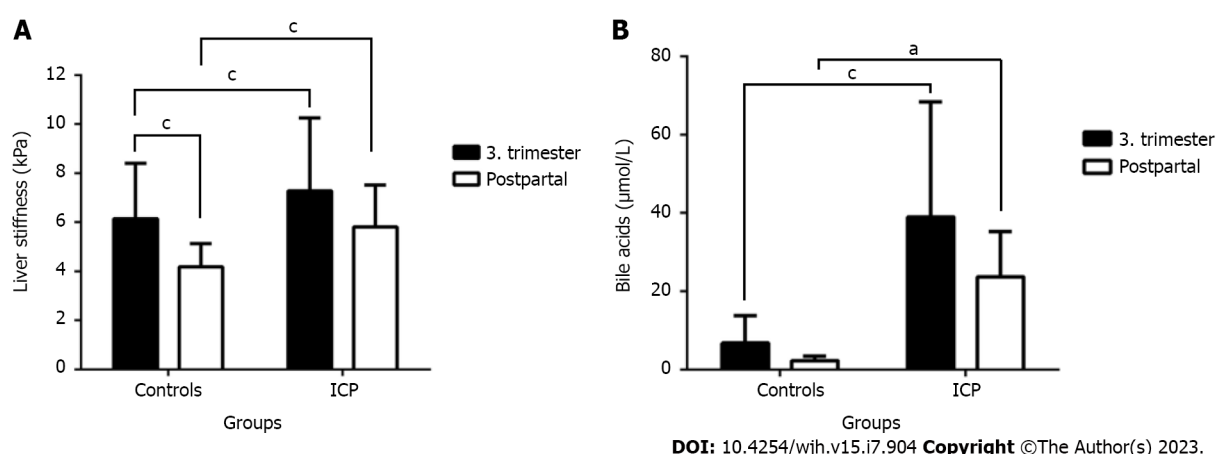
Figure 2 The 3. trimester and postpartal in the intrahepatic cholestasis of pregnancy and control group (ᵃ*P* < 0.05, ᶜ*P* < 0.001). A: Liver stiffness (mean and standard deviation); B: Bile acids (mean and standard deviation). ICP: Intrahepatic cholestasis of pregnancy.

kPa was observed in 7.1% of the control and 15.6% of the ICP group. LS of > 12.5 kPa considered above the cutoff value for cirrhosis was measured in 3.0% of the control and ICP groups. In confirmation to our initial study[26], however, postpartum LS decreased significantly in both groups to 5.8 ± 1.7 kPa in the ICP group and 4.2 ± 0.9 kPa in the control group, respectively. Finally, hepatic steatosis, as measured by the CAP, was normal in most women. It slightly but significantly increased in the ICP group during delivery, from 206 to 213 dB/m, whereas no significant changes were observed in the controls (Table 1). In summary, although LS generally increases during pregnancy, the liver is significantly stiffer in women with ICP before and after delivery than in controls without hepatic complications.

Parameters associated with LS elevation
Next, we performed Spearman's rho correlation analysis with clinical and laboratory parameters to identify potential confounders associated with elevated LS. Table 2 shows the results of the ICP, control, and total cohorts. In the ICP cohort, only a few parameters were significantly correlated with LS, namely, serum levels of bile acids and the liver apoptosis marker M30. Bilirubin levels hardly met the level of significance, whereas leukocyte count and Quick's test results were negatively correlated. No association between LS and bile acid levels was observed in the control group, and M30 Levels were weakly but significantly correlated with LS. In contrast, as described recently[26], LS significantly correlated with the duration of pregnancy, onset of gestational diabetes, body weight, mean arterial diastolic pressure, and AP and M65 levels in the total cohort. Interestingly, AST levels, which are usually highly associated with LS in liver diseases[33], were significantly correlated in the total cohort but not in the ICP or control groups alone. No correlation was observed between GGT and ALT levels in the ICP cohort. Finally, no association was observed between markers of hemolysis or anemia. In conclusion, in women with diagnosed ICP, bile acids are tightly associated with elevated LS and markers of liver apoptosis, but not with conventional liver function tests, except for bilirubin.

Table 2 Spearman Rho correlation with liver stiffness for women with intrahepatic cholestasis of pregnancy, controls and all

Parameter	Spearman rho correlation with liver stiffness		
	ICP, *n* = 100, r	Control, *n* = 450, r	All, *n* = 550, r
Bile acids total (μmol/L)	0.368[c]	0.085	0.438[d]
M30 (U/L)	0.881[b]	0.313[b]	0.385[c]
Quick (%)	-0.276[b]	0.210[a]	-0.010
Leukocytes (1/nL)	-0.223[a]	0.012	-0.203[b]
Bilirubin total (mg/dL)	0.213[a]	-0.091	0.124
MAD (mmHg)	-0.379	0.137[b]	0.154[c]
Gestational diabetes (1 or 0)	0.376	0.269[d]	0.301[d]
Pruritus (1 or 0)	0.353	-0.015	0.246[d]
Spleen size (cm)	0.340	-0.032	0.014
Uric acid (mg/dL)	0.308	0.261[a]	0.411[d]
AST (U/L)	0.146	0.103	0.327[d]
Creatinine (mg/dL)	0.281	0.057	0.163
Body weight (kg)	0.241	0.301[d]	0.317[d]
Platelets (1/nL)	-0.119	0.000	-0.074
AP (U/L)	0.237	0.329[c]	0.378[d]
Urea (mg/dL)	0.234	-0.064	0.041
Hemoglobin (g/dL)	-0.112	-0.022	-0.104
CAP (dB/m)	0.097	0.083	-0.006
M65 (U/L)	0.357	0.389[c]	0.452[d]
ALT (U/L)	0.048	0.047	0.273[d]
GGT (U/L)	-0.010	0.114	0.150

[a]*P* < 0.05.

[b]*P* < 0.01.

[c]*P* < 0.001.

[d]*P* < 0.0001.

Note that parameters are sorted first by levels of significance in the intrahepatic cholestasis of pregnancy group (p) and then the absolute correlation coefficient in descending order. Most relevant parameters are on top. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; AP: Alkaline phosphatase; ICP: Intrahepatic cholestasis of pregnancy; CAP: Controlled attenuation parameter, MAD: Mean arterial diastolic pressure; M65: Soluble cytokeratin 18; M30: Caspase-cleaved cytokeratin 18 fragment.

LS is independently associated with elevated bile acids in ICP

Figure 2B shows the levels of bile acids in both controls and women with ICP before and after delivery. Bile acid levels, which are a major criterion for the diagnosis of ICP were about six times elevated in the ICP cohort and promptly decreased after delivery. A slight but significant decrease was observed in the control group, suggesting that pregnancy causes bile acid elevation. Notably, bile acid levels were markedly elevated in the ICP cohort after delivery. Supplementary Table 2 lists the parameters associated with elevated bile acid levels. Bile acids are significantly associated with clinical features of pruritus and gestational diabetes. Among the laboratory parameters, bile acids were mostly correlated with the liver apoptosis marker M30 and the levels of ALT, AST, and bilirubin. Haptoglobin levels, leukocyte counts, and coagulation test results (Quick) were negatively associated with them. No association was observed between liver steatosis (CAP), M65 levels, AP, or splenic size. In the total cohort, bile acids were best associated with ALT, AST, and LS, in descending order. In multivariate analysis, LS remained the only parameter independently associated with elevated bile acid levels (Supplementary Table 3). In conclusion, in patients with ICP, bile acids are closely associated with liver damage in the form of apoptosis, and LS is independently associated with bile acid levels. In the third trimester, a LS of 6.5 kPa significantly discriminates between ICP and controls (*P* = 0.033) although with a modest AUROC of 0.65 (0.58–0.72, *P* = 0.033).

DISCUSSION

In this study, we noninvasively measured LS through TE and steatosis using CAP in a large cohort of pregnant women with diagnosed ICP, primarily through elevated bile acids. Our data clearly show that LS is higher in women with ICP than those in controls. Although LS decreased rapidly after delivery, as described recently[26,27], it remained significantly higher in women with ICP despite identical follow-up observation times. Women with ICP predominantly had elevated ALT levels, followed by those with elevated AST and bilirubin levels. In addition to AP levels, no differences were observed in GGT levels. Although all parameters, including LS, improved after delivery, GGT levels increased significantly in the ICP cohort. In addition, hepatic fat content, as measured using CAP, although within the normal range, was lower in patients with ICP than in controls. Finally, the liver apoptosis marker M30 showed the highest association with bile acid levels in univariate regression analysis, whereas LS remained the strongest independent predictor of bile acid levels > 20 mol/L in multivariate regression analysis.

In confirmation of earlier reports[26-28], the present study demonstrates that noninvasive assessment of LS through elastography is feasible and well accepted in pregnant women. In contrast to reports from internal medicine departments [34], elastography can be performed in all women. Second, we showed that LS is significantly elevated in women with ICP and higher than that in controls, which is remarkable, as we and others showed that LS is generally and reversibly elevated in the third trimester[26,27]. Consequently, and comparable to the previously reported LS elevation in women with preeclampsia, LS can be considered a feasible and noninvasive tool for screening, identifying, and following women with pregnancy-related liver complications.

What are the confounding factors for LS elevation in women with ICP? In contrast to initial beliefs, LS can be elevated because of many confounding factors, including inflammation, arterial and venous pressure elevation, and physiological conditions such as meal intake[22,24]. Mechanic cholestasis has been demonstrated to reversibly increase LS[35]. Of note, however, continued elevation of LS owing to these confounders has a negative impact on the liver, and the first long-term mortality data demonstrated that LS is one of the best long-term predictors of liver-related and all-cause mortality[22]. LS measurement can also be used to identify and monitor pregnant women with preexisting liver cirrhosis and predict hepatic decompensation[28]. The first study in women with uncomplicated pregnancies showed that elevated LS was significantly correlated with AP, leukocytes, gestational age, and an increase in body weight[26]. In the present study, in women with ICP, the confounders were completely different, and LS was tightly associated with elevated serum levels of bile acids and serum markers of liver apoptosis (M30). This is particularly interesting with regard to the fact that in liver diseases, such as alcoholic liver disease or viral hepatitis, LS elevation is typically associated with transaminase levels, namely, AST but not ALT[33]. Although we show here that LS is an independent predictor of elevated bile acids, the performance of LS in predicting ICP was only moderate and lower than that in the previous smaller ICP cohort[26].

To the best of our knowledge, this is the first study to show an exceptionally strong association between the established serum liver apoptosis marker (M30) with levels and bile acid levels in women with ICP and its association with elevated LS. In the multivariate analysis, LS remained the only parameter independently associated with elevated bile acid levels. In patients with liver disease, the association between liver apoptosis and LS elevation has already been shown both at the histological level[36] and using serum markers, such as M30[29]. The tight association of bile acids with LS and M30 levels in pregnancy is new. Bile acids are synthesized in hepatocytes as essential components for bile formation[9,17]. Owing to their detergent nature, however, they are highly cytotoxic and can disrupt cellular membranes if not protected, *e.g.*, by phospholipids[9,17,19]. Specifically, serum cholic acid becomes the primary bile acid in women with ICP, in contrast to normal pregnant and non-pregnant women, whose proportion is similar to that of chenodeoxycholic acid[37]. Typical examples are cholestatic liver diseases, such as primary biliary cirrhosis, which ultimately cause chronic bile duct inflammation, and later cirrhosis and cancer. Even simple mechanical cholestasis through obstruction of the major bile ducts by biliary stones can cause severe tissue damage.

In the last three decades, many gene mutations have been discovered that can cause cholestasis through the impairment of hepatocyte or cholangiocyte transport proteins relevant to bile formation[8-10,18-20]. These findings have resulted in a group of diseases known as progressive familial intrahepatic cholestasis. The normal GGT levels in our ICP cohort are a strong argument for a genetic cause. Hormonal changes/normalization after delivery with subsequent normalization of bile acid export through the hepatocellular apical membrane are considered important for the role of sex hormones in ICP[9]. In line with this, we observed a postpartum increase in GGT in our ICP cohort, suggesting a reinduction of GGT with the onset of bile flow.

Surprisingly, hepatic steatosis, as measured by CAP, which is now widely explored in patients with fatty liver[38], did not show any conclusive data in women with normal pregnancy or with ICP. The reason for this remains unclear because we expected that at least some women would present with steatosis, which can be a severe complication of pregnancy. We also briefly discuss some of the limitations of our study, which are mostly due to the challenging setting of performing clinical studies during late pregnancy, particularly in women with suspected complications. Serum was not available for all women to allow for the subsequent measurement of markers such as M30 and M65. In addition, we managed to measure LS sequentially before and after delivery in only a few cases in the same person. Another limitation with regard to postpartum follow-up measurements was that we only included women 24 h after delivery. This short time may explain why some parameters did not reach statistical significance.

CONCLUSION

In conclusion, we showed in a large cohort that women with ICP show significantly elevated LS compared with women

with uncomplicated pregnancies. In contrast to the large body of evidence in the liver literature, elevated LS in ICP is primarily correlated with the accumulation of bile acids known to be highly toxic to hepatocytes, and liver apoptosis, as measured through M30 levels, but not with transaminases, bilirubin, or GGT. We also showed for the first time that typically low GGT levels in ICP increase after delivery. Consequently, we believe that screening for LS in pregnancy is not "another diagnostic tool" to further complicate the already intensive surveillance during pregnancy, but could provide a novel non-invasive strategy to early identify women at risk for complications.

ARTICLE HIGHLIGHTS

Research background

Intrahepatic cholestasis of pregnancy (ICP) is a rare but severe hepatic complication for both mother and unborn child. Diagnosis is normally based on elevated serum levels of bile acids. Unfortunately, these blood tests usually take several hours, even at maximum care facilities. So far, there are no screening test for liver disease in pregnancy besides serological testing for viral hepatitis in the third trimester.

Research motivation

Measurement of liver stiffness (LS) through elastographic techniques has become the novel gold standard for the non-invasive diagnosis of liver cirrhosis often avoiding invasive liver biopsies. LS is not only highly correlated to the hepatic fibrosis stage but can also be elevated due to other important confounding factors such as inflammation, congestion or cholestasis. For these reasons, liver elastography could be an ideal diagnostic tool to address hepatic complications during pregnancy.

Research objectives

The aim of the present study was to specifically explore LS in a large cohort of women with ICP before and after delivery compared to a control group with uncomplicated pregnancy.

Research methods

LS and the hepatic steatosis marker controlled attenuation parameter (CAP) were measured in 100 pregnant women with ICP using transient elastography (Fibroscan, Echosens, Paris, France). In 17 cases, LS could be measured after delivery. A large cohort of women with uncomplicated pregnancy served as control group. Routine laboratory, levels of bile acids and the apoptosis marker M30 were also measured.

Research results

In the third trimester, women with ICP show a significantly increased LS at 7.3 ± 3.0 kPa compared to controls (6.2 ± 2.3 kPa, $P < 0.0001$). LS decreases significantly 24 h after deliver and remains higher in ICP (5.8 ± 1.7 kPa *vs* 4.2 ± 0.9 kPa, $P < 0.0001$). In ICP, LS is mainly correlated with levels of bile acids and the apoptosis marker M30. No correlation was seen with GGT and GGT even increased after delivery in women with ICP.

Research conclusions

In conclusion, LS is significantly elevated in ICP which is most likely due to toxic bile acid accumulation and hepatocyte apoptosis. In association with conventional laboratory markers, LS provides additional non-invasive information to rapidly identify women at risk for ICP.

Research perspectives

In the future, elastography should be further validated in order to early identify women at risk for complications. Moreover, elastography studies should be combined with genetic risk assessment, as several mutations of bile transport proteins are involved in the development of ICP.

FOOTNOTES

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

ORCID number: Juliane Nees 0000-0003-4886-9808; Franziska J Ammon 0000-0002-8877-9711; Johannes Mueller 0000-0002-5205-4129; Herbert Fluhr 0000-0001-9300-4868; Sebastian Mueller 0000-0002-3577-8491.

Corresponding Author's Membership in Professional Societies: European Association for the Study of the Liver, No. 3538; European Society for Biomedical Research on Alcoholism.

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