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Clinical Outcome of Liver Damage in Pregnancy: A Single Centre Cohort Study

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Abstract

Background & Aims: Liver damage can occur due to pregnancy specific or superimposed conditions or underlying liver disease. Maternal and neonatal outcomes may differ accordingly.

Methods: Between 2008 and 2018, we collected clinical data of all consecutive pregnant women referred to our Liver Unit for abnormal liver function tests (LFTs). Liver damage was classified as cholestatic (pruritus, raised gamma-GT and/or aminotransferases and bile acids), viral (IgM anti-HAV, HBsAg and/or anti-HCV+), metabolic (pre-existing diabetes, metabolic syndrome, or obesity) and others. Data of new-borns were collected at birth and after delivery**.** ABCB4 (rs8187797 C>G) and ABCB11(rs2287622 T>C) genetic polymorphisms have been performed in a subgroup of patients.

Results: Among 6,310 mothers with live-born deliveries, 109 women with abnormal LFTs were included. Patients were categorized as: cholestatic (n=32), viral (n=22), metabolic (n=46), and others (n=9). Pregnant women with known liver disease were 62%. Patients with cholestasis showed pruritus and significantly higher ALT levels at third trimester (271.7 ± 220.5 IU/l) as compared to viral (21.2 ± 8.2 IU/l) and metabolic pattern (83.5 ± 96.2 IU/l) ($p< 0.001$). Liver damage of any aetiology did not affect outcome of pregnancy, even if 63% of pregnant received caesarean section. Majority of new-borns (88.9%) had an Apgar score ≥ 8 and a mean weight within the normal range for the 37-38 weeks of gestation. ABCB11 (CC) was significantly more detected in the cholestatic group ($p=0.01$), whereas no difference was shown for ABCB4.

Conclusions: In our consecutive cohort of pregnant, incidence of liver damage was relatively low and did not affect maternal and neonatal outcomes.

Keywords Clinical Outcome; Pregnancy; Liver Damage; ABCB11

Introduction

Pregnancy is a para-physiological condition, which usually occurs without any complication, even if liver diseases could complicate about 3% of pregnancies. Among the most common disorders, related or not to pregnancy, we find intrahepatic cholestasis of pregnancy (ICP), viral and metabolic disease, and the latter could further influence the patient's clinical course after delivery [1-2].

ICP is a condition related to pregnancy, involving mainly during the second and third trimester [3]. Alteration of liver function tests (LFTs), raised bile acids, pruritus and jaundice represent the main features [4]. In Europe, incidence ranges between 0.5% and 1.8%, with the highest peak in Scandinavia [5]. Its multi-factorial pathogenesis have been related with change in the ABCB4 and ABCB11 transporter genes in about 15% of cases [6,7], however because of ICP usually occurs in twin pregnancies and/or in infertile women, a theory regarding sex hormones has been also considered [8]. Adverse outcomes of pregnancy, as preterm delivery, pre-labour rupture of membranes, stillbirth, hospitalization of new-born, have been associated with raised bile acids and LFTs [9-10]. Although severe ICP is usually associated with adverse outcome [11] and management through ursodeoxycholic acid (UDCA) improves symptoms, foetal monitoring represents the only way to avoid adverse pregnancy and perinatal outcomes [12].

Viral hepatitis is a frequent condition usually pre-existing to pregnancy. Major and minor hepatotropic viruses are responsible for 40% of viral hepatitis and jaundice during pregnancy in Western Countries. Clinical course appears benign, with nausea, vomiting, headache, and less commonly cholestasis [13]. Complications, such as elevate risk of superimposed ICP, low weight at birth, preterm delivery and hospitalization of newborn in neonatal intensive care unit, are rarely present [14,15].

Non-alcoholic fatty liver disease (NAFLD) represents the hepatic expression of metabolic syndrome (MetS). Pregnant women with pre-existing NAFLD commonly present with LFTs alteration. During gestation, a decrease in insulin-sensitivity of 50–60% can occur, and thus, increases the risk of insulin-resistance (IR) [16] and gestational diabetes (GDM), unmasking underlying metabolic disorders. In addition, given that, in Western countries, nearly one-third of women at the childbearing age are overweight or obese, there is an increased risk of metabolic dysfunctions during pregnancy [17]. Even if seems that NAFLD do not affect maternal outcomes, data has been inferred from literature on obese and diabetic pregnant. On this regard, maternal obesity and GDM influence foetal outcomes, with excessive foetal growth of the new-born, development of juvenile obesity and finally metabolic disorders [18].

In this complex scenario, the alteration of LFTs may be attributable to various causes of a cholestatic, viral or metabolic nature, depending on whether abnormal LFTs are associated with raised bile acids, pruritus and/or jaundice or obesity. Thus, we aim to firstly evaluate in a cohort of pregnant women with alteration of LFTs consecutively referred, the impact of liver damage on pregnancy, maternal and neonatal outcomes. Further, being nearly one-third of our patients diagnosed as suffering from cholestasis of pregnancy, we assess ABCB4 and ABCB11 genetic polymorphisms and related it to clinical outcomes.

Methods

Design of the study

Between September 2008 and September 2018, we prospectively enrol consecutive pregnant women with alteration of LFTs admitted to Obstetrics and Gynecology Unit of our Academic hospital. Both women with new diagnosis of alteration of LFTs during pregnancy and with pre-existing liver disease were referred. Furthermore, some patients with gestational diabetes and abnormal LFTs were simultaneously followed at Endocrinology and Metabolic Disease Unit.

Patients visit were at first (V1), second (V2) and third (V3) trimester of gestation; follow-up visit (FUV) have been done within the 4-6 weeks after delivery and no later than 3 months.

Clinical and biochemical data were registered for each visit (V1, V2, V3 and FUV); a computerized database was constructed.

Study Population

The study was performed in accordance with the principles of the Declaration of Helsinki and its appendices, and with local and national laws. Approval was obtained from the AOUP Policlinico "P.Giaccone" of Palermo, Institutional Review Board and Ethics Committee, and written informed consent was obtained from all patients and controls.

Between September 2008 and September 2018, we prospectively collected socio-demographic and clinical data of 109 pregnant women with increasing of LFTs or liver disease pre-existing to pregnancy. Liver damage was classified as cholestatic, viral, metabolic and cryptogenic (no definite aetiology) (Figure 1).

Cholestatic pattern of disease or ICP was defined when all but one the following criteria were satisfied. 1. history of generalized pruritus with or without jaundice with a raised serum bile acid above the upper limit of normal for the local laboratory, developed at the end of second or third trimester of pregnancy, in the absence of any dermatological or other systemic medical conditions causing pruritus; 2. Increase in laboratory indices of cholestasis and/or serum aminotransferases; 3. Spontaneous resolution of clinical symptoms and normalization of laboratory indices after delivery; 4. Exclusion of other aetiology of liver disease (mainly viral, autoimmune and metabolic diseases)[1].

Viral disease was defined by presence of HBsAg and/or anti-HCV positive. Viral load of HBV and HCV was determined to assess the risk of vertical transmission. Other hepatotropic viruses as HAV, CMV, EBV and HSV were also tested for assess perinatal outcomes [1,13].

Metabolic liver disease was diagnosed if gestational diabetes mellitus (GDM) onset during pregnancy or diabetes was pre-existing or if MetS was present. GDM was defined as any degree of glucose intolerance with onset or first recognition during pregnancy. MetS was defined by the presence of at least three or more than the following criteria pre-existing before pregnancy: 1. Abdominal obesity (waist circumference: man >102 cm, woman >88 cm); 2. Hyperglycaemia (fasting plasma glucose concentration ≥110 mg/dL); 3. Arterial hypertension (systolic blood pressure ≥130 mmHg and diastolic blood pressure ≥85 mmHg); 4.Dyslipidaemia (HDL<40 mg/dl in men, <50 mg/dl in woman, Triglycerides>150 mg/dl)[18].

Patients with known alcohol abuse, history of drug addiction or positivity of transglutaminase antibodies in absence of celiac disease were excluded, as well.

Clinical and biochemical data

Clinical and biochemical data of the mothers were registered at first (V1), second (V2) and third (V3) trimester of gestation and during follow-up (FUV); a computerized database was constructed. Included clinical data: age, weight in kilograms, height in meters, BMI, race, systolic and diastolic arterial pressure, presence of clinical symptoms as pruritus, nausea, vomiting, diarrhoea, cutaneous rash; concomitant disease and pharmacological history. Detailed information on reproductive life were collected: number of previous pregnancy (parity), live birth rate, miscarriages and termination.

Biochemical data included serum level of aminotransferases, gamma-glutamyltranspeptidase, alkaline phosphatases, total bilirubin, INR, albumin, glucose, blood urea nitrogen, creatinine, total cholesterol, completely blood count were assessed at each visit. Autoantibodies (TTG- ANA- AMA- LKM-ASMA) and viral markers, (HBsAg and Anti-HCV) were recorded. During the first trimester, toxo, rubeo, CMV and HAV were assessed, through IgG and IgM, evaluating a previous or acute infection. In particular, serology for CMV, rubeo and toxoplasma was checked every visit for IgG negative pregnant.

Liver ultrasound was performed to investigate the presence of steatosis and biliary diseases. NAFLD should be considered in differential diagnosis of abnormal LFTs. Steatosis was present if there was a Bright Liver Echo Pattern (BLEP), with thickly crammed echoes, increased liver brightness, hyper echogenicity compared to renal parenchyma, possible evidence of signs of deep attenuation of the echoes and altered visualization of intrahepatic vessel walls [19].

All patients were followed until the delivery and at least up three months later during the post-partum period. We assessed timing of delivery, type of delivery (natural or caesarean section), pre-labour rupture of membranes (PROM). For the most of them, FUP visit have been planned usually around 6 weeks after delivery.

For the new-borns, Apgar Index and birth weight were collected in the database. Apgar score was calculated through five vital parameters, as cardiac rate, respiratory rate, muscular tone, reflexes, colour of skin. Scores can be distinguished as follows: normal (8-10); moderately risk, that need revaluation (4-7); severe risk, with quickly need of therapy (<4).

Gene polymorphisms for ABCB4 and ABCB11

Blood samples from all pregnant were harvested and stored at -80°C to perform genetic analysis. DNA was purified using the QIAmp blood Mini Kit (Qiagen, Mainz, Germany); DNA samples were quantified using spectrophotometric determination. Drug Metabolism Genotyping Assay for ABCB4 (rs8187797 C>G) and for ABCB11 (rs2287622 T>C) was carried out using the TaqMan SNP allelic discrimination method (Applied Biosystems, Foster City, CA, USA). The genotyping call was done with SDS software v.2.3 (StepOnePlus Real Time PCR, Life Technologies, Foster City, CA, USA).

Blood samples of pregnant women with alteration of LFTs were matched with those of pregnant controls with same age and timing of delivery and normal LFTs, without liver disease.

Statistical analysis

Continuous variables were summarized as mean ± standard deviation and categorical variables were expressed as frequencies and percentages.

For continuous values, comparisons between groups were made by ANOVA univariate and post hoc analysis was performed by Bonferroni's test. Chi square test and Fisher's exact was performed for non-continuous variables. P<0.05 was considered statistically significant.

Results

Out of 6,310 mothers with live-born deliveries at our Academic Hospital during the inception period, 109 (mean age 31.2± 6.2 years) entered the study (see Figure 1).

Patients were categorized according to the pattern of liver damage as cholestatic (n=32), viral (n=22), metabolic (n=46), and others (n=9). Clinical features of all 109 pregnant women are showed in Table1 and Figure 2.

Cholestatic Damage

Patients with cholestatic damage were clinically diagnosed as ICP if they showed typical pruritus with a raised serum bile acid above the upper limit of normal for the local laboratory and significantly higher ALT levels at second/third trimester of pregnancy. In this group, ALT was higher (median 271.7 IU/l) as compared to viral (median 21.2 IU/l) and metabolic pattern (median 83.5IU/l) (p< 0.001). Almost all pregnant women with ICP had no previous liver disease or other associated diseases; pruritus (88% of patients with cholestatic pattern) was present only in this group. History of familiarity for ICP was present in 5 women; only 3 women (9.3% within this pattern) were treated with hormonal therapy to induce pregnancy. In 8 out of 32 (25%) pregnant women with ICP a gestational diabetes was diagnosed.

Twenty-two (68%) women out of 32 underwent to caesarean section (20% of the total); six (18%) had a premature rupture of the membranes, PROM (5% of the total). Delivery was at time of 37.6 ± 3.0 weeks of gestations; a mean weight of new-borns was 3055 ± 465 gr. Apgar index for 4 new-borns was

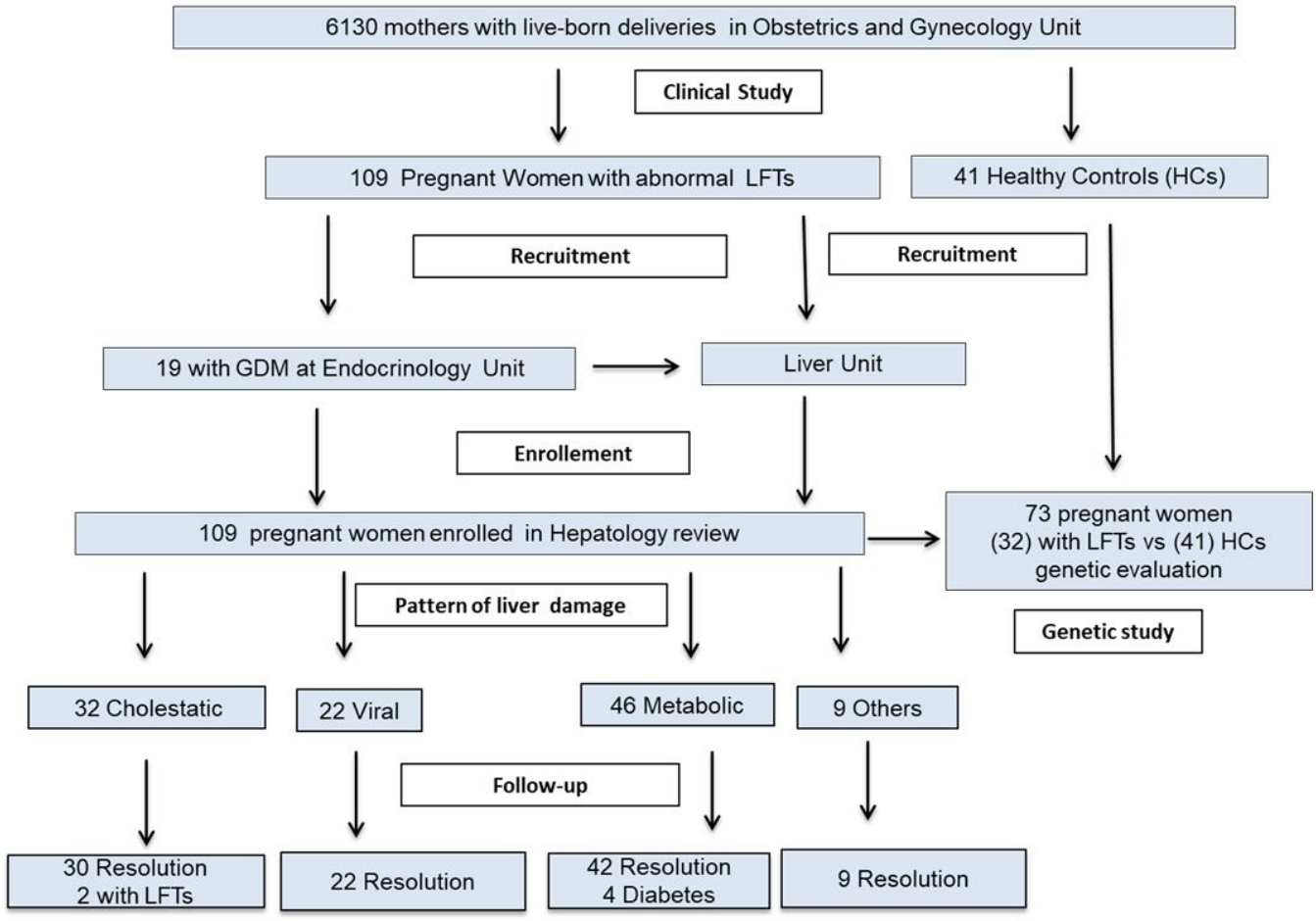

Figure 1: Flow-chart describing recruitment, enrollment, pattern of liver damage and follow-up of 109 pregnant women with abnormal LFTs

Table 1: Clinical and biochemical features of pregnant presenting with liver damage

VARIABLES	N = 109
AGE(years)	31.2 ± 6.2
NATIONALITY Caucasian Non caucasian	 96 (88.1%) 13 (11.9%)
PREVIOUS LIVER DISEASE Yes No	 57 (52.3%) 52 (47.7%)
PATTERN OF LIVER DAMAGE Cholestatic Viral Metabolic Others	 32 (29,3%) 22 (20,4%) 46 (42.2%) 9 (8.3%)
ALT(IU/L) II Trimester III Trimester	 86.7 ± 185.2 137.8 ±174.2
PROM	11 (10.1%)
DELIVERY Caesarean section Vaginal	 74 (67.9%) 35 (32.1%)
TIMING OF DELIVERY (weeks)	37.4 ± 3.5
NEWBORN PARAMETERS Apgar score < 9 Weight (gr)	 13 (11.9%) 3,124 ± 569
MULTIPLE ABORTION	8 (7.3%)
DM before pregnancy	10 (9.1%)
GDM	19 (17.4%)

PROM: pre-labour rupture of membranes. DM: diabetes mellitus. GDM: Gestational diabetes mellitus

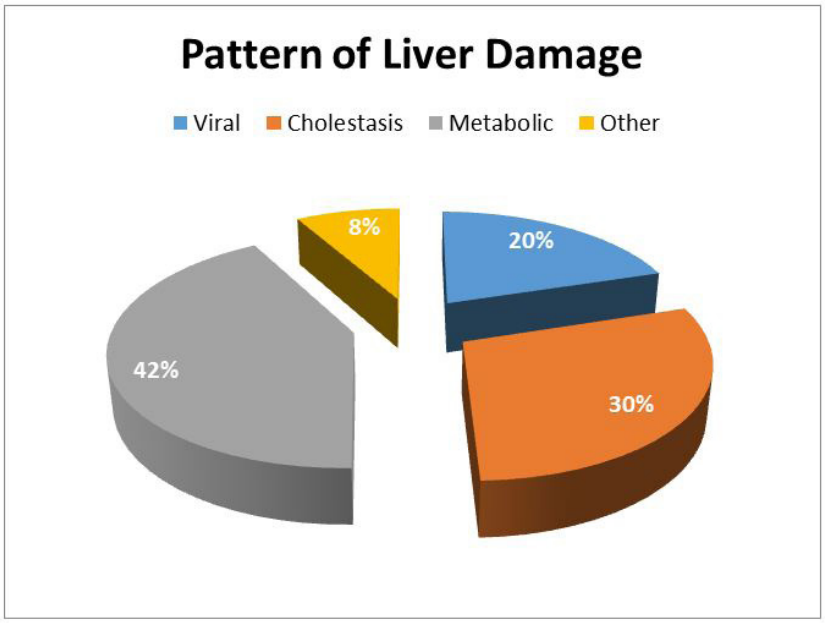

Figure 2: Different pattern of liver damage in 109 pregnant women referred to our Unit for abnormal LFTs

Table 2: Comparison of clinical features of pregnant within different patterns of liver damage

VARIABLES	**CHOLESTATIC (32)**	**VIRAL (22)**	**METABOLIC (46)**	**OTHERS (9)**	***P*-value**
AGE(years)	31.9 ± 5.4	26.7 ± 6.1	31.4 ± 6.8	31.9 ± 5.8	0.60
NATIONALITY Caucasian Non caucasian	27 (84.4%) 5 (15.6%)	16 (72.7%) 6 (27.3%)	40 (87.0%) 6 (13.0%)	12 (100%) 0	0.89
COMORBIDITY Hypertension Diabetes mellitus	1 (3.1%) 0	0 1 (4.5%)	0 8 (17.4%)	0 1 (11.1%)	0.58 **0.02**
PREVIOUS LIVER DISEASE Yes No	1 (3.1%) 31 (96.9%)	19 (86.4%) 3 (13.6%)	37 (80.4%) 9 (19.6%)	0 9 (100%)	**<0.001**
SYMPTOMS Pruritus Nausea	25 (78.1%) 0	0 2 (9.1%)	0 1 (2.2%)	0 0	**< 0.001** 0.21
ALT (UI/L) II Trimester III Trimester	198.9 ± 349 271.7 ± 220.5	24,6 ±13.9 21.2 ± 8.2	45.9 ± 35.6 83.5 ± 96.2	120.7 ± 90.8 96.3 ± 6.6	0.06 **< 0.001**

DELIVERY MODALITY **PROM** Caesarean section Vaginal	6 (18.7%) 22 (68.7%) 10 (31.3%)	1 (4.6%) 16 (70.0%) 6 30.0%)	3 (6.5%) 29 (56.5%) 17(32.6%)	1 (1.1%) 7 (77.8%) 2 (22.2%)	0.2 0.8
TIMING OF DELIVERY (weeks)	37.6 ± 3.0	38.3±1.3	37.4±3.7	34.9 ± 6.6	0.17
NEWBORN PARAMETERS Apgarscore< 8 Weight (gr)	4 (12.5%) 3,055 ± 465	1 (4.6%) 3,235± 423	4 (8.7%) 3,206 ± 618	4 (44.4%) 2,747± 888	**0.01** 0.2
MULTIPLE ABORTION	1 (3.1%)	1 (4.6%)	5 (19.9%)	1 (11.1%)	0.5
DM before pregnancy	0	1 (4.6%)	8 (17.4%)	1 (11.1%)	**0.06**
GDM	8 (25%)	3 (13.6%)	4 (8.7%)	4 (44.4%)	**0.036**

PROM: prelabor rupture of membranes. DM: diabetes mellitus. GDM: Gestational diabetes mellitus

lower than 8. Almost all patients with ICP have been treated with UDCA at dosage of 450-600 mg improving pruritus, ameliorating LFTs and bilirubin, with a complete resolution within 6-8 weeks after delivery. Features of the pregnant with ICP are showed in Table 2.

Viral damage

Patients with viral damage were younger (26.6 ± 6.1 years) than other pregnant women included in our entire cohort, with a previous chronic viral infection that did not receive a specific antiviral therapy. Among these, 11 women had HBV infection, 9 had HCV infection and 1 woman showed HCV/HIV co-infection. Rubeo test, evaluating a previous infection, was positive in 57 women (23 with cholestatic pattern, 11 with viral and 19 with metabolic one). A previous infection of toxoplasma was present in 13 pregnant (6cholestatic, 5 viral, 2 metabolic), CMV (IgG) was positive in 33 pregnant (12 with ICP, 7 viral, 12 metabolic).

Only one pregnant woman had HSV acute infection, with no need of therapy and with good rapid resolution. About 30% of pregnant women with viral hepatitis were not Caucasian, but black-african. Overall, aminotransferases values appeared within the normal range, in both second and third trimester of gestation. Clinical manifestations were insignificant with only three cases of nausea and vomiting. Maternal and foetal outcome were not influenced by this pattern, even if the majority of pregnant with viral infection underwent caesarean section to avoid vertical transmission (63%). Delivery was at time of 38.3±1.3 weeks of gestations; a mean weight of new-borns was 3,235± 423 gr. All new-borns from HBV patients were treated with HBV-Immunoglobulin and the first dose of anti HBV vaccination according the EASL Practice Guidelines on HBV and AISF Position Paper on Pregnancy [25]. Follow-up after delivery, 4-6 weeks later, showed normal clinical and biochemical data and patients were treated with available therapy (mostly for HCV). All data regarding viral pattern of damage are reported in Table 2.

Metabolic damage

Metabolic pattern was the most frequent in our cohort (46 out of 109). It was associated with NAFLD, MetS and diabetes. Eight pregnant women out of 46(17.4%) were diagnosed with diabetes mellitus before pregnancy. Liver disease of metabolic origin (NAFLD) was present in 37out of 46 women. Women with metabolic pattern had less symptoms during pregnancy than other groups. This pattern did not influence maternal and foetal outcome, although 29 out of 46 (63%) women underwent elective caesarean section to avoid labour dystocia due to high obesity. In this group, mean timing of delivery was at 37.4±3.7 weeks of gestations, whereas new-borns showed a mean weight of 3,206 ± 618 gr.

Follow-up of these women after delivery (6-8 weeks later), showed a clinical and biochemical resolution, but in four cases (8.6%) pregnancy unmasked the onset of diabetes mellitus requiring therapy (metformin or insulin).

Comparison among different groups

There were no differences between groups in terms of demographic characteristics (age, nationality). Previous liver diseases were significantly more frequent in viral and metabolic group (p< 0.001), especially presence of diabetes mellitus before pregnancy in the metabolic group (p< 0.003). Regarding clinical and biochemical data, patients within ICP presented more symptomatic, with pruritus (p<0.001) as compared with other group, with need of UDCA therapy (p<0.001); further, aminotransferases were higher than other group, especially in the third trimester of gestation (p <0.001); by analysis, ALT were statistically significant as cholestatic versus viral (p<0.0001), cholestatic versus metabolic (p<0.02) and versus other (p<0.0001). However, abnormal LFTs of any aetiology did not affect outcome of pregnancy, regardless of its pattern. No significant differences were present on type and timing of delivery (about 60% of caesarean section), even if PROM was more frequent in patients with ICP than in others (6 patients out of 11). Majority of new-borns (88%) had an Apgar score ≥ 8 and a mean weight at birth within a normal range for the weeks of pregnancy (3,124±569 gr).

Expression of ABCB4 and ABCB11 gene polymorphism

Table 3: Comparison between ABCB11 and ABCB4 polymorphism in pregnant women with ICP and controls

	ICP (N=19)	**CONTROLS (N=41)**	***p***
ABCB4*			
CC/CG	18 (94.7%)	39 (95.1%)	0.95
GG	1 (5.3%)	2 (4.9%)	
ABCB11*			
TT	3 (15.8%)	10 (24.4%)	0.5
TC	5 (26.3%)	21 (51.2%)	0.09
CC	11 (57.9%)	10 (24.4%)	**0.01**

Table 4: Clinical parameters of pregnant women with ICP according to different ABCB11 alleles

VARIABLES	**ABCB11-CC (N=12)**	**ABCB11 -TT (N=2)**	**ABCB11-TC (N=5)**	***p***
PRURITUS	12 (100%)	1 (50.0%)	3 (60.0%)	**0.04**
ALT (UI/l) (mean ± SD)	351.2 ± 258.4	351.4 ± 198.8	393.28 ± 248.2	0.7
PREGNANCY OUTCOME				
IUFD	1 (8.3%)	-	-	0.7
IUGR	1 (8.3%)	-	-	0.7
PROM	1 (8.3%)	-	3 (60.0%)	**0.04**
APGARE SCORE < 8	4 (33.3%)	-	1(20.0%)	0.6

IUFD: intrauterine foetal death. IUGR: intrauterine growth retardation.
PROM: pre-labour rupture of membranes

Thirty-two patients with liver damage, whose 19 with ICP, were assessed by performing genetic testing and compared them with 41 healthy pregnant, matched to age and time of delivery (Figure 1). We evaluated single nucleotide polymorphisms in ABCB4 (rs8187797 C>G) and ABCB11 (rs2287622 T>C) genes. There was a significant higher presence of ABCB11 (CC) genotype in patients with ICP, as compared with healthy pregnant (p 0.01), while no difference has been found regarding ABCB4 polymorphism. The genotypes frequencies are consistent with Hardy-Weimberg equilibrium (Table 3).

Furthermore, we evaluated women with ICP pattern and different ABCB11 genotype, such as CC, TC, TT. We found that all pregnant women carrying the ABCB11-CC homozygosis showed symptomatic disease with pruritus (p=0.04), whereas those carrying ABCB11-TC showed more PROM (p=0.04); no significant differences regarding incidence of severe complications of pregnancy such as intrauterine foetal death and intrauterine growth restriction were detected (Table 4). All pregnant women tested for ABCB11 were Caucasian except four, who were categorized as follow: ABCB11-TC (2), ABCB11-CC (1) and ABCB11-TT (1).

Management of pregnant women with alteration of LFTs

Rarely at the end of the 2nd trimester of gestation, but usually at the 3rd, and particularly after the 32nd week, some

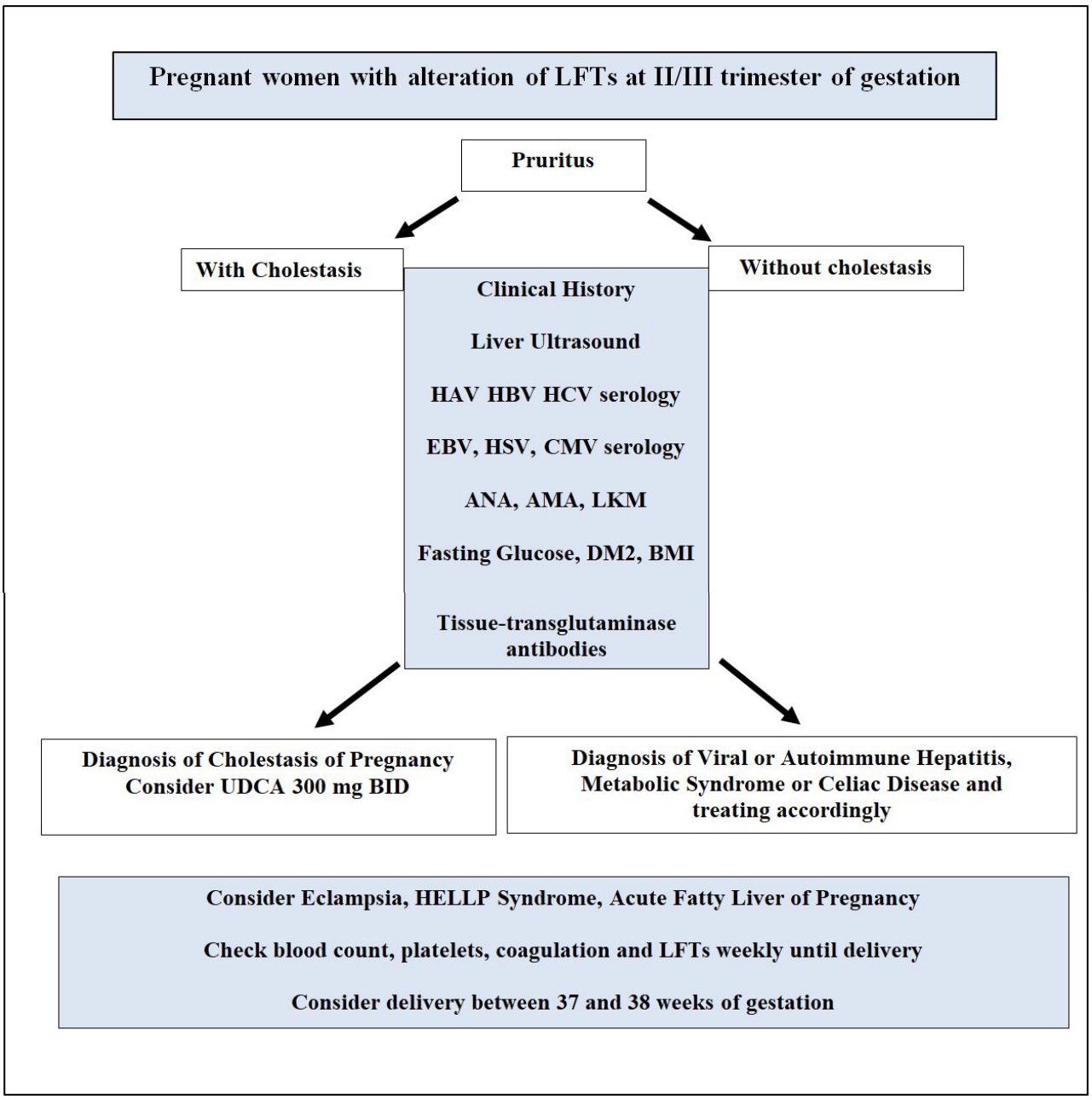

Figure 3: Diagnostic algorithm for pregnant with alteration of LFTs at II/III trimester of gestation

pregnant women may experience abnormal LFTs, which may or may not be associated with pruritus. The latter usually present throughout the body, and in particular in the palms of the hands and soles of the feet, regardless of the value of the bile acids, must lead to suspicion of the onset of cholestasis in pregnancy. However, to validate the correct diagnostic hypothesis, but above all, to carry out a careful screening in perspective of possible perinatal infections, it is advisable to evaluate pregnant women for major and minor hepatotropic viruses, to exclude an autoimmune liver disease by searching non-specific autoantibodies, to assess fasting glycaemia, in the case of a possible gestational diabetes. Finally, among the causes of raised aminotransferases, it is also worthwhile investigating anti-transglutaminase antibodies, as pregnancy could be the trigger of celiac disease. Lastly, a liver ultrasound for the study of the liver structure and biliary tree, the measurement of anthropometric parameters and the calculation of the BMI, helps in the overall evaluation of the pregnant woman. Unquestionably, all this must always be considered in the light of any other anamnestic data and/or pre-existing disease. After excluding viral and metabolic disease, and thus, considering the possibility of intrahepatic cholestasis, it is suitable to start therapy with UDCA at a dosage of 300 mg, BID, continuing the weekly monitoring of platelets and blood count, LFTs and coagulation parameters until delivery (preferred between 37th and 38th weeks of gestation). Close monitoring of the mother and the unborn child from the 36th week of gestation and up to at least 2 weeks after delivery is strictly suggested (Figure 3).

Discussion

In an attempt to assess the impact of liver damage on maternal and neonatal outcome during pregnancy, we carried out a cohort study including consecutive pregnant women referred to our tertiary referral centre for increasing of LFTs. We found that the incidence of liver disease in our cohort was about 2%, according data reported from literature [3-5]. In fact, out of 6310 women who underwent liver function tests measurement, only 109 showed an alteration of these parameters compatible with a liver disease. Pregnant with pre-existing chronic liver disease were 62% (mostly of viral or metabolic aetiology). Pregnant with a cholestatic pattern, diagnosed as ICP, showed significantly higher serum ALT levels at third trimester of gestation. Abnormal LFTs of any aetiology did not affect outcome of pregnancy, regardless of its pattern, even if about 60% of pregnant within each group had caesarean section. Majority of new-borns had an Apgar score ≥ 8 and a mean weight at birth within the normal range for the 37-38 weeks of gestation (3,122 ± 592 gr). ABCB4 and ABCB11 genetic analysis of pregnant ICP showed a significant higher presence of ABCB11 (p=0.01), whereas no difference for was found for ABCB4. Further, ABCB11 polymorphism occurred significantly in pregnant with pruritus and pre-labour rupture of membranes.

Considering the different pattern of liver damage, only the cholestatic one developed clinical symptoms, as pruritus [4]. In fact, among all liver diseases in pregnancy, ICP represents one of the most dangerous even if not so serious. In our study, we found that ICP pregnant women showed an increased risk of spontaneous preterm birth and poor outcomes of the baby, such as foetal distress and stillbirth. These data are in agreement with literature [9]. Our ICP pregnant, treated with UDCA, improved pruritus, but there is no sufficient evidence, whether UDCA improves neonatal outcomes [12]. In fact, UDCA does not hold a specific indication to be prescribed during pregnancy, but it is considered to be safe, thus usually it has been taken until delivery and stopped following, when pruritus and LFTs usually return to normal (days or almost few weeks) [1,20].

Different mutation in the alleles potentially associated to ICP have been studied during pregnancy. However, ABCB4 and ABCB11 gene polymorphisms are frequent findings especially in northern Europe [7,21-24]. In our study, assessing pregnant women with liver damage and healthy controls, matched for age and timing of delivery, we found a significant higher presence of ABCB11 (CC) in 19 women with ICP than in healthy patients (p 0.01), whereas no difference was shown for ABCB4. In addition, regarding ABCB11 genetic polymorphism, we found that ICP pregnant women with homozygosis CC were symptomatic, whereas those with heterozygosis TC had more often pre-labour rupture of membranes, showing sometimes complications associated to outcome of pregnancy and new-borns with an Apgar score less than< 8. However, further genetic studies from larger cohort are need to support these clinical data.

In our study, we found that women with viral pattern had aminotransferase values within the normal range either in the second or in the third trimester. Maternal and foetal outcome usually are not influenced by this pattern, and the real problem in cases of viral disease is the acute infection with high viral load and thus the reliable risk of mother to child transmission [13]. In our cohort, almost all pregnant infected with HBV or HCV, underwent caesarean section and all new-borns from HBV positive mothers were treated with HBV-Immunoglobulin and the first dose of anti-HBV vaccination [26]. Although we included almost pregnant women with chronic viral disease (only 1 with

acute HSV infection), we do not proceed with liver biopsy or liver stiffness measurement to staging the pre-existing liver disease. As the indication for liver biopsy in this category of patients is the same as non-pregnant women and data safely proceed with measurement of liver stiffness by transient elastography are just published [27-28], we do not perform any fibrosis staging approach to avoid possible adverse events related to invasiveness of procedure.

In our cohort, metabolic pattern was the most common and it was frequently associated to metabolic syndrome and, thus diabetes mellitus, reflecting its high prevalence in the general population of western Countries [16]. In this category, some conditions as liver steatosis, diabetes and arterial hypertension, as expression of metabolic syndrome were also present and nearly one quarter of our pregnant with metabolic pattern were diabetic. In general, prevalence of diabetes in pregnancy appears to be remarkably variable in different countries and within the same country, being probably related to diagnostic and screening criteria [29-31], resulting in about 16-25% of the general population [32-34]. Usually, metabolic liver disease during pregnancy shows a benign course with the absence of unfortunate maternal and neonatal outcomes, even if maternal events (pre-eclampsia, induction of labour, pre-term labour), caesarean section and neonatal complications (macrosomia, neonatal intensive care unit admission, respiratory distress, neonatal hypoglycaemia and jaundice) may appear [34].

In our study, pregnant with metabolic disease were asymptomatic; 29 out of 46 (63%) underwent caesarean section, without maternal or neonatal complications; either mean timing of delivery or mean weight of new-borns were within the normal range. It is noteworthy that, in this category multiple abortion was more frequent, as compared with other groups. In obese/diabetic pregnant women, there is a greater risk of large gestational age and small gestational age new-borns, connected one to up-regulation of glucose transporters, to dyslipidaemia (hypertriglyceridemia, low levels of HDL), while the other to an abnormal placental development with suboptimal perfusion and altered passage of nutrients from the mother to the foetus. Furthermore, for these mothers there is also a greater risk of congenital malformations [35]. Given that, it is advisable that pregnant with NAFLD has to be screen for diabetes and further, to be monitor for the development of hypertension, especially those overweight or obese. Diet and weight reduction are pivotal issues in these patients, and early diagnosis and properly management could change neonatal outcomes.

Follow-up of our obese pregnant women after delivery showed clinical and biochemical resolution, but in some of our cases pregnancy unmask diabetes mellitus, with need of therapy. Accordingly, after delivery, mothers with gestational diabetes have a 7–13 folds increase risk of progression to type 2 diabetes with need of treatment [34] and are at increased risk for cardiometabolic disorders compared to mothers with normal glycaemia during pregnancy [36,37]. Finally, within metabolic group, we did not find any neonatal complications and outcomes were favourable. This is partially in contrast with literature, but it is probably due to the few numbers of women included. Offspring born to mothers with gestational diabetes, which in our cohort was present in 19%, are at increased risk of obesity, and early onset diabetes [38-39]. The risk of developing type 2 diabetes is increased by 3–4 folds in children born to mothers who had gestational diabetes with a cumulative incidence of 15% by the age of 30 years [40].

In sum, given our experience in an attempt to properly follow pregnant women presenting with raised amino-transferases at III trimester of gestation, regardless of bile acids increases, we designed a simply algorithm to manage this issue, which usually internists, especially hepatologists face during obstetric consultancy.

Indeed, we reasonably believe that liver diseases in pregnancy are infrequent and not so serious, even if sometimes they might have relevant consequences on the maternal and neonatal outcomes. Thus, it is important to recognize symptoms and laboratory alterations and regularly follow women during pregnancy and immediately after delivery, to avoid adverse outcomes.

Compliance with Ethical Standards

Ethical approval and Informed Consent

We declare that the study was performed in accordance with the principles of the Declaration of Helsinki and its appendices, and with local and national laws. Approval was obtained from the AOUP Policlinico "P.Giaccone" of Palermo, Institutional Review Board and Ethics Committee, and written informed consent was obtained from all patients and controls**.**

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Conflict of interest

We declare that we have no conflict of interest.

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