UNIVERZITA PAVLA JOZEFA ŠAFÁRIKA V KOŠICIACH

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Pregnancy-related liver disorders

JOANA PIMENTA FALCÃO MARQUES

DIPLOMA THESIS

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Thesis supervisor: MUDr. Martin Janičko, PhD.

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Abstrakt

Ochorenia pečene sa vyskytujú približne v 3% tehotenstiev. Tieto ochorenia sa dajú zaradiť k dvom typom porúch, a to k ochoreniam v priamom súvise s tehotenstvom a nesúvisiacich s tehotenstvom. Ochorenia pečene v priamej súvislosti s tehotenstvom sú najčastejšími príčinami dysfunkcie pečene vyskytujúcej sa počas tehotenstva a sú spojené s morbiditou a mortalitou plodu ako aj matky. Ochorenia pečene, ktoré sa vyskytujú iba počas tehotenstva, sú viazané na špecifické trimestre a zahŕňajú: hyperemesis gravidarum, intrahepatálnu tehotenskú cholestázu, preeklampsiu, HELLP syndróm a akútnu tehotenskú steatózu pečene.

Rýchla diferenciálna diagnostika špecifickej tehotenskej hepatopatie je potrebná u žien, ktoré majú už v anamnéze poruchu funkcie pečene. Základným kameňom je včasné rozpoznanie ochorenia, a pri ťažkom priebehu určitých typov ochorení je jediným riešením urgentný pôrod dieťaťa. Nedávne pokroky v diagnostike a liečbe znížili morbiditu a mortalitu týchto ochorení, napriek tomu je stále potrebných viac výskumov a klinických skúšok v tejto oblasti.

Táto diplomová práca má za cieľ poskytnúť kompletný prehľad a revíziu mechanizmov výskytu týchto ochorení a zdôrazňuje najdôležitejšie aspekty diagnostiky a manažmentu pacienta v klinickej praxi.

Abstract

Liver disorders occur in approximately 3% of all pregnancies. They fall into pregnancy-related and pregnancy-unrelated disorders. Pregnancy-related liver disorders are the most frequent causes of liver dysfunction occurring during pregnancy and are associated with both fetal and maternal morbidities and mortality. Liver disorders unique to pregnancy are most often trimester-specific and include: hyperemesis gravidarum, intrahepatic cholestasis of pregnancy, preeclampsia, HELLP syndrome and acute fatty liver of pregnancy.

Differential diagnosis between liver diseases presenting during pregnancy can be challenging in most of the cases. The cornerstone is early diagnosis and at the severe end of the disease spectrum, promptly delivery. Recent advances have improved morbidity and mortality rates of these diseases, nevertheless more research and clinical trials are required.

This diploma thesis aims to carry out a complete review and update underlying mechanisms of their occurrence and highlighting most relevant to clinical practice to

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Cieľ: To describe liver disease associated with pregnancy

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Declaration on honor

I hereby certify that this diploma thesis is entirely the result of my own work and I have faithfully and properly cited all sources used in the thesis.

In Košice on 21st June 2017

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List of Symbols and Abbreviations

kg	kilogram, SI mass unit
g	gram, 10^{-3} kilograms
mg	milligram, 10^{-6} kilograms
L	Liter, SI volume unit
dl	deciliter, 10^{-1} liters
ml	milliliter, 10^{-3} liters
µL	microliter, 10^{-6} liters
mol	mole, SI amount of substance unit
mmol	millimole, 10^{-3} moles
µmol	micromole, 10^{-6} moles
mmHg	millimeter of mercury
AFLP	Acute Fatty Liver of Pregnancy
AHP	Asymptomatic Hypercholanemia
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
APPT	Activated Partial Thromboplastin Time
AST	Aspartate Transaminase
AT1-AA	Angiotensin II Type-1 Receptor Autoantibody
BA	Bile Acids
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CA	Cholic Acid
CBC	Complete Blood Count
CD	Cluster of Differentiation
CDCA	Chenodeoxycholic acid
CRP	C-Reactive Protein
CO	Cardiac Output
CT	Computed Tomography

DCA	Deoxycholic Acid
DIC	Disseminated Intravascular Coagulation
FA	Fatty Acids
FAO	Fatty Acid Oxidation
FAOD	Fatty Acid β-Oxidation Disorder
GFR	Glomerular Filtration Rate
GGT	Gamma-Glutamyl Transferase
GSTA	Glutathione S-Transferase Alpha
Hb	Hemoglobin
hCG	Human Chorionic Gonadotropin
HELLP	Hemolysis, Elevated Liver Enzymes, Low Platelets
HG	Hyperemesis Gravidarum
HUS	Hemolytic-Uremic Syndrome
ICP	Intrahepatic Cholestasis of Pregnancy
ICU	Intensive Care Unit
IL	Interleukin
INR	International Normalized Ratio
ISSHP	Study of Hypertension in Pregnancy
IU	International Units
LCA	Lithocholic Acid
LCHAD	Long-Chain 3-Hydroxyacyl CoA Dehydrogenase
LFTs	Liver Function Tests
LHD	Lactate Dehydrogenase
MHC	Major Histocompatibility Complex
MMP	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
MTP	Mitochondrial Trifunctional Protein
NICE	National Institute for Health and Care Excellence
NK	Natural Killer
NVP	Nausea and Vomiting in Pregnancy

PCT	Phosphocholine Transferase
PE	Preeclampsia
PlGF	Placental Growth Factor
PT	Prothrombin Time
PUQE	Pregnancy-Unique Quantification of Emesis
RBC	Red Blood Cells
sFlt-1	Soluble fms-like tyrosine kinase-1
STOX1	Storkhead box 1 gene
SVR	Systemic Vascular Resistance
TG	Triglycerides
TNF- α	Tumor Necrosis Factor Alpha
tPA	Tissue Plasminogen Activator
TTP	Thrombotic Thrombocytopenic Purpura
UDCA	Ursodeoxycholic Acid
USG	Ultrasonography
VEGF	Vascular Endothelial Growth Factor
WBC	White Blood Cells
WHO	World Health Organization

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Introduction

Pregnancy is a dynamic process in which physiologic adaptations occur in the mother in response to the demands. These physiologic events can result in changes in liver biochemical profile which are normal in pregnancy. However, up to 3% of all pregnancies are complicated by liver disorders with different clinical outcomes ranging from self-limiting to rapidly fatal (1,2).

Liver disorders during pregnancy can be related or unrelated to pregnancy. Unrelated disorders can be further differentiated into preexisting disorders, that might worsen during pregnancy and those presenting -*de novo* (3) (see table 1).

Disorders specific to pregnancy include hyperemesis gravidarum (HG), intrahepatic cholestasis of pregnancy (ICP), preeclampsia (PE), hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome and acute fatty liver of pregnancy (AFLP). Liver dysfunction unrelated to pregnancy can occur any time, contrarily to liver disorders specific to pregnancy, which display characteristic trimester-specific clustering of occurrence. Moreover, they have been shown to be the most common reasons for abnormalities in liver test results during pregnancy and can carry a high mortality rate for both mother and baby. A rapid evaluation of pregnant women to distinguish between those is essential to facilitate appropriate management, which at the severe end of the spectrum require urgent delivery (4).

This diploma thesis will be focusing on pregnancy-related liver disorders by summarizing possible causes, underlying mechanisms of their occurrence and a methodological approach to their diagnosis and treatment.

Table 1. Liver diseases during pregnancy

Pregnancy-related liver diseases	Pregnancy-unrelated liver diseases
• **Hyperemesis gravidarum** • **Intrahepatic cholestasis of pregnancy** • **Preeclampsia and eclampsia** • **HELLP syndrome** • **Acute fatty liver of pregnancy**	*Pre-existing liver diseases* • Cirrhosis and portal hypertension • Hepatitis B and C • Autoimmune liver disease • Wilson's disease *Liver diseases co-incident with pregnancy* • Viral hepatitis • Biliary disease • Budd-Chiari syndrome • Drug-induced hepatotoxicity

1. Pregnancy-induced physiologic changes

During pregnancy, significant anatomical and physiological changes occur in order to accommodate to the developing fetus. Cardiovascular changes are characterized by an increased maternal heart rate, cardiac output (CO), together with a marked fall in systemic vascular resistance (SVR) (5). Arterial blood pressure (BP) remains unaffected or demonstrates some tendency toward lower diastolic pressure. Plasma volume increases progressively throughout pregnancy, peaking in the second trimester (50%). Expansion in plasma volume is greater than the increase in red blood cell (RBC) mass, which results in fall of hemoglobin (Hb) concentration, hematocrit and RBC count. However, mean corpuscular volume and mean corpuscular hemoglobin concentration do not change. Platelet count falls progressively but remains in normal reference values. *Gestational thrombocytopenia* (100 000 to 150 000 cells/μL), occurs in 5 to 10% of pregnant women, mostly in late gestation, with no apparent risk to either mother or fetus (6,7).

Regional blood flow to kidney, lung, skin and uterus are increased in normal pregnancy. Renal blood flow changes, result in an increased glomerular filtration rate (GFR) (30 to 50%), with consequent decrease in serum blood urea nitrogen (BUN) and creatinine levels, together with increased renal protein excretion. Rising estrogen levels stimulate renin-angiotensin-aldosterone system, increasing tubular reabsorption of sodium. In contrast, distal tubular reabsorption of glucose decreases, hence glycosuria maybe a normal finding (7).

Physiologically, water retention is greater in pregnancy which is partly mediated by fall in plasma osmolality. This, together with increased venous pressure bellow uterine level, as a consequence of partial inferior vena cava obstruction, favors edema, which becomes worse at late pregnancy.

Lastly, pregnancy causes an increase in iron, folate and vitamin B12 requirements (6).

1.1. Hepatobiliary changes in pregnancy

Physical examination of liver is compromised during pregnancy, since it is shifted upwards and posteriorly, especially during the third trimester, yet it does not change in size. Increased plasma volume does not influence hepatic blood flow as SVR is lower and the liver receives a lower percentage of the CO (28%) (8,9).

Finding which might suggest liver disease such as spider nevi and palmar erythema are often normal during pregnancy. These are attributed to the increased circulating hormonal levels of estrogens. Moreover, increased levels of progesterone in pregnant women, have been associated with dilation of gallbladder and biliary duct system by its action on smooth muscle (by cholecystokinin inhibition). In addition, during pregnancy hepatic cholesterol synthesis increases, however bile acids (BA) levels remain within normal limits during pregnancy. Even so, several studies have documented increased BA levels in late pregnancy, compared to first trimester values. Overall, overproduction of selected BA (especially chenodeoxycholic acid) and decreased concentration of biliary water, reduce the ability of bile to solubilize cholesterol. The aforementioned alterations result in an increased lithogenecity of bile, stasis and higher prevalence of cholelithiasis among pregnant women (8).

Furthermore, hormonal changes according gestational age, can also affect metabolism by altering expression of cytochrome P450 system. Expression of hepatic CYP1A2 is decreases while CYP2D6 and CYP3A4 increases (10).

Coagulation pathway and its changes during pregnancy are complex and out of the scope of this diploma thesis. In general, physiological changes in pregnant women result in a hypercoagulable state. Procoagulant activity is enhanced by elevation of fibrinogen, factors V, VII, VIII, IX, X, XII and von Willebrand factor. Antithrombin levels are decreased. In contrast, activated partial thromboplastin time (APTT) and prothrombin time (PT) remain unchanged (4,9). Although fibrinolysis has been suggested to be augmented during pregnancy, recent studies have produced conflicting results, suggesting reduced fibrinolytic activity. Fibrinolytic process is normally mediated by tissue plasminogen activator (tPA) that converts plasminogen into plasmin, ensuring fibrin degradation. Interestingly, tPA activity has been shown to be decreased in pregnant women along with raised levels of plasminogen activator inhibitors 1 and 2. These events are countered by increased levels of plasminogen and decreased level of another plasmin inhibitor (α2 antiplasmin), ensuring hemostatic balance during normal pregnancy. Overall, body is adapting to minimize blood losses in delivery, however, these same changes increase mother’s risk of thrombotic events from the first trimester until 12 weeks postpartum (6).

Table 2. Typical reference ranges for liver enzymes by pregnancy and trimester (11)

Liver enzyme	Not pregnant	Pregnant	1st trimester	2nd trimester	3rd trimester
ALT (IU/L)	0–40	—	6–32	6–32	6–32
AST (IU/L)	7–40	—	10–28	11–29	11–30
Bilirubin (µmol/L)	0–17	—	4–16	3–13	3–14
GGT (IU/L)	11–50	—	5–37	5–43	3–41
ALP (IU/L)	30–130	—	32–100	43–135	133–418
Albumin (g/L)	35–46	28–37	—	—	—
Bile acids (µmol/L)	0–14	0–14	—	—	—

ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase.

Physiological changes occur in liver function tests (LFTs) during pregnancy (see table 2). Important liver parameters such as alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) remain unchanged. However, superior limit is slight altered due to hemodilution effect. Expansion in plasma volume also affects serum albumin and bilirubin concentrations. Serum alkaline phosphatase (ALP) levels are higher and peak in the third trimester when noticeable placental ALP and fetal bone isoenzyme are produced. This makes it has a poor diagnostic tool in pregnancy. To a lesser extent, 5-nucleotidase activity also increases in the second and third trimesters.

Lastly, lactate dehydrogenase (LDH) levels are unchanged throughout gestation (6,8).

2. Approach to liver diseases occurring during pregnancy

Abnormal liver studies can be an early indicator of serious pathology. Differentiating liver disorders related to pregnancy from those unrelated is essential for management of these diseases and to achieve good outcomes. Standard workup in a patient presenting with abnormal liver enzymes, as with any non-pregnant individual, comprises several steps that one must follow. A complete history (including past LFTs), physical exam and standard serological workup should be performed as indicated by clinical presentation. LFTs can change significantly during normal pregnancy, making challenging interpretation of it. They might fall under 3 categories:

i. Tests of the liver's capacity to transport organic anions and to metabolize drugs- serum bilirubin, urine bilirubin and urobilinogen;
ii. Tests that detect injury to hepatocytes (serum enzyme tests) – aminotransferases, ALP, GGT and 5-nucleotidase; and
iii. Tests of the liver's biosynthetic capacity- serum proteins, albumin, prealbumin, PT, α-fetoprotein, serum ceruloplasmin, procollagen III peptide and α1 antitrypsin (12).

Thus, approach to a pregnant woman with abnormal LFTs, can be simplified by following questions:

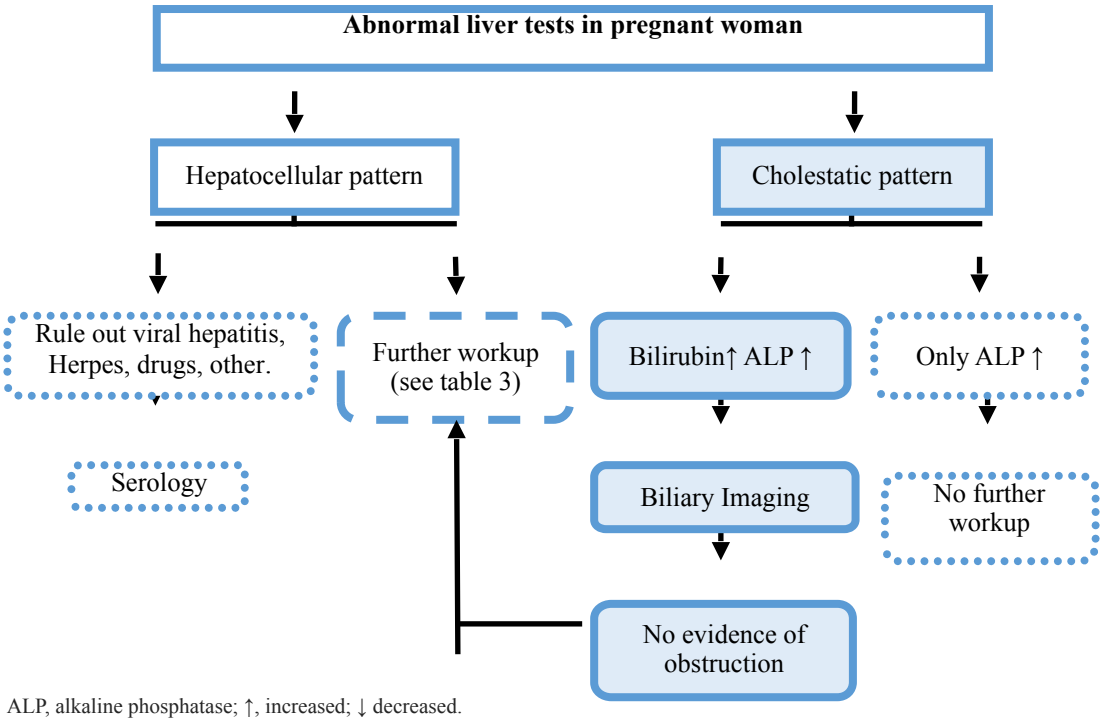
1) *What is the pattern of abnormal LFTs?* (see figure 1) (see table 3)

Three main patterns of LFTs results are recognize: hepatocellular, cholestatic and mixed. Raised aminotransferases levels out of proportion to ALP suggests hepatocellular injury, with inflammation or necrosis of hepatocytes. Levels of ALT are more specific as AST can be found ubiquitously throughout the body (8). Slight AST or ALT elevations (within 1.5 times the upper limits of normal) do not necessarily indicate liver disease, however these women should be followed up. Additional, mild to moderate increase in transaminases might suggest specific disorders like HG, AFLP, ICP whereas marked increase is often seen in severe PE, HELLP syndrome and liver rupture. Concerning pregnancy-unrelated liver disorders, cholelithiasis and alcoholic hepatitis usually present with mild elevation, in contrast with drug-induced hepatotoxicity, and viral hepatitis, that can result into extensive damage to hepatocytes with striking raised levels of transaminases (13). One should rule out hepatitis, by appropriate serological tests.

Cholestatic pattern suggests obstruction of biliary flow, that results in hepatobiliary disturbances, with high levels of ALP out of proportion to aminotransferases. Accessing to 5-nucleotidase, GGT, and bilirubin levels, which are increased in cholestatic pattern, can be helpful to confirm pathological ALP levels in pregnant women. Raised serum BA are present in almost any disease of the hepatobiliary system, yet serum BA are a sensitive but nonspecific marker of hepatic and biliary disease, when presenting alone (8). Severe biliary obstruction can lead to high amounts of serum conjugated bilirubin, resulting in a dark urine and pale stools (9). Imagining of biliary system may be helpful in cases of suggesting obstruction. Ultrasonography (USG) remains the safest imaging. If further detailed imaging is needed, magnetic resonance imaging (MRI) without contrast can be safely done (2).

Mixed pattern is usually presenting in cases of expanding masses, punching into hepatic structures, resulting in a concomitant elevation of both transaminases and cholestasis parameters.

Figure 1. Workup abnormal liver tests in pregnant woman. *Adapted* (5)



ALP, alkaline phosphatase; ↑, increased; ↓ decreased.

Table 3. Patterns of abnormal liver function tests (LFTs) (11).

Pattern of LFTs changes	Likely diagnosis	Further workup
ALT ↑ (1.5–8-fold) **Total serum BA ↑ (1.5–15-fold)** **Total bilirubin usually normal**	ICP	Hepatitis C serology Antimitochondrial and ASMA Abdominal USG
ALT ↑ (2–5-fold) **Total serum BA usually normal** **Total bilirubin usually normal**	PE with hepatic impairment	BP, ↑ in most Urine analysis for protein, + Creatinine ↑ Platelets ↓
ALT ↑ (2–30-fold) **Total serum BA usually normal** **Total bilirubin ↑ (1.5–10-fold)**	HELLP syndrome	BP ↑ in most Urine analysis for protein, + Creatinine ↑ in most Platelets ↓ LHD Hb ↓
ALT ↑ (3–15-fold) **Total serum BA usually normal** **Total bilirubin ↑ (4–15-fold)**	AFLP	BP (↑ in most) Urine analysis for protein, + in most Creatinine ↑ Platelets ↓ WBC count ↑ Plasma glucose ↓
ALT ↑ (2–5-fold) **Total serum BA usually normal** **Total bilirubin usually normal**	HG	Serum Na^+ ↓ Serum K^+ ↓ Thyroxine ↑ TSH ↓

ALT, alanine aminotransferase; BA, bile acids; ICP, intrahepatic cholestasis of pregnancy; PE, preeclampsia; HELLP, hemolysis, elevated liver enzymes and low platelets; AFLP, acute fatty liver of pregnancy; HG, hyperemesis gravidarum; ASMA, anti-smooth muscle antibodies; USG, ultrasonography; BP, blood pressure; LDH, lactate dehydrogenase; Hb, hemoglobin; WBC, white blood cells; TSH, thyroid stimulating hormone; ↑, increased; ↓ decreased; + positive.

2) *In which trimester is pregnant women presenting abnormal LFTs?*

Gestational age can be a clue to identify liver diseases in pregnancy, as pregnancy-related disorders present in a specific trimester in most of the cases. Contrastingly, pregnancy-unrelated liver disorders can occur throughout the gestation (4).

3) *Does patient present additional signs?* (see table 4)

Laboratory findings must be interpreted according presenting symptoms. Liver involvement usually presents with fatigue, jaundice, pruritus, abdominal pain, nausea and vomiting. As patient presents with signs of liver dysfunction, one might search for constellations of signs typical for each disease, together with past/present history and severity of complains. During physical examination measurement of BP, weight, presence of jaundice and edema, might help to decide further investigations (11).

Table 4. Typical presentation of pregnancy-related liver disorders (11)

Symptom	Likely diagnosis	Other possible diagnoses
Pruritus (3rd trimester)	ICP	Preeclampsia, AFLP, biliary obstruction, pre-existing hepatobiliary disease (PBC, PSC), DILI
Epigastric pain **Nausea and vomiting (2nd and 3rd trimesters)** **Headache, visual disturbance**	Pre-eclampsia, HELLP syndrome, AFLP	Gallbladder disease, cholangitis, viral hepatitis
Nausea and vomiting (1st trimester)	Hyperemesis gravidarum	Viral hepatitis
Jaundice	Viral hepatitis	HELLP syndrome, gallbladder disease, cholangitis, DILI, rarely ICP, AFLP, pre-eclampsia
Pale stools and dark urine	Biliary obstruction secondary to gallstone disease	ICP, cholangitis, viral hepatitis, other rare causes of biliary obstruction

ICP, intrahepatic cholestasis of pregnancy; AFLP, acute fatty liver of pregnancy; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; DILI, drug induced liver injury; HELLP, hemolysis, elevated liver enzymes, and low platelets.

3. Pregnancy-related liver disorders

3.1. Hyperemesis gravidarum

3.1.1. Definition

Nausea and vomiting in pregnancy (NVP) is a common symptom among pregnant women. About 50 to 80% (14) of pregnant women experience these symptoms in a mild and self-limiting way and improved without intervention in the second trimester. However, some women develop a severe form of these symptoms. This condition, known as HG, is characterized by intractable vomiting leading to dehydration, electrolyte abnormalities and significant weight loss (at least 5% of body weight) requiring hospitalization. HG is mostly self-limited and presents often during the first trimester (typically 4-10 weeks).

HG is not a liver disease by itself, but leads to liver dysfunction in 50% of cases (4).

3.1.2. Incidence and risk factors

HG is a condition present in 0.8 to 3.6% of all pregnancies and the most common cause of pregnancy-related hospitalization in the first half of gestation (15). Asian women seem to be more affected then Caucasian women (16). It's a multifactorial condition and has been associated with many risks factors (17). These include: younger age (<20), multiple pregnancy, nulliparity, obesity, metabolic disturbances, trophoblastic and psychological disorders (18). Specific diseases that have been showed to increase risk of HG include hyperthyroid disorders, gastrointestinal problems, pre-existing diabetes and asthma. Maternal smoking and age (>30) have been associated with decreased risk (19).

3.1.3. Etiopathogenesis

Etiology is not yet fully understood, but it is believed to be multifactorial. The most relevant etiological factors are hormonal, immunological and genetic (20).

High levels of human chorionic gonadotropin (hCG), have been shown to correlate with the severity of HG, which stimulate the secretory function of the gastrointestinal tract and thyroid function (3). Transient hyperthyroidism may occur in 60% of gestations owing to the thyroid-stimulating activity of hCG (4). Other hormonal factors proposed in the etiology of HG are elevated estrogen levels, decreased levels of prolactin, and hyperactivity of the hypothalamic-pituitary-adrenal axis (16).

Available data indicate that HG is associated with a hyperactivity of the immune system (21). Increased levels of interleukin 6 (IL-6) (22), tumor necrosis factor alpha (TNF-α) (23), immunoglobulins G and M, and complement proteins C3 and C4 have been shown in patients with HG. Furthermore, lymphocytes count, natural killer (NK) cells and extrathymic T-cells are higher than normal (24).

Recent studies also point for the potential role of *Helicobacter pylori* (*H. pylori*) in HG. *H. pylori* was found, histologically, in gastric mucosa of 95% of pregnant women with HG. Further studies identified *H. pylori* genome in saliva of 61.8% HG patients compared with 21.6% of pregnant women without symptoms (18). Although these associations have not determined if *H. pylori* infection is a cause or not, administration of antibiotics in these women improved their symptoms (25).

3.1.4.Clinical presentation

The clinical symptoms are mostly unspecific and uncharacteristic (20). The main symptom is intractable vomiting that results in loss of volume, weight loss, and electrolytes abnormalities.

HG typically starts in the first trimester , peaks in approximately by the ninth week and resolves by the twentieth week in 90% of women (26). However, it is not uncommon for HG to continue into the late second and third trimesters.

Frequent findings include metabolic acidosis, caused by a poor nutritional intake or metabolic alkalosis by the loss of hydrochloric acid and hypokalemia (2). Metabolic ketoacidosis and ketonemia may present with a “fruity smell on the breath” and ketonuria (18). Pyrexia, gastric pain, headache or neurologic signs point to other causes, although the latter finding may, in rare cases, result from severe and prolonged NVP.

Laboratory findings include raised BUN and creatinine, and decreased phosphate and magnesium. These disorders resolve with the cessation of vomiting.

Hepatic involvement occurs in 50 up to 60% of cases. The most common alteration is raised transaminases levels, with higher values of ALT when compared to AST (2). Clinical jaundice rarely occurs, but if does, bilirubin levels rarely exceed 100 μmol/L (27). The persistence of these alterations should raise alternative diagnoses (2).

3.1.5.Diagnosis

According the recent guidelines from the Royal College of Obstetricians and Gynecologists “NVP should only be diagnosed when onset is in the

first trimester of pregnancy and other causes of nausea and vomiting have been excluded". HG should be diagnosed when there is protracted NVP with the triad of more than 5% pregnancy weight loss, dehydration and electrolyte imbalance (26).

HG diagnosis is mainly by exclusion, from other conditions associated with emesis such as: other pregnancy associated conditions (emesis gravidarum, PE, HELLP syndrome and AFLP), gastrointestinal, urogenital, metabolic and neurologic causes (see table 5), and based on clinical symptomatology.

Table 5. Differential diagnosis in sustained nausea and vomiting in pregnancy (28).

Causes	Differential diagnosis	Diagnostic pointers
Pregnancy associated	Emesis gravidarum (<5 times/day)	Mostly in the morning, watchful waiting
	HG (>5 times/day)	Ketonuria, ketonemia
	Pre-eclampsia and HELLP syndrome	Clinical symptoms
	Acute fatty liver	Clinical symptoms, serology, ultrasonography
GIT	Gastroenteritis	Clinical symptoms, watchful waiting, stool culture
	Hepatitis	Raised transaminases
	Appendicitis	Early pregnancy: typical points for pain on pressure Late pregnancy: no typical leading symptoms
	Pancreatitis	Clinical symptoms, serology, amylases, lipases
	Ileus and subileus	Clinical symptoms, plain abdominal radiography (even in pregnancy)
	Hepatic or cholecystitis disorders	Serology, ultrasonography of upper abdomen
	Stomach ulcer or duodenal ulcer	Gastroscopy
	Stomach cancer	Gastroscopy
	Diaphragmatic hernia	Gastroscopy
Urogenital	Pyelonephritis	Clinical symptoms, urinary status, creatinine
	Nephrolithiasis	Ultrasonography
	Degenerative uterine fibroids	Ultrasonography
	Uremia	Urinary status, creatinine
Metabolic	Diabetic ketoacidosis	Clinical symptoms, urinary status
	Addison's disease	Clinical symptoms, serology
	Hyperthyroidism	fT3, fT4, TSH
	Thyrotoxicosis	Clinical symptoms, serology
Neurologic	Wernicke's encephalopathy	Medical history, clinical course, if required MRI
	Vestibular disorders	Nystagmus, impaired hearing
	Korsakoff's psychosis	Medical history, clinical course
	Migraine	Medical history
Other causes	Food poisoning	Medical history
	Iron medication	Medical history
	Drug poisoning	Medical history

HG, hyperemesis gravidarum; HELLP, hemolysis, elevated liver enzymes and low platelets; GIT, gastrointestinal; MRI, magnetic resonance imaging; fT3, free triiodothyronine; fT4, free thyroxine; TSH, thyroid stimulating hormone.

History and complete examination together with investigations are essential for differential diagnosis. USG (ultrasonography) scan should be scheduled to confirm viability and gestational age and to exclude multiple pregnancy or trophoblastic

disorder (29). Complete blood count (CBC), serum electrolytes, urinalysis and hepatic, renal and thyroid parameters should be performed. Liver biopsy is not recommended.

To classify the severity of NVP two validated and objective scores have been used: Pregnancy-Unique Quantification of Emesis (PUQE) score and Rhodes Index. The PUQE score is based on shorter disease-specific questionnaire and can be used to determine whether the NVP is mild, moderate or severe (see Annex A) and to track progress with treatment (26). Rhodes Index was originally validated to measure emesis in patient undergoing chemotherapy, but has subsequently been used for NVP (30).

3.1.6.Complications

3.1.6.1. Maternal complications

Pregnant women suffering from HG are at risk for complications related to excessive vomiting and dehydration with its subsequent consequences of malnutrition, electrolytes abnormalities and metabolic disturbances (29).

Nowadays, in its extreme forms HG may cause severe maternal malnutrition. End-organ damage may follow it manifesting as oliguria and abnormal liver tests, however permanent liver damage and associated death is rare (17).

Hematemesis, esophageal rupture, Mallory–Weiss tears and pneumothorax, were described in severe cases due to prolonged vomiting (31,32).

The mean nutrients dietary intake of most nutrients falls below 50% in women with HG that ultimately may lead to vitamin deficiencies and low protein status. Suboptimal biochemical status of vitamin A, B1 (thiamine), B2 (riboflavin), B6 (pyridoxine), B12 (cyanocobalamin) and retinol-binding protein have been shown in 60% of HG patients (33). Wernicke's encephalopathy is rare but can be caused by thiamine deficiency and precipitated by carbohydrate-rich food and glucose infusions (34).

Profound hyponatremia may result in personality changes, muscle cramps and weakness, ataxia, drowsiness and lately convulsions (15). Furthermore, central pontine myelinolysis, characterized by damage of corticospinal and corticobulbar pathways, was associated with rapid correction of plasma sodium levels and reported to co-exist with Wernicke's encephalopathy in HG patients (33).

Hypokalemia, when profound, together with hypovolemia may result in rare consequences, such as rhabdomyolysis, pancreatitis, and acute renal failure with resolution of symptoms after electrolyte and hydration repletion (35).

The combination of pregnancy, dehydration and further immobility in HG women increases the risk of thromboembolic events (33).

HG has been reported at increased risk for cognitive, behavioral and emotional dysfunction in pregnancy. Moreover, authors described an increased risk for depression, anxiety and mental health difficulties (17).

3.1.6.2. Fetal and perinatal complications

HG has been reported to be associated with an increased risk for adverse pregnancy outcomes such as low birth weight, preterm birth and small-for-gestational age infants (36-38) . However, not all studies found adverse outcomes in HG (39,40). A systematic review identified no association with Apgar scores, congenital anomalies, or perinatal death (39).

Pregnancy outcome in HG patients are determine mainly by maternal characteristics but other factors associated with HG, such as insufficient weight gain during pregnancy, maternal stress responses during pregnancy, or *Helicobacter pylori* (*H. Pylori*) infection involving the placenta, could adverse birth outcomes associated with HG (17).

Risk of psychological and behavioral problems in adulthood is increased in two studies concerning the neurodevelopment (15). Moreover, a recent study showed that insulin sensitivity in children of hyperemetic mothers is decreased (41). Others found that early pregnancy weight loss was associated with increased BP in offspring (42). Lastly, vitamin K deficiency due to nutritional maternal deficiencies was purposed as risk factor for fetal intracranial hemorrhage owing its potential on fetal blood coagulability (43).

3.1.7.Treatment

HG is often a self-limited disorder and its treatment is essentially supportive and should be adapted to the severity of the clinical picture. The main objectives are to control nausea and vomiting, correction of fluids and electrolyte imbalances and, if needed, thromboprophylaxis (3,29).

Mild cases can be controlled only with nutritional counselling and some changes in lifestyle (18). Pregnant women should be encouraged to avoid stimuli that cause nausea. Meals should be light and frequent, high in carbohydrates and low in fat content (1). When correction of dehydration is necessary, intravenous fluid therapy is performed.

Treatment of NVP with vitamin B6 or vitamin B6 plus doxylamine is recommended as first-line therapy, when available. Second-line therapy includes anti-emetic drugs such as antihistamines, namely promethazine and prochlorperazine. Metoclopramide, a dopamine antagonist is also safe and approved, as well as ondansetron and corticosteroids for refractory cases (3,44).

Supplementation with folates and thiamine should be weighted, especially in cases of vomiting with longer evolution time (29,34,44). Since in HG the administration of glucose enhances the risk of Wernicke encephalopathy, this should be accompanied by administration of thiamine (34) .

In severe cases, nasogastric tube feeding and/or total parenteral nutrition may be required (14).

Currently, alternative options such as ginger root and acupuncture are available for treatment, and results seem favorable (29).

3.2.Intrahepatic cholestasis of pregnancy

3.2.1.Definition

ICP is the most common pregnancy-specific liver disease. It presents in most of the cases with the triad of pruritus, typically of the palms and soles, abnormal LFTs, and raised serum BA levels (45). Biochemical abnormalities and symptoms usually resolves after delivery but recurrences in later pregnancies and with the use of combined hormonal contraceptives were often described (27). It classically presents in the third trimester but it can present as early as in 7 weeks of gestation (2). It has been associated with increased rates of adverse fetal and pregnancy outcomes.

3.2.2.Epidemiology

ICP is reported to have an incidence between 0.2 and 2%, however, it varies geographically and according ethnicity (45). Its prevalence is higher in South America, particularly in Chile (ranging from 3 to 5%) (3), and northern Europe, notably in

Sweden where there was a higher incidence of cholelithiasis and hepatitis C virus in ICP women (27). The incidence in Europe and USA is lower (about 1%), and rarely reported in African countries (3). Higher incidence occurs in multiple pregnancies (20 to 22%) and after *in vitro* fertilization (2.7%) (45).

3.2.3.Risk factors

Several risk factors are listed in the literature. Those include history of previous cholestasis during oral contraceptive use, family history of cholestasis, multiparity, multiple pregnancy, advanced maternal age, personal and/or family history of cholelithiasis, and hepatitis B virus infection (2,45).

Risk of recurrence in future pregnancies has been shown to range from 40 to 60% (46).

3.2.4.Etiopathogenesis

ICP has a multifactorial etiology to which genetic, hormonal and environmental factors contribute (47).

Genetic defects of canalicular transporters have been identified for the development of ICP, which may be further influenced by reproductive hormones (specially progesterone and estrogen). The etiology of fetal complications is likely to be related to the destructive effects of toxic BA, which accumulate in the fetal compartment.

Hormonal effects are based on following four circumstances: Firstly, ICP usually starts in the last trimester (highest maternal estrogen and progesterone concentrations) (48). Secondly, the incidence is higher in multiple pregnancies (49,50), in pregnancies following *in vitro* fertilization (2.7% *vs* 0.7% in normal conception) (49). Thirdly, studies have shown ICP in pregnant women given oral progesterone to prevent preterm labor; and finally, ICP resolves promptly after delivery and recurs in half of the patients during subsequent pregnancies (3).

Rodent studies have shown that estrogen has a key role in the development of cholestasis by causing reduced expression of hepatic biliary transport proteins and through internalization of the bile acid transporter bile salt export pump (45). Moreover, sulphated progesterone metabolites can act as partial agonists of farnesoid X receptor, thereby reducing the function of the main hepatic BA receptor and subsequent impairment of hepatic BA homeostasis (51).

The genetic etiology of ICP is largely unknown. However, several studies have identified genetic variations in genes encoding biliary transport proteins and BA receptor (primarily farnesoid X receptor). Positive familial history was reported in UK in 14% of pregnant women with ICP. Some studies have suggested an autosomal-dominant, sex-limited pattern (50). Overall, ten different mutations have been identified (2). About 15% of cases have genetic mutations in ABCB11, ABCB4 (3) and in a smaller amount of cases in ATP8B1 (45). 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 (52). ABCB4 encodes the multiresistant protein 3 (MRP3), a phosphatidylcholine flippase that transports phatidylcholine to the inner leaflet of the canicular membrane (45). Mutations in this gene may affect BA transport and lead to a rise in BA concentrations. These have been linked to a severe form of ICP (52).

Various single nucleotide polymorphisms in ABCB11 (encodes bile salt excretory protein) and ABCB4 genes are associated with ICP. ATP8B1, which encodes phosphatidylserine flippase 1, is also a candidate gene for ICP but small number of cases were identified (45).

Some of the exogenous factors implicated in the genesis of ICP are selenium deficits, seasonality and hepatitis C virus infection. Low serum selenium levels were reported in ICP pregnant women, and it is known that their dietary intake is low in Chile and Finland, countries where the incidence of this syndrome is higher (48). Interestingly the incidence peaks in winter months in these countries, when is known that selenium levels are lower. Selenium deficiency may cause defective bile formation or secretion since it is a cofactor for several oxidative hepatic enzymes (45). Not to mention that vitamin D levels are likely to be lower in the winter months, and deficiency of this vitamin has been reported in women with ICP (53).

3.2.5.Clinical presentation

Pruritus is a cardinal symptom of ICP, being present in 95% of the cases (54). It usually arises in the third trimester, after 25 weeks (55), and starts in the palms and soles. It often becomes worsen and generalized (27). The pathophysiology of the pruritus is still unknown but it has been suggested that results from bile salts deposition on nerve endings (56). The pruritus is most severe at night and can cause insomnia resulting in a considerable discomfort for pregnant women. Other causes of itching

must be excluded such as atopic dermatitis, allergic or drug reaction, prurigo of pregnancy and atopic eruption of pregnancy (45). Skin excoriations can be find flowing scratching (54).

Jaundice affects about 10 to 15% of pregnant women with ICH and manifests two to four weeks after the onset of pruritus (45,55). It is usually mild, associated with bilirubin levels not higher than 6 mg/dL (102.6 μmol/L) and keeps stable throughout gestation. Rarely, severe cases can present with pale stools and dark urine (27).

Pascual *et al*., describes the phenomena of asymptomatic hypercholanemia (AHP) of pregnancy present in 10% of pregnant population (57). It consists in elevated serum BA in pregnancy in the absence of symptoms and other biochemical markers of ICP. Furthermore, Castaño *et al*., described AHP in 40% of pregnant women in Argentina with normal pregnancy outcomes (58).

In severe cases ICP can cause steatorrhea with decreased absorption of fat-soluble vitamins and weight loss (59). This can further result in vitamin K insufficiency and prolonged PT (60).

There is usually spontaneous resolution of pruritus within the first 48 hours postpartum and analytical changes resolve within the next two to eight weeks (3).

3.2.6.Complications

ICP is usually associated with a good maternal prognosis. Malabsorption of vitamin K may lead to an increase in PT, and if not corrected it can lead to life-threatening postpartum hemorrhages, however it is a rare event. In women affected by ICP, cholestasis may recur with the use of oral contraceptives and/or with hormone replacement therapy (45).

ICP is associated with several adverse outcomes with considerable perinatal morbidity and mortality.

There are no concrete explanations for the occurrence of fetal complications, but these seem to be related to the raised maternal serum BA. BA induce contraction of the placental chorionic veins and increase the sensitivity of the myometrium to the action of oxytocin (27).

Several studies have shown that the risk of fetal complications increases 1 to 2% for each increase of one μmol/L of serum BA. The risk is significant higher when serum BA values exceed 40 μmol/L (61,62).

The main complication is prematurity. Incidence varies between 19 and 60%. Intrauterine death (0.4 to 4.1%) may also occur, as well as fetal distress (22 to 33%), meconium staining of the amniotic fluid (approximately 15%) (27) and fetal bradycardia (>14%) (55).

The rate of abortion and malformations is not increased in this disease (27).

3.2.7.Diagnosis

Laboratory findings, clinical presentation, and exclusion of other causes, make the diagnosis of ICP.

Liver tests, including direct bilirubin, PT, transaminases, GGT and serum BA (45) should be performed. The study of these pregnant women should also include the serology for the exclusion of hepatitis A, B, C and cytomegalovirus virus infection, as well as evaluation of renal function and CBC with platelets. Abdominal USG allows visualization of hepatic morphology and main bile ducts (63). USG usually reveals no dilatation of the intrahepatic nor extrahepatic bile ducts, but fasting and ejection volumes of the gallbladder are greater than in normal pregnant women. Thirteen percent of women with ICP are found to have gallstones in USG examination (27).

Generally, liver biopsy is not required for the diagnosis of ICP to be established. When performed, it reveals centrilobular cholestasis without associated inflammation and deposition of bile agglomerates in hepatocytes and canaliculi (48).

The differential diagnosis includes other forms of hepatic diseases. In patients with very high serum levels of aminotransferases, hepatitis A, B, C, severe PE, the HELLP syndrome, acute fatty liver of pregnancy, and drug toxicities should be excluded (3).

Increased serum BA levels have been used widely to diagnose ICP. Classification of ICP according the levels of BA seems to correlate with severity of fetal outcomes (64). A BA levels between 10-14 μmol/L (6 μmol/L if fasted woman) and 40 μmol/L are classified as mild, whereas >40 μmol/L is described as severe ICP. The primary BA cholic acid (CA) and chenodeoxycholic acid (CDCA) are the end products of hepatic cholesterol metabolism. These are conjugated with taurine or glycine before transport across canicular membrane. Within terminal ileum and colon, conjugated BA undergo bacterial modifications to form the secondary BA – deoxycholic acid (DCA) and lithocholic acid (LCA). In normal pregnancy, it is accepted and increase of levels of BA up to 1.5 μmol/L. More accurate parameter is CA/CDCA ratio which is reported to be normal between 0.68 and 1.9 at 38-40 weeks of gestation (45). Conjugated primary BA,

particularly tauroconjugates of cholic and chenodeoxycholic acid have been purposed as the most suitable biochemical marker for both diagnosis and monitoring of ICP, whereas CA/CDCA ratio, as being the most sensitive indicator of early diagnosis of the disease (27). Moreover, analysis of urine from women with ICP shows an increased excretion of CA and CDA, but lower values of DCA and LCA. The BA profile shows a shift from glycine to taurine conjugation and increased proportions of sulfated species. Nevertheless, a recent study in the Chinese population has shown, that unlike findings in other populations, total serum BA levels was not a reliable indicator of ICP. Instead, the authors of this study have found more specific biomarkers for this condition: MMP-2 and MMP-9 (metalloproteinases). Both markers were elevated in serum of ICP pregnant women and did correlate with the disease severity. MMPs also play a role in remodeling within the liver and have been studied as potential markers of fibrosis and cirrhosis. Elevated levels were also found in hepatitis C virus, cirrhosis and hepatocellular carcinoma (65). Prior studies in China also reported a better correlation to severity of ICP with indicators such as cholyglycine (a serum BA), serum bilirubin levels, in addition to AST and ALT levels (66,67).

In most of the cases liver transaminases are slightly elevated. This may occur before or after rise of serum BA, indicating in both scenarios hepatocellular damage. ALT seems to be the more sensitive indicator than AST (68).

The levels of serum bilirubin are elevated in the most severe form of ICP, and the level of total bilirubin is associated with preterm labour (69). The activity of GGT is increased in about 10 up to 15% but is commonly normal (48).

Glutathione S-transferase alpha (GSTA) is a phase II detoxification enzyme found within hepatocytes and in high concentrations within the liver. Therefore, plasma GSTA is expected to raise following acute liver damage. Dann *et al.*, has identified high serum GSTA levels as a useful indicator of liver dysfunction also in ICP and might help to distinguish ICP from pruritus gravidarum (70).

In a recent study, high serum autotaxin activity was suggested as a sensitive marker and correlated with the onset of ICP-related pruritus (71).

Other routine laboratory parameters can also predict an adverse perinatal outcome in ICP. Raised mean platelet volume is associated with preterm delivery in ICP patients (69). The neutrophil-to-lymphocyte ratio is elevated in pregnancies complicated with ICP and may be a predictor of the severity of ICP (72). Finally, a recent study showed

that RBC distribution width, was associated with ICP and was suggested as a diagnostic and prognostic marker in ICP (73).

3.2.8.Treatment

Treatment of ICP is mostly symptomatic to pregnant women, and ensure, as far as possible, a good fetal prognosis.

Currently, ursodeoxycholic acid (UDCA), a hydrophilic BA, is the treatment of choice at the dose of 500 mg twice daily or 15 mg /kg/day. UCDA shows a good effect in reduction of pruritus, improvement in liver tests and allows the prolongation of pregnancy, thus reducing prematurity. It is well tolerated by woman and is not associated with adverse maternal and/or fetal reactions. In higher doses, woman may complain of gastrointestinal upset and diarrhea, but is a rare finding (27).

Cholestyramine (8 to 16 g/day) is another viable option (62). This therapeutic choice reliefs pruritus, but does not decrease BA levels and there may even be worsening of vitamin K deficiency (2).

S-adenosyl-L-methionine and dexamethasone, may also be used. However, those are far less effective when compared to UDCA.

All pregnant women diagnosed with ICP should be supplemented with vitamin K (3).

Lastly, in severe cases, induction of labour between 37 and 38 weeks of gestations may be necessary (74). The decision must be taken on a case by case basis, considering the risks of prematurity and the possible complications inherent to this pathology.

3.3.Hypertension-associated liver dysfunction of pregnancy

Hypertensive pregnancy disorders include a broad range of clinical conditions, including chronic hypertension; gestational hypertension (transient hypertension of pregnancy or chronic hypertension identified in the latter half of pregnancy; formerly known as pregnancy-induced hypertension) (75); PE -*de novo* or superimposed on chronic hypertension (76) . These disorders are considered to be major causes of maternal, fetal, and neonatal morbidity, and may be life-threatening for both mother and fetus (44).

HELLP syndrome used to be categorized as a separate syndrome, but current thinking considers it as a severe manifestation of PE (77). However, is still controversial so will be discussed separately from PE.

Finally, hypertensive disorders in pregnancy may be associated with serious hepatic manifestations, including infarction, hemorrhage, and rupture.

3.3.1.Preeclampsia

3.3.1.1. Definition

PE is defined by the International Society for the Study of Hypertension in Pregnancy (ISSHP) as the onset of hypertension in previously normotensive pregnant women after the 20th week of pregnancy, of at least 140/90 mmHg, combined with new onset of one or more of the following : proteinuria (greater than 300mg in 24 hours); and/or signs of maternal organ dysfunction (renal, hepatic or neurologic involvement) and/or signs of uteroplacental dysfunction (fetal growth restriction) (78),. Detailed diagnostic criteria are further reviewed in subchapter "*Diagnosis"*. PE must resolve completely by the 6th postpartum week (79). It is considered as multisystemic disease that affects every maternal organ, predominantly the vascular, renal, hepatic, cerebral and coagulation systems (77). If PE presents with one or more convulsions, it becomes defined as eclampsia. PE can be classified as mild or severe which influences following approach to this condition. Severe PE is associated with one or more of the following findings: severe hypertension, BP ≥160 mmHg systolic, or ≥110 mmHg diastolic, on two different occasions at least 4 hours apart; proteinuria ≥500 mg in a 24-hour urine collection (dipstick [3^+] or [4^+]); or symptoms of organ dysfunction such as headache, hyperreflexia, oliguria, epigastric or right upper quadrant pain, impaired liver function, hepatomegaly, or thrombocytopenia (80).

Controversy remains as to whether fetal growth, without any other maternal feature of PE, should be considered to define it. Some authors support that this should apply, given that PE is more often itself a primary placental disorder (78).

Liver dysfunction in pregnancy complicated by PE and eclampsia ranges from 20 to 30 % (81).

3.3.1.2. Epidemiology

Hypertensive disorders affect 5 to 10% of all pregnancies. According to the World Health Organization (WHO), they are the main single cause of maternal death, accounting for approximately 16% of them in industrialized countries.

Pregnancy complicated by PE accounts for approximately 10% of all gestations and accounts for >50000 maternal deaths worldwide per year (4,82). In developed countries, the maternal mortality is practically nil, while in developing countries it can reach 15 to 20%. Fetal mortality is reported to range from 1 to 2% (1).

In 2002, incidence in Slovakia was estimated in 0.29/ 1 000 pregnancies. Average maternal age was about 23 years old, 86.7% of deliveries were cesarean and without fetal nor maternal deaths (83).

3.3.1.3. Risk factors

Complication rates are directly related to the severity and duration of elevated BP. Patients with severe hypertension in the first trimester have a greater than 50% risk of developing superimposed PE (75). All hypertensive patients should undergo increased surveillance, laboratory tests throughout pregnancy, USG scans to follow intrauterine growth, and antenatal testing (84).

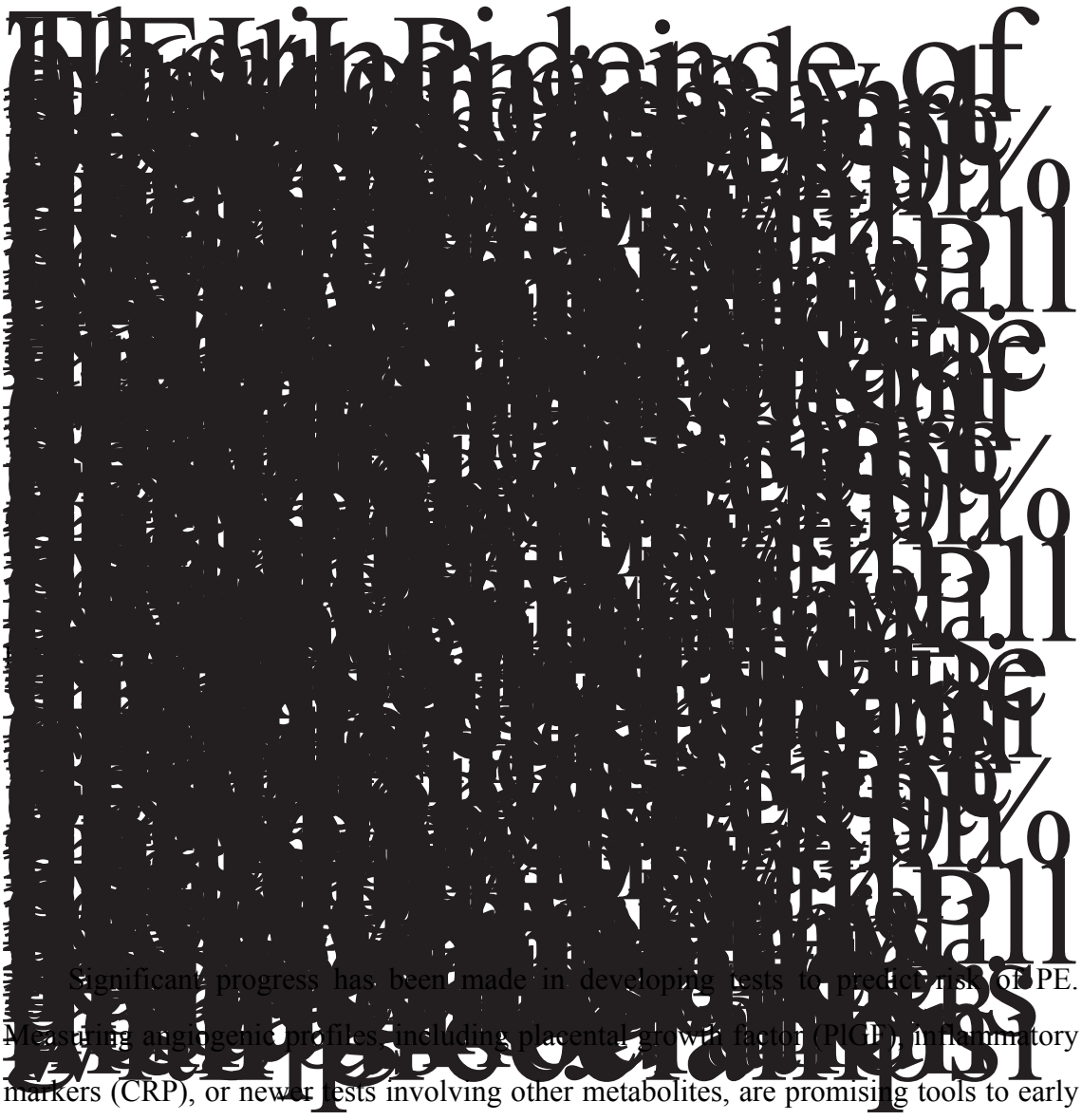
Early identification of PE, and prevention when possible, it's the main principle in an adequate management. Maternal characteristics that are associated with an increased risk of PE are:

- Past PE history (especially when severe or early-onset);
- Underlying antiphospholipid antibody syndrome;
- Pre-existing medical conditions - chronic hypertension, chronic renal disease, or insulin-dependent diabetes mellitus (85);
- Multiple pregnancy (79).

Other factors less strongly associated with PE include:

- Primigravidity;
- Interval between two pregnancies greater than 5 (86);
- Primipaternity – changed paternity (87);
- Short duration of sexual relationship (88);
- Obesity;
- Women who become pregnant with donor eggs, embryo donation, or donor sperm (79);

- African American race;
- Advanced maternal age, >40 years old;
- Family history of PE (89).



Significant progress has been made in developing tests to predict risk of PE. Measuring angiogenic profiles, including placental growth factor (PlGF), inflammatory markers (CRP), or newer tests involving other metabolites, are promising tools to early management of this condition (79,82).

3.3.1.4. Etiopathogenesis

Mechanisms responsible for the pathogenesis of PE are not fully understood. Growing evidence point to the central role of placenta in the development of hypertension, that explains the remission of this condition after delivery (90). Thus, placental ischemia/hypoxia is thought to lead to widespread dysfunction of the maternal vascular endothelium. Angiogenic unbalance leads to generalized vasoconstriction throughout the vascular system, ultimately compromising renal regulation of arterial pressure (77). Numerous factors including genetic, behavioral, and environmental factors have been implicated in the pathogenesis of PE (91) (see figure 2).

During normal placental development, fetal cytotrophoblasts induce changes in uterine spiral arteries that leads to changes and differentiation of the endothelium. Overall, this remodeling ultimately results in a conversion of the high-resistance, small diameter vessels into a high-capacitance, low-resistance vessels to accommodate to the increased maternal circulation needed for adequate placental perfusion (77). Multiple studies have suggested that in PE somehow this process is prevented, resulting in hypoperfusion and some degree of placental hypoxia (75).

Pathogenesis of this process has been discussed and different approaches have been proposed. These include: immune-mediated, angiogenic factors unbalance, cardiovascular maladaptation and vasoconstriction, genetic predisposition, platelet activation and vascular endothelial damage or dysfunction.

Current thinking enhances the role of immunological factors (92). Variability of immune system genes that code for major histocompatibility complex (MHC) molecules and NK cell receptors (93), was recently suggested to play an important role on human placentation. Colucci *et al*., proposed that specific combinations of fetal MHC and maternal NK cell receptors genes in humans, correlate with the risk of PE, recurrent miscarriage and fetal growth restriction (94). Thus, interaction between maternal NK cell and trophoblastic cells seems to fail in PE, as well as macrophage-derived TNF-α (95). Evidence support of this theory is that the risk of PE is highest in a first pregnancy (77).

Other specific factors recently implicated in placentation include: 1) the Notch signaling pathway; 2) the transcription factor storkhead box 1 (STOX1); 3) components of the renin-system (96); and 4) the intracellular serpin proteinase inhibitor-9. Notch signaling pathway is thought to play an important role in vasculogenesis by modulating differentiation and function during cell-cell contact. A recent report has shown that the absence of *Notch2* gene in mice is associated with reduced spiral artery diameter and placental perfusion (97). Doridot *et al.*, reported that aberrant and mutated forms of STOX1 induced in mouse leads to a phenotype that mimics PE, with severe hypertension during gestation and rise in levels of soluble fms-like tyrosine kinase-1 (sFlt-1) (98).

While abnormal placentation leads to placental insufficiency and fetal growth restriction, the development of PE may not occur. Further systemic changes are needed (92,93). Thus, immune maternal tolerance appears to play an important role in the

pathogenesis of PE (92). Despite the fact that the immune system is altered during a normal pregnancy compared to the non-pregnant state, pregnant women with PE have an even greater alteration, with an increase in innate immune activation in the periphery as well as in the uteroplacental unit (99). Secretion of inflammatory factors from immune cells such as $CD4^+$ T helper-1 cells, cytolytic NK cells, and B cells further worsens endothelial dysfunction, as well as contribute to decreased maternal vascular remodeling of the placental unit beginning early in the first trimester of a PE pregnancy (92). Specifically, growing evidences suggest that the imbalance between two $CD4^+$ T cell subtypes, regulatory T cells and T helper 17 cells, is involved in the pathophysiology of PE (93). As the pregnancy progresses the disease worsens and such factors may also contribute to the maternal vascular and renal dysfunction and hypertension observed in the later trimesters of PE. The lack of vascular remodeling contributes to an increase in uterine artery resistance, reduced placental blood flow, low oxygen and nutrient delivery and the development of placental ischemia with further intrauterine growth restriction of the fetus (100). The reduction in the supply of oxygenated blood to the uteroplacental unit causes an increase in oxidative stress and hypoxia stimulated factors (79). These factors include sFlt-1, the angiotensin II type-1 receptor autoantibody (AT1-AA), and cytokines such as TNF-α and IL-6 (101).

Recently, C-reactive protein (CRP), an acute phase reactant, sensitive indicator of tissue damage and inflammation, has been identified as a possible potent predictor of endothelial dysfunction in hypertensive disorders and PE in pregnancy. Furthermore, prolonged exposure to increased CRP levels has been linked with increased cardiovascular risks later in life (82,102). CRP is stimulated by TNF-α and IL-8/6 during inflammatory process, and predominantly synthetized and secreted by liver. Kolesárová *et al*., measured CRP levels in 61 women in risk of developing gestation hypertension, in 3 phases during pregnancy (before 20^{th} week, after 20^{th} week and postpartum). Ten women, developed gestational hypertension, which were found to have 3 times higher average values of CRP before 20^{th} weeks of gestation, when compared to those that didn't developed hypertension. Parchim *et al*., demonstrated that additional placental CRP production in women with PE (by syncytiotrophoblast cells) induced phosphocholine modification, carried by a phosphocholine transferase (PCT), which likely are pathogenic factors contributing to PE. As CRP binds to surface phosphocholine, it activates the complement system. PCT posttranslational modification of neurokinin B, a known pathogenic molecule of PE, are known at this moment to

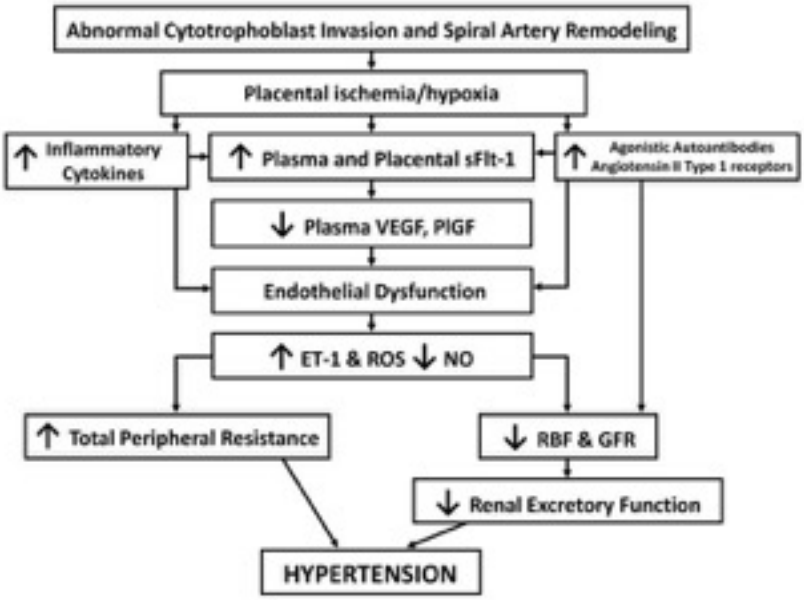
occur in only 2 places, testis and placenta. As the neurokinin 3 receptor is thought to be also involved in hypertension in pregnancy and phosphocholinated neurokinin B preferentially binds to this receptor, this pathway was explored. In a classical setup, the authors infused CRP at concentrations comparable to those found in the circulation of preeclamptic women into mice, which led to hypertension, glomerular damage, and associated proteinuria and atherosclerotic changes within placentas. Furthermore, author experimented neurokinin 3 receptor blockade, into hypertensive mice, that resulted in improvement of tissue lesions within the kidney and within the placenta, as well as sFlt-1 levels on CRP exposure (82).

Numerous studies have focusing in how sFlt-1 mediates PE. Recent studies show that the loss of vascular endothelial growth factor (VEGF) and P1GF (103) contributes towards to hypertensive changes. Elevated levels of sFlt-1, which works as a receptor for both VEGF and PlGF, act as a potent inhibitor of VEGF. Therefore, increased levels of sFlt-1 seem to prevent VEGF and PlGF availability to stimulate angiogenesis and maintain endothelial integrity (100). Moreover, smoking is known to inhibit sFlt-1, and clinical trials have shown reduce risk of PE in smokers (104). Thus, sFlt-1 represents for many researchers a potential therapeutic target, by reducing its levels to minimize PE severity and prolongation of pregnancy in early-onset disease (79).

Furthermore, inflammatory factors enhance the formation of endothelin (105) and superoxide (101), increased vascular response to angiotensin II, and decreased synthesis of vasodilators such as nitric oxide (90). Clinical expression of this process will involve high BP (75) with multi-organ dysfunction, especially in cases of early onset PE.

Therefore, identifying the connection between placental ischemia and maternal cardiovascular abnormalities is an important area of research (79).

Figure 2. Pathophysiological events in preeclampsia (90).



sFlt-1, fms-like tyrosine kinase 1; ET-1, endothelin-1; ROS, reactive oxygen species; NO, nitrous oxide; RBF, renal blood flow; GFR, glomerular filtration rate; VEGF, vascular endothelial growth factor; PlGF, placental growth factor.

Signs and symptoms, such as headache, tinnitus, scotoma, vomiting, nausea, epigastric and right upper quadrant pain, oliguria, hyperreflexia, and convulsions are believed to result from an imbalance between thromboxane and prostacyclin and the consequences of subsequent hypoxia and ischemia resulting from the obstruction of blood flow (106,107).

Blood concentration is further increased by volume changes due to loss of protein in the urine because of the presence of glomerular endotheliosis. Eclamptic states are explained based on focal vasospasms (108) of cerebral vasculature together with increased brain synthesis of neurotransmitters in pregnant women suffering from PE. Significant increase in the production of excitatory amino acids such as glutamate, binds to the N-methyl-D-aspartate receptor, allowing the opening of calcium channels in the cell membrane, with calcium entry into hypoxic cells triggering the typical tonic-clonic convulsions of eclampsia (106).

It should also be mentioned that studies have suggested that the time of onset of PE results in two different maternal hemodynamic states, explained as a consequence of different time-dependent adaptations to PE (109). Early PE (before 34 weeks) appears to be linked mainly to failed placental vascular remodeling and expresses through a high SVR - CO response, whereas late PE (after 34 weeks) might be more linked to maternal constitutional factors and is characterized by a low SVR - high CO (110).

To conclude, it seems well-founded that development of PE is mediated through the degree of placental pathology and the maternal inflammatory response to it.

3.3.1.5. Clinical presentation

The non-specific and variable presentation of the PE reflects its multi-organ involvement (2). PE presents in a severe form in 25% of cases (9).

Thus, when present, clinical features of PE include vomiting, edema, visual disturbances such as diplopia and scotomas, brisk tendon reflexes, and vigilance disorders (as a result of cerebral edema) (111). Severe cases might present with: oliguria, as the result of acute renal failure; pulmonary edema, presenting with shortness of breath (4,112); and uterine contraction and vaginal bleeding in case of placental abruption (111).

If liver is involved this will be presenting with band-like epigastric or right upper quadrant pain likely from stretching of Glisson's capsule from hepatomegaly (9).Vomiting and tenderness may accompany the pain (113). Pregnant women affected by PE most often do not present with clinical symptoms of liver dysfunction. However, liver tests often present some degree of disarrangement and in 70% of deaths from PE hepatic involvement is documented (44). Liver injury results from vasoconstriction and fibrin precipitation within the liver (3). AST and ALT elevations can be striking. In 20 to 30% of PE cases, transaminases increase up to ten to twenty times of reference value (2,76). Nevertheless, it is documented that 50% of PE women will have at least a single abnormal liver function test during gestation (114). ALP is elevated above normal pregnancy ranges and mild bilirubin elevations are also common.

In women with PE, function tests performed better in predicting adverse maternal than fetal outcomes. The presence of increased liver enzymes was associated with an increased probability of maternal and fetal complications, but normal liver enzyme levels do not rule out disease, as specificity was often higher than sensitivity (115). Therefore liver involvement suggest severe form of PE, thus alerting the physician that immediate management of hypertension is needed (2), or, if possible, early delivery (3). Recent analysis of a large cohort of births found that increased aminotransferase during the first 20 weeks of pregnancy was associated with a higher risk for severe PE in the second half of pregnancy but no clinical cutoff value was identified (116). Increased levels of LDH and uric acid in the setting of severe PE might indicate a progression to HELLP syndrome (117).

DIC and intravascular hemolysis in severe cases present with abnormal PT, APPT and fibrinogen levels.

Neurological impairment presents with cerebral edema, seizures and cortical blindness (112).

3.3.1.6. Complications

PE is itself a risk factor for both early and late complications affecting both mother and fetus (see table 6). Early-onset PE have been shown at greater risk of both fetal and maternal mortality when compared with late-onset (118).

Some of the early maternal complications observed are placental abruption (1 to 4%), renal failure (1 to 5%), pulmonary edema (2 to 5%) and stroke (rarely) (1,119).

Currently, consistent relationship is being established between PE and the risk of long-term cardiovascular and metabolic morbidities. Women whose pregnancy has been complicated by PE have been shown to have 3.7 times higher risk of developing hypertension later in life, 2.2 times increased risk of coronary heart disease and 1.8 times higher risk of stroke. The risk of developing diabetes was shown has 2.9 times higher (119,120).

Abnormal liver functional tests as a result of dysfunctional hepatic vascular bed can, in severe cases, progress to maternal portal hypertension (85). If hypertension is left untreated, hematoma below Glisson's capsule can develop and cases of hepatic rupture have been reported (3). These severe complications are referred for pregnant women suffering from PE, HELLP syndrome and AFLP. Thus, hepatic rupture, infarction and hematoma will be discussed at the end of this chapter.

Table 6. Maternal and fetal complications in severe preeclampsia (119).

Maternal complications	Neonatal complications
Placental abruption (1–4%)	Preterm delivery (15–67%)
DIC/HELLP syndrome (10–20%)	Intrauterine growth restriction (10–25%)
Pulmonary edema/aspiration (2–5%)	Hypoxia-neurologic injury (1%)
Acute renal failure (1–5%)	Perinatal death (1–2%)
Eclampsia (1%)	Long-term cardiovascular morbidity associated with low birthweight (fetal origin of adult disease)
Liver failure or hemorrhage (1%)	
Stroke (rare)	
Death (rare)	
Long-term cardiovascular morbidity	

DIC, disseminated intravascular coagulation; HELLP, hemolysis, elevated liver enzymes, low platelets.

The main fetal complications are the intrauterine growth restriction (10 to 25%), prematurity (15 to 67%) and death (1%) (119,120). Newborns of pregnancies complicated by PE are more likely to have newborn complications such as: low Apgar score, febrile seizures and encephalopathy. Long-term cardiovascular morbidities such as stroke and hypertension, associated with low birthweight (fetal origin of adult disease,) in child born to preeclamptic pregnant women has been described (119).

3.3.1.7. Diagnosis

The classification and diagnostic criteria for hypertension in pregnancy has been subject of discussion and divergence in the medical and scientific community. While in the past proteinuria was mandatory criteria for the diagnosis of PE, recent studies increasingly point to diagnostic failures considering this permission. In this way, ISSHP published an international recommendation to help classifying and diagnose these disorders. Table 7 summarizes those criteria.

Table 7. International Society for the Study of Hypertension in Pregnancy (ISSHP) definition of preeclampsia (78).

Hypertension - SBP ≥140 mmHg or DBP ≥90 mmHg, after 20 weeks gestation and the coexistence of one or more of the following new onset conditions:
1. **Proteinuria** - spot urine protein/creatinine >30 mg/mmol or >300 mg/day or at least 1 g/L [2+] on dipstick testing.
2. Other maternal organ dysfunction: • **renal insufficiency** - creatinine >90 μmol/L; 1.02 mg/dL. • **liver involvement** - elevated transaminases and/or severe right upper quadrant or epigastric pain. • **neurological complications** - examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata. • **hematological complications** - thrombocytopenia, DIC, hemolysis.
3. Uteroplacental dysfunction - **fetal growth restriction**.

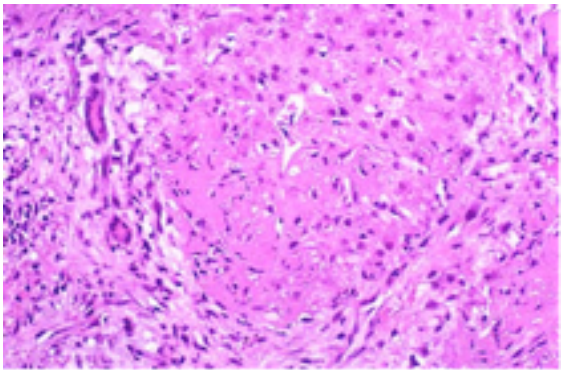
SBP, systolic blood pressure; DBP, diastolic blood pressure; DIC, disseminated intravascular coagulation.

Despites that a clinical definition remains the most appropriate, there is a growing recognition among medical society about the diagnosis based on biomarkers, particularly angiogenetic factors (91).

All hypertensive patients should undergo increased surveillance, laboratory tests throughout pregnancy, USG scans to follow intrauterine growth and antenatal testing. To exclude PE, even if asymptomatic with mild hypertension (140 to 159 and 90 to 109 mmHg) and no dipstick proteinuria, laboratory investigations must be performed, to

exclude maternal organ dysfunction. PE with hepatic involvement elevates the diagnosis to severe PE. Liver biopsy is rarely necessary, but histological findings include periportal hemorrhage and sinusoidal fibrin thrombi, ischemic lesions, microvesicular fat deposits and hepatocellular necrosis (2) (see figure 3).

Figure 3. Necrosis of periportal hepatocytes with abundant fibrin deposition (85).



Indeed, it is indisputable that identification of PE, and prevention when possible, is the main principle in an adequate management.

Many diagnostic parameters have been explored during early pregnancy as predictors of development of PE; these include, amongst others:

- Uterine artery doppler studies;
- Measurement of angiogenic factors - Endoglin, sFlt-1 and sFLt-1/PlGF ratio (91,121);
- ADAM-12 (Disintegrin and metalloproteinase domain-containing protein 12) (122), pregnancy-associated plasma protein A, placental protein 13, homocysteine, asymmetric dimethylarginine, uric acid and leptin;
- Urinary albumin or calcium (91).

3.3.1.8. Management and treatment

Once the diagnosis is made the therapeutic approach can be focused on four objectives: 1) to prevent and treat seizures; 2) to control hypertension; 3) to determine the gestational age at which to end pregnancy; and 4) to diagnose maternal and fetal complications (106).

Management of women with PE is done according to the severity of the clinical picture and expect evolution (79).

Early delivery and subsequent fetal prematurity, is associated with higher fetal mortality. Conservative management may result in maternal complications. Thus, the decision to deliver involves balancing the risks of worsening PE against those of prematurity. WHO (see Annex B) and National Institute for Health and Care Excellence (NICE) guidelines to manage hypertensive disorders in pregnancy, are, currently, used by physicians worldwide (84,123).

If the pregnancy is at term, the decision is easy: the baby should be delivered. Usually, delivery is not indicated for women with mild PE until 37 to 38 weeks of gestation. If remote from term, the mother should be admitted for evaluation (79).

In cases of severe PE pregnant women should be hospitalized and an initial strategy involves stabilization, fetal assessment and decision on the time of delivery. Prophylactic measures should be taken to prevent seizures and to control the BP. Magnesium sulphate is indicated for the prevention of seizures, as well as placental abruption (84).

To control BP there are several options to prevent maternal morbidity. Nonetheless, antihypertensive medications have no effect on disease progression or preventing eclampsia. Medications must be given with caution: if BP is lowered too fast, it can have a dramatic effect on uteroplacental perfusion and can cause an already compromised fetus to rapidly decompensate and become bradycardic. Preferred medications are hydralazine (5-10 mg intravenous bolus every 10-15 minutes), labetalol, nicardipine, and sodium nitroprusside. In acute situations, the use of intravenous labetalol or hydralazine are preferred.

Delivery before 34 weeks of gestation is recommended if one or more of the following emerge:

- Inability to control maternal BP despite antihypertensive drugs;
- Maternal pulse oximetry <90% or pulmonary edema unresponsive to initial diuretics;
- Progressive deterioration in liver function, GFR, hemolysis or platelet count;
- Ongoing neurological symptoms or eclampsia;
- Placental abruption; and
- Reversed end-diastolic flow in the umbilical artery Doppler velocimetry, a non-reassuring cardiotocography, or stillbirth (78).

Before 34 weeks of gestation if stabilization of both woman and fetus is achieved, but even though delivery is necessary, betamethasone must be administered (every 12

hours) to accelerate fetal pulmonary maturation. Delivery must be performed within the next 48 hours (120).

The NICE and WHO guidelines on the management of hypertensive disorders during pregnancy recommend that all women with a high risk of developing PE should start low-dose aspirin (75 mg) from 12 weeks until delivery (75,84). Women considered to be at high risk are the following:

- Hypertensive disease in the previous pregnancy;
- Chronic kidney disease;
- Autoimmune disease such as systemic lupus erythematosus and antiphospholipid syndrome;
- Type 1 or Type 2 diabetes mellitus; and
- Chronic hypertension.

NICE guidelines add that the same recommendation holds for women with more than one moderate risk factor for developing PE. These include:

- Primigravida;
- Age 40 years or older;
- Pregnancy interval of more than 10 years;
- Body mass index $\geq$35 kg/m^2;
- Family history of PE; and
- Multiple pregnancy (75).

Moreover, in areas where dietary intake of calcium is low, prophylactic treatment with calcium supplementation during pregnancy, is recommended in all women, but especially those at high risk of developing PE. Antioxidants like vitamin C and E can also be used (84,124).

Diuretics are usually contraindicated, however in case of pulmonary edema they are necessary. Intravenous hydration for oliguria must be given cautiously to avoid pulmonary edema, ascites and cardiopulmonary overload. If there is no evidence of pulmonary edema, a trial of fluid resuscitation (500 ml over an hour) should be given (123).

In severe PE, intensive monitoring, correction of coagulopathy and thrombocytopenia should continue for 24 to 72 hours after delivery, as patients are still susceptible to seizures, hemorrhage and other complications including renal failure, hepatic infarction, rupture and multi-organ failure (108).

3.3.2.HELLP Syndrome

3.3.2.1. Definition

HELLP refers to a syndrome characterized by hemolysis, elevated liver enzymes, and a low platelet count. As previous mentioned, it is currently suggested that is part of PE complications. Nonetheless, 15 up to 20 % of patients with HELLP syndrome do not have precedent hypertension or proteinuria, leading some authorities to believe that HELLP is a separate disorder from PE (125).

3.3.2.2. Epidemiology

The incidence of HELLP syndrome ranges from 0.5 to 0.9% of all pregnancies and from 10 to 20% of those with severe PE/eclampsia. Seventy percent of cases occur between weeks 27 and 37, with 20% occurring within 48 hours postpartum (117). Features of PE occur in the majority of patients presenting HELLP syndrome. Proteinuria accompanies more than 75% of cases, while 50 to 60% present with hypertension (126).

Maternal mortality associated with HELLP syndrome is around 2%, while perinatal mortality is 33%. The risk of recurrence is estimated at 3 % (13).

Dugátová *et al*., reported an incidence of HELLP syndrome in Slovakia of 0.63/ 1 000 births between 2012 and 2014. Twenty percent presented criteria to complete HELLP syndrome, while 80% to partial HELLP syndrome (127).

3.3.2.3. Risk factors

A previous history of PE or HELLP is a risk factor for HELLP syndrome. Sisters and offspring of women with a history of HELLP syndrome are also at increased risk of developing the syndrome (128). A variety of genetic variants associated with an increased risk of HELLP syndrome have been reported, but have no role in clinical management (129). HELLP syndrome is more frequent among Caucasian and mid-age women.

In contrast to PE, nulliparity is not a risk factor for HELLP syndrome. More than half of affected patients are multiparous (130).

3.3.2.4. Etiopathogenesis

The pathogenesis of HELLP syndrome is unclear. Maternal predisposing factors such as gene variants as well as environmental factors, are suggested etiological

mechanisms (125). Meanwhile gene variations associated with increased risk for HELLP syndrome include glucocorticoid receptor gene, toll-like receptor 4 gene, VEGF gene, FAS gene, CD95 and the coagulation factor V Leiden (117).

If HELLP syndrome it is a form of severe PE, it likely originates from abnormal placental development and consequent events described earlier. As an independent syndrome, it has been attributed to abnormal placentation, similar to PE, but with greater hepatic inflammation and greater activation of the coagulation system than in PE (131). Indeed, levels of fetal messenger RNAs (coding for both sFlt1 and Endoglin) in maternal blood at 15–20 gestational weeks are significantly more abnormal in HELLP than in PE. Maternal blood levels of anti-angiogenic sFlt1 are similar, but endoglin and Fas Ligand levels are possibly higher in HELLP than in PE. Moreover, inflammatory response in vascular endothelium is significantly higher in HELLP. Activated coagulation and complement, with high levels of activated leucocytes, inflammatory cytokines, TNF-α, and active von Willebrand factor, induce thrombotic microangiopathy with platelet–fibrin thrombi in microvessels (129). The angiopathy results in consumption of circulating platelets, causes hemolysis in affected microvessels and reduces portal blood flow in the liver.

Placental Fas Ligand damages hepatocytes, which is toxic for these cells. Fas Ligand triggers the production of TNF-α which may induce hepatocyte apoptosis and necrosis. Hepatocyte damage in HELLP is enhanced by the microangiopathy which impairs portal blood flow. Kidney dysfunction is usually moderate in HELLP and probably caused by the glomerular endotheliosis. In women with HELLP and postpartum renal failure, renal biopsy reveals thrombotic microangiopathy and acute tubular necrosis.

In about one half of women with HELLP, activation of coagulation factors and platelets precipitates disseminated intravascular coagulation (DIC), which in severe scenarios contributes to life-threatening multiorgan failure (77,129,130).

Mitochondrial fatty acid β-oxidation disorders (FAODs) are a heterogeneous group of defects in fatty acid (FA) transport and mitochondrial β-oxidation, and have been also suggested to play an important role in the pathogenesis of HELLP syndrome, PE and AFLP. They are inherited as autosomal recessive disorders (132). Fetal long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency has been particularly identified in children born to mothers with HELLP syndrome (133).

Role of ADAMTS-13 (ADAM metallopeptidase with thrombospondin type 1 motif 13) deficiency, a von Willebrand factor cleaving protease as a predisposing factor for women with HELLP syndrome is under study. Several studies shown low serum maternal level of this protease in women with HELLP syndrome, although its specificity and sensitivity is still suspicious (134).

3.3.2.5. Clinical presentation

Majority of women presents with symptoms in third trimester of pregnancy (28-36 weeks). However 30 % of cases occur in postpartum period usually within the first 48 hours (4). In an illustrative study with 437 women who had 442 pregnancies complicated by the HELLP syndrome, approximately 80% were diagnosed prior to 37 weeks of gestation and fewer than 3% developed the disease between 17 and 20 weeks of gestation (135).

There are no pathognomonic clinical signs and some women with HELLP may be asymptomatic (9). The most common symptom is abdominal pain and tenderness in the midepigastrium, right upper quadrant, or below the sternum (see table 8). Many patients also have nausea, vomiting, and malaise. Less common signs and symptoms include headache, visual changes and jaundice. Excessive weight gain and generalized edema precede the syndrome in approximately 50% of the cases. Underestimating signs, like abdominal pain, nausea, vomiting, and malaise for viral illness, have resulted in maternal death and severe morbidity (136).

Table 8. Reported frequency of signs and symptoms of HELLP syndrome

Symptoms	Frequency
Proteinuria	>85% (3)
Hypertension	82 to 88 %
Right upper quadrant/epigastric pain	65-100 %
Nausea, vomiting	35 – 100% (3,137)
Headache	30 to 60%
Visual changes	20 %
Generalized edema	50% (136)
Jaundice	5 % (4)

Hypertension (BP ≥140/90 mmHg) and proteinuria are present in majority of cases, but it is important to remember that either or both may be missing in women with otherwise severe HELLP syndrome (108,138).

Elevation of fibrin degradation products, low fibrinogen and a secondary prolonged PT are evidences of ongoing DIC (3).

3.3.2.6. Complications

Serious maternal morbidity may be present at initial presentation or develop shortly thereafter. This includes DIC, placental abruption, acute renal failure, pulmonary edema and retinal detachment (108).

Severe hepatic consequences are well recognized and include hepatic infarction, subcapsular hematomas and intraparenchymal hemorrhage (9).

DIC is one of the most serious complications of HELLP syndrome. It is present in about 38% of cases and closely related to placental abruption (136).

Isler *et al*., studied maternal mortality associated with HELLP syndrome. Out of 54 maternal deaths, 60% had class 1 disease, 35.6% had class 2 disease and 4.4% had class 3 disease. Events associated with maternal deaths included cerebral hemorrhage (45%), cardiopulmonary arrest (40%), DIC (39%), adult respiratory distress syndrome (28%), renal failure (28%), sepsis (23%), hepatic hemorrhage (20%), and hypoxic ischemic encephalopathy (16%). Delay in diagnosis of HELLP syndrome was reported in 22 of 43 deaths (51.1%) (139).

Peri-natal complications range from 20 to 30%. Most of the perinatal complications, e.g. asphyxia, respiratory disease and others, seem to be related to prematurity rather than an inherited defect of metabolism (114). A peri-natal infant mortality has been reported due to prematurity or secondary to mother complications (2).

3.3.2.7. Diagnosis

The diagnosis of HELLP syndrome is based upon the clinical presentations and presence of laboratory abnormalities comprising the acronym (hemolysis, elevated liver enzymes, and low platelet count). Thus, laboratory work-up should include (5):

- CBC with platelet count;
- Peripheral smear;
- AST, bilirubin (136);

- Serum creatinine; and
- Urine protein to creatinine ratio.

Recognized classifications include Tennessee and Mississippi systems (see table 9). In the Tennessee system classification, the result can be either complete/true or incomplete/partial HELLP syndrome. Complete HELLP syndrome stands for all the following criteria:

- Microangiopathic hemolytic anemia with characteristic schistocytes (also called helmet cells) on blood smear (see figure 4). Other suggestive signs are: raised LDH (≥600 IU/L), low haptoglobin (≤25 mg/dL) and raised total bilirubin (≥20.5 μmol/L) (140).
- Thrombocytopenia (≤100 000 cells/μL) - secondary to vascular endothelial damage and fibrin deposition in vascular walls.
- Elevated aminotransferases. Serum AST >2 times upper limit of normal for local laboratory (usually >70 IU/L). Some investigators obtain ALT levels instead of, or in addition to, AST levels. An advantage of the AST is that it is a single test that reflects both hepatocellular necrosis and RBC hemolysis (117).

Pregnant/postpartum women encompassing one or two components described before are considered to have incomplete/partial HELLP syndrome (9,109).

Other laboratory findings are hemoconcentration and metabolic acidosis. The PT or international normalized ratio (INR) remains normal until evidences of DIC or severe liver injury. Hypoglycemia and prolongation of PT may distinguish AFLP from the HELLP syndrome. Serum uric acid levels higher than 464 μmol/L are associated with increased maternal and fetal morbidity and mortality (2).

In the Mississippi-Triple Class System, a further classification of the disorder is based on the nadir platelets for assessment of the severity of the pathologic process of HELLP. Class 1 requires severe thrombocytopenia (≤50 000 cells/μL), evidence of hepatic dysfunction (AST and/or ALT ≥70 IU/L), and evidence suggestive of hemolysis (total serum LDH ≥600 IU/L); class 2 requires similar criteria except thrombocytopenia is moderate (>50 000 to ≤100 000 cells/μL); and class 3 includes patients with mild thrombocytopenia (>100 000 but ≤150 000 cells/μL), mild hepatic dysfunction (AST and/or ALT ≥40 IU/L), and hemolysis (total serum LDH ≥600 IU/L) Class 1 denotes worse prognosis and longer hospital stay. The trends in platelet count and serum LDH levels are predictive of speed of recovery (117).

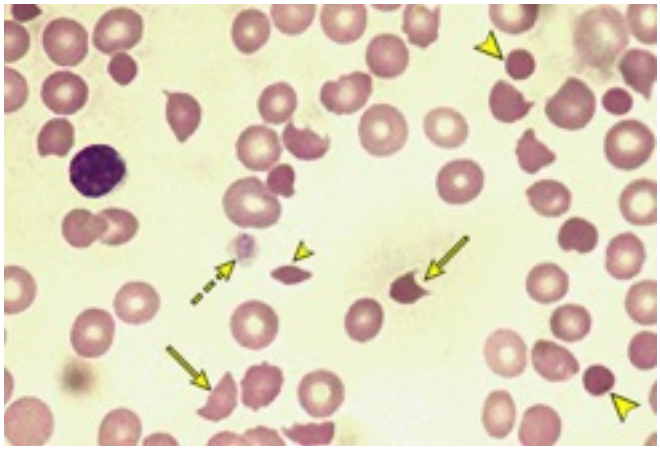
Liver biopsy is not indicated as the diagnosis is based on clinical criteria and is considered a high-risk procedure because of the thrombocytopenia and possible hemorrhage. Microscopic findings may be non-specific or like those of PE with small to diffuse areas of hemorrhage and necrosis in early stages in Zona 1 and later extending through all the lobule (2,4).

Table 9. Tennessee Classification and Mississippi classification for HELLP syndrome (9,109).

HELLP class	**Tennessee Classification**	HELLP class	**Mississippi classification**
Complete syndrome	MAHA on blood smear ↓haptoglobin LDH ≥600 IU/L Platelets ≤100 000 cells/μL ↑bilirubin AST or ALT ≥ 70 IU/L	**1**	Platelets ≤50 000 cells/μL AST and/or ALT ≥70 IU/L LDH ≥600 IU/L
Incomplete syndrome	Any one or two of the above	**2**	Platelets ≤100 000 cells/μL ≥50 000 cells/μL AST and/or ALT ≥ 70 IU/L LDH ≥ 600 IU/L
		3	Platelets ≤150 000 cells/μL ≥100 000 cells/μL AST and/or ALT ≥40 IU/L LDH ≥600 IU/L

HELLP, hemolysis, elevated liver enzymes, low platelets; MAHA, microangiopathic hemolytic anemia; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; IU; international units.

Figure 4. Peripheral blood smear from a patient with a microangiopathic hemolytic anemia with marked red cell fragmentation (141).



The smear shows multiple helmet cells (arrows) and other fragmented red cells (small arrowhead); microspherocytes are also seen (large arrowheads). The platelet number is reduced; the large platelet in the center (dashed arrow) suggests that the thrombocytopenia is due to enhanced destruction.

Liver imaging , such as computed tomography (CT) and MRI, may be important for the detection of complications such as hematoma and hepatic infarction (4).

Differential diagnosis of HELLP syndrome include: AFLP, gastroenteritis, hepatitis, appendicitis, gallbladder disease, immune thrombocytopenia, lupus flare, antiphospholipid syndrome, hemolytic-uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP) (see table 10) (114).

Table 10. Comparison of frequency of signs, symptoms, and laboratory findings in TTP, HUS, HELLP and AFLP (108,117,142,143).

Symptoms	HELLP	AFLP	TTP	HUS
Abdominal pain	++	++	++	++
Low ADAMST-13 activity	–/+	?	+/++	–
Anemia	+	+	++	++
Elevated LDH	++	+/++	++ very high	++ very high
Elevated transaminases	++	++	–/+	–/+
Fever	–	+	+	–
Headache or visual disturbance	++	–/+	++	–
Hypertension	++	+	+/++	++
Jaundice	+	++	–	–
Nausea and vomiting	++	++	++	++
Proteinuria	++	–	+ / hematuria	++
Thrombocytopenia	++	+	++	++
von Willebrand factor	–	?	++	++
Hypoglycemia	–/+	++	–	–

TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome, HELLP: hemolysis, elevated liver enzymes, low platelets; AFLP, acute fatty liver of pregnancy; LDH, lactate dehydrogenase; +, prevalence of finding in affected patients.

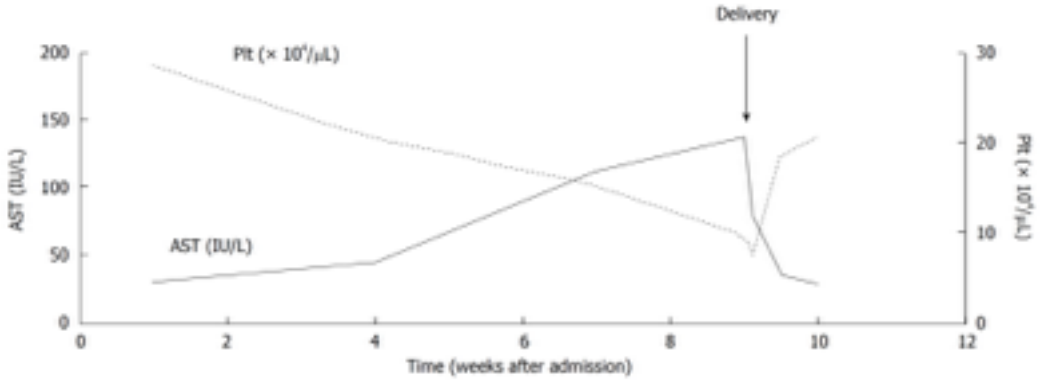
3.3.2.8. Management and treatment

Delivery is the only effective therapy for PE, eclampsia and HELLP. Management and decisions about HELLP syndrome, follow the same approach described in PE.

Caesarean section should be performed in women who develop HELLP syndrome before 30 weeks' gestation or if oligohydramnios is diagnosed. Regional anesthesia is indicated for cases with platelets count below 100 000 cells/μL. However, epidural anesthesia is contraindicated if the platelets count is below 75 000 cells/μL. Some authors also claim that regional anesthesia is contraindicated if the platelet count is below 100 000 cells/μL. Platelet transfusion prior to caesarean section has been suggested for class 1 HELLP syndrome, and for those with vaginal delivery and platelets count below 20 000 to 25 000 cells/μL (136). The laboratory changes are more

pronounced in the first 48 hours postpartum (see figure 5), so both mother and the newborn should be transferred to an Intensive Care Unit (ICU), with control of BP (144). Moreover, risk of maternal complications, especially renal failure and pulmonary edema, are increased in this period compared with antenatal period. In HELLP syndrome, in most of the cases the maternal platelets count continues to decrease immediately post-partum with an increasing trend on the third day.

Figure 5. Clinical course of hemolysis, elevated liver enzymes, and low platelets syndrome (HELLP) syndrome (121).



AST, Aspartate aminotransferase; Plt, Platelet.

The administration of postpartum corticosteroids (10 mg of dexamethasone every 12 hours) has been shown to be useful for raising maternal platelet count, lowering transaminases, and controlling BP (117,136). Progressive postpartum clinical, deterioration with elevation of bilirubin or creatinine for more than 72 hours may, require fresh frozen plasma (144). In the case of continuing hemolysis, persistent thrombocytopenia and hypoproteinemia, post-partum RBC and platelets administration, as well as albumin supplementation should be considered. Fluid supplementation should be done carefully, in cases of persistent oliguria (5) .

3.3.3.Hepatic hematoma, rupture and infarction

Incidence of hepatic hematoma and rupture in pregnancy is extremely low reaching about 1 case in 45 000 to 225 000 deliveries It is usually preceded by an intraparenchymal hemorrhage that progresses to a contained subcapsular hematoma, most often in the right lobe (75% of cases) (145). Persistent and growing hepatic hematoma perforate the capsule of Glisson, causing peritoneal irritation with secondary hypovolemic shock and cardiovascular collapse. Hepatic hemorrhage and rupture are most often complications of PE, eclampsia, HELLP syndrome. Therefore, often presents

in second or third trimester, with 30% of cases presenting postpartum. Is has also been reported in cases of AFLP (146). In 14% of cases there was no clear diagnosis of underlying condition. Is has been suggested that any trauma to the abdomen, either external or internal, including uterine contractions, is a risk factor for hepatic rupture. Furthermore, cocaine abuse in women with HELLP syndrome seem to be more susceptible (147).

Maternal mortality is estimated at 57 to 75%. Fetal mortality ranges from 62 to 77%, mostly from placental rupture, intrauterine asphyxia, or prematurity (146).

Patients usually present with sudden abdominal pain, that radiates to right shoulder, associated with nausea, abdominal distention, vomiting, fever (76) and hypotension. Ascites and right-sided pleural effusions have been also described. Laboratory findings include high transaminases levels (often >3000 U/L), thrombocytopenia, abnormal coagulation values (147), leukocytosis and anemia. CT and MRI are the best method for diagnosis (see Figure 6a) (145).

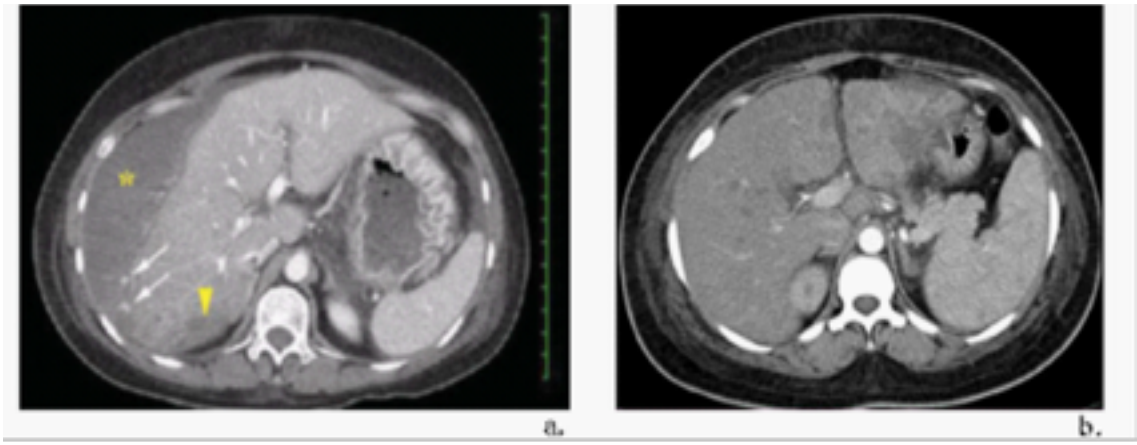
Antepartum women should be delivery as soon as possible. If woman is stable and without findings of liver rupture, hematoma can be managed conservatively with support coagulation factors, fluid management, antibiotics (to prevent infections) and blood transfusions, as required. Hemodynamically unstable patients, with persistent bleeding, increasing pain, or continued expansion of the hematoma, should receive recombinant factor VII and undergo urgent angiography with hepatic artery embolization and/or surgical intervention. Surgical interventions include: packing of the liver with collagen sponges, absorbable mesh, or fibrin glue; direct hepatic pressure; hepatic artery ligation; and partial hepatic resection (145,147). Liver transplantation may be necessary but has only rarely been performed in this setting (76).

Most of the times, patients have complete resolution, with normal CT scans, approximately 4 to 6 months later (147)

Hepatic infarction can be followed by hepatic hemorrhage. It is a rare event, and has been reported in HELLP and PE associated with an underlying antiphospholipid syndrome. Clinical findings include marked elevation in serum aminotransferases (usually 1000 to 2000 IU/L or higher), right upper quadrant pain, pyrexia, leukocytosis and anemia (148). The diagnosis is supported by characteristic hepatic imaging (MRI or CT) (see Figure 6b). There may be associated signs of liver failure. In the majority of

cases the liver complete resolute, but if in cases with extensive infarct, death from multi-organ failure or hepatic rupture can occur (108).

Figure 6. Abdominal CTs of pregnant women with **(a)** HELLP syndrome (145) and **(b)** antiphospholipid syndrome (149).



(a) Abdominal CT imaging performed postpartum in a woman with severe HELLP syndrome and right-upper quadrant pain. A large subcapsular hematoma (asterisk) is seen confluent with intrahepatic infarction and hematoma (arrowhead). Numerous flame-shaped hemorrhages are seen at the hematoma interface (arrows).
(b) Contrast-enhanced CT reveals patchy hepatic infarctions in a pregnant woman with associated antiphospholipid syndrome.

3.4. Acute fatty liver of pregnancy

3.4.1. Definition

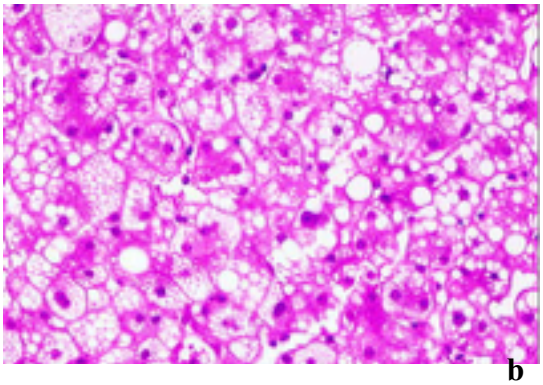
Acute fatty liver of pregnancy is a rare condition, first described in 1937 (2). It is a primary mitochondrial liver disease (150) and represents a medical and obstetric urgency (3). Noticeable concomitance between HELLP, PE and AFLP has been established in the last few years. Growing evidences support those as part of the same disease spectrum (151).

AFLP is characterized by the presence of microvesicular hepatic steatosis, which might rapidly progress to hepatic failure (3). It usually occurs in the third trimester of gestation, more specifically between 30 and 38 weeks. However, there were described cases at the end of second trimester as well as in early-postpartum period.

Most affected women recover within the first few weeks after delivery and remain without sequelae (60).

3.4.2. Epidemiology

The incidence of AFLP varies one in 7 000 to 15 000 pregnancies. Historically, geographic location, and ethnicity have not been considered as significant factors



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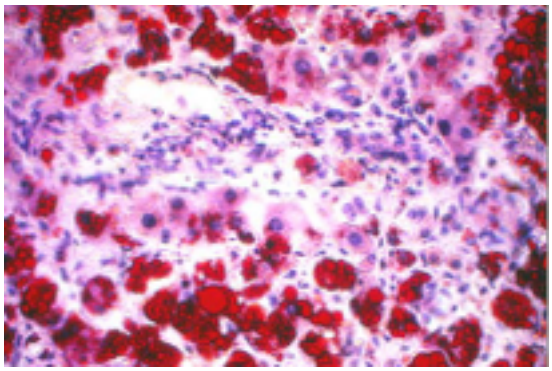

PUQE form:

Pregnancy-Unique Quantification of Emesis and nausea

Circle the answer that suit the best your situation for the last 24 hours.

1. On average in a day, for how long do you feel nauseated or sick to your stomach?

> 6 hours 5 points	4-6 hours 4 points	2-3 hours 3 points	≤1 hour 2 points	Not at all 1 point

2. On average in a day, how many times do you vomit or throw up?

≥7 times 5 points	5-6 times 4 points	3-4 times 3 points	1-2 times 2 points	Not at all 1 point

3. On average in a day, how many times have you had retching or dry heaves without bringing anything up?

≥7 times 5 points	5-6 times 4 points	3-4 times 3 points	1-2 times 2 points	Not at all 1 point

Total score (sum of replies to 1, 2, and 3): mild NVP ≤6; moderate NVP, 7-12; severe NVP ≥13.

Quality of life question:
On a scale of 0 to 10, how would you rate your well-being:_____
0 (worst possible) 10 (As good as you felt before pregnancy)