Research Square

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The Comparison of Total Bile Acid Concentration and Alcohol Dehydrogenase Activity as Markers of Intrahepatic Cholestasis of Pregnancy

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Abstract

Background: Intrahepatic cholestasis of pregnancy (ICP) is the liver disorder in the second or early third trimester of pregnancy. It is characterized by pruritus with increased serum total bile acids concentration (TBA) and increased liver enzymes. It is important to recognize the disease in its early stage. We aimed to investigate the serum alcohol dehydrogenase (ADH) activity and compare it with the concentration of total bile acid (TBA) in women with ICP..

Methods: Serum samples were taken from 80 pregnancies with ICP in the second or third trimester of pregnancy and from 80 healthy pregnant women. For measurement of ADH we used the spectrofluorometric and photometric methods.

Results: The results shows a statistically significant increase in the activity of ADH I and ADH total (about 60% and 41%, respectively). Activity of ADH I well correlated with aminotransferases (alanine ALT and aspartate AST) and TBA concentration. The total ADH activity was also positively correlated with ALT, AST and TBA.

Conclusion: We can state that the activity of class I ADH isoenzyme in the sera of patients with ICP is increased and seems to be a good indicator of liver cell destruction during ICP and is comparable with the value of other markers.

Background

Intrahepatic cholestasis of pregnancy (ICP) is a reversible cholestasis beginning in the second or third trimester. It affects 0.2 % – 15.6 % pregnant women but higher numbers of ICP cases have been reported in South America and Scandinavian. The etiology of ICP is poorly understood and is thought to be multifactorial, both genetic and environmental factors contribute to ICP pathogenesis [1]. Intrahepatic cholestasis of pregnancy is associated with an increased risks of perinatal complications, including spontaneous preterm labor, fetal respiratory distress syndrome or sudden intrauterine death [2]. The clinical manifestations of ICP include cutaneous pruritus, formation of jaundice and abnormal hepatic function [3]. ICP is usually detected quite late, therefore it is necessary to search for a new intrahepatic cholestasis of pregnancy markers which would enable the diagnosis of ICP at earlier stages of the disease. Currently, the diagnosis of ICP is based on only one symptom - pruritus, and is additionally supported by an elevated concentration of total bile acids (TBA) and/or liver enzymes [4]. However, there are no clinically useful diagnostic methods. Many studies show that changes in enzyme activity in the hepatocytes in the course of liver diseases are reflected by the change of its activity in the serum [5–6]. In our previous investigations we have found that the activity of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) were significantly higher in the serum of patients with different liver diseases (e.g. hepatitis C, autoimmune hepatitis, fatty liver disease) than in healthy subjects [7–9].

In this study we investigated the effect of ICP on the serum activity of alcohol dehydrogenase isoenzymes. These results were also compared with the concentration of total bile acids and activities of

enzymes which are commonly accepted as markers of liver cell damage (e.g. alanine aminotransferase, aspartate aminotransferase, concentration of bilirubin).

Methods

Material

Serum samples were taken for routine biochemical investigations from 80 pregnancies (range age 19–40 years) complicated by ICP in the second or third trimester of pregnancy hospitalized in the 2nd Department of Obstetrics and Gynecology, Medical University of Warsaw (Poland). Diagnosis was performed on the basis of clinical and laboratory investigations (total bile acid concentration, transaminases activities) Exclusion criteria were: chronic liver disease, Hellp syndrome and hepatic viral infection type A, B or C. Tested group were compared with 80 healthy pregnant women (range age 20–38 years) in the second or third trimester of pregnancy. None of the women consumed any alcohol.

Methods

Determination of class I ADH isoenzyme

The measurements were performed on a Shimadzu RF-6000 spectrofluorophotometer (Shimadzu Europa GmbH, Germany) at excitation wavelength 316 nm and emission of 370 nm. Class I of alcohol dehydrogenase isoenzyme activity was measured using fluorogenic substrates (4-methoxy-1-naphthaldehyde) in reduction reaction according to Jelski et al [10]. The assays were performed in a reaction mixture containing 0.1 M of sodium phosphate buffer, pH 7.6 (2.69 mL), a substrate (150 µL of 300 µM)), nicotinamide adenine dinucleotide reduced form (NADH) (100 µL of 1 mM) and serum (60 µL [11].

Determination of total ADH activity

The reduction of NDMA was monitoredat 440 nm on a Shimadzu UV/VIS 1202 spectrophotometer (Shimadzu Europa GmbH, Germany). Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline (NDMA) as a substrate. The reaction mixture (2 mL) contained 1.8 mL of a 26 µM solution of substrate in 0.1 M of sodium phosphate buffer, pH 8.5 and 0.1 mL of a mixture containing 0.25 M n-butanol and 5 mM NAD and 0.1 mL of serum [12].

Determination of other enzymes and total bile acids concentration

Alanine (ALT) and aspartate (AST) aminotransferases, γ-glutamyltransferase (γ-GT), alkaline phosphatase (ALP) activities, and total bile acids (TBA) concentration were measured with the kits from Abbott on an ALINITY biochemical analyzer (Abbott).

STATISTICAL ANALYSIS

All data were statistically analyzed using SPSS24.0 software from IBM. Measurement data conforming to the normal distribution are expressed as $x \pm s$, Statistical analysis was performed employing the $\chi 2$ test, Wilcoxon's test, and Pearson's correlation coefficients. Differences were considered significant at $p < 0.05$.

Results

In comparison with the control level (1.77 mU/L), the serum activity of class I ADH in ICP increased about 60% (2.83 mU/L). This increase was statistically significant ($p < 0.05$). The total activity of alcohol dehydrogenase was significantly higher in women with intrahepatic cholestasis of pregnancy than in healthy pregnant women (about 41.3 %). The median total activity of ADH was 1095 mU/l in patients with ICP, 643 mU/l in pregnant women. The activities of other enzymes, commonly accepted as markers of liver cell injury (alanine and aspartate aminotransferases), were high, with more apparent elevation of alanine than aspartate aminotransferase (Table 1). The activities of enzymes tested as markers of cholestasis (γ-glutamyltransferase and alkaline phosphatase) were increased but the differences were not statistically significant. The total bile acids concentration was significantly higher .in patients with ICP than in healthy pregnant women.

Table 1
Serum activities of cholestasis markers in ICP women and healthy pregnant

TESTED GROUP	ADH I Median. Range. Mean.	ADH Total Median. Range. Mean.	AST Median. Range. Mean.	ALT Median. Range. Mean.	ALP Median. Range. Mean.	γ-GT Median. Range. Mean.	Total Bile Acids Median. Range. Mean.
Pregnancies with ICP (n = 80)	2.83 0.78–5.28 2.65	1095 336–2614 1038	86 46–143 79	118 41–253 107	151 76–266 142	42 18–65 38	68.15 21.48–107.72 56.52
Healthy Pregnant Women (n = 80)	1.77 0.64–4.29 1.46 p < 0.05*	643 259–1368 621 p < 0.05*	55 13–69 34 p < 0.05*	62 32–93 51 p < 0.05*	144 63–238 131 p = 0.362	39 16–58 35 p = 0.402	5.72 1.27–9.43 4.85 p < 0.05*

ADH I and ADH Total are expressed as mU/L.

AST, ALT, ALP, **γ-GT** are expressed as U/L

Total Bile Acids are expressed as μmol/L

*- statistically significant differences between suitable groups

p, pregnancies with ICP vs healthy pregnant women

Serum ADH I activity was positively correlated with the activity of total ADH ($r = 0.412$, $p < 0.01$) (Table 2). The activity of this class was correlated with aminotransferases level ($r = 0.534$, $p < 0.01$ for ALT and $r = 0.487$, $p < 0.01$ for AST). ADH I was also positively correlated with total bile acids concentration ($r = 0.727$, $p < 0.01$). In case of enzymatic markers of cholestasis, alkaline phosphatise and γ-glutamyltransferase did not correlate with ADH I.

Table 2
Correlation coefficient between activities alcohol dehydrogenase and other markers of cholestasis in ICP.

	ADH Total	AST	ALT	ALP	γ-GT	Total Bile Acids
ADH I	r = 0.412	r = 0.487	r = 0.534	r = 0.153	r = 0.066	r = 0.727
	p < 0.01*	p < 0.01*	p < 0.01*	p = 0.132	p = 0.261	p < 0.01*
ADH Total	—	r = 0.392	r = 0.426	r = 0.106	r = 0.024	r = 0.638
		p < 0.01*	p < 0.01*	p = 0.173	p = 0.308	p < 0.01*
r is the correlation coefficient						
*Linear dependence						

The total ADH activity was positively correlated with aminotransferases activities and with TBA concentration ($p < 0.01$).

Discussion

Intrahepatic cholestasis of pregnancy is a pregnancy-specific disease that significantly increases the risk of fetal complications. It is also known as obstetric cholestasis (OC) [13]. It has been reported that inflammation is responsible for hepatocyte damage, dysfunction of biliary transport system, increased toxicity of bile acids and altered apoptosis [14]. Inflammation seems to be a risk factor for etiological pathology of intrahepatic cholestasis of pregnancy. Similarly, inflammation-related hepatic cell degradation products are probably responsible for the injury to the cellular components of the intercellular matrix, which is very important in maternal-fetal interaction. These events may be the reason for obstetrical complications such as prematurity, chronic impaired fetal perfusion and preeclampsia [15]. The pathogenesis of ICP is investigated. ICP laboratory diagnostics is also underway. The lack of agreement on diagnostic criteria contributes to the differences in the management of ICP. A diagnosis of intrahepatic cholestasis of pregnancy is confirmed by an elevated serum level of total bile acids and symptoms including pruritus and jaundice in the late second or third trimester of pregnancy without any sign of chronic liver disease. Elevated serum total bile acid levels (≥ 10 μmol/l) and increased serum aminotransferases with pruritus are the characteristic findings of this pregnancy-specific disease. Bile acids are a large family of molecules that have a steroidal structure and are synthesized from cholesterol in the liver and actively secreted along into the bile. Bile acid levels are increased in the serum and liver in patients with cholestasis and, perhaps because of their detergent action, they may damage hepatocytes. Thus, increased bile acid levels in hepatocytes may account for some of the liver damage in cholestatic liver diseases [16]. If TBA values are higher than 40 micromoles/L, there is an increased risk of fetal complications, although there seems to be no correlation between the severity of maternal symptoms and the level of the total bile acids [17]. No typical diagnostic biomarker, other than the TBA, is currently

available. However, serum TBA could not be used to distinguish the ICP patients with low pruritus from normal pregnant women, and even more, normal serum TBA concentrations have been observed in some cases with ICP [18–19]. New diagnostic and prognostic ICP biomarkers are also urgently required. A new group of potential markers are sphingolipids–structural components of cell membranes. These molecules participate in regulation gene expression as well as cell signaling on such phenomena as cell growth and death. They are regulators of hepatic homeostasis, modulators of liver regeneration and markers of liver injury [20] Mikucka et al. reported that sphingolipids can potentially become screening markers as well as markers for monitoring the treatment of ICP in pregnant women. However, their study do not suggest the use of sphingolipids can substitute TBA as earlier or better markers of ICP [21]. In preliminary studies Zou et al. indicated the usefulness of microRNA as ICP biomarkers [22]. MicroRNAs (miRNAs) are small, single-stranded non-coding RNAs (18–24 nt in length) which affect various biological processes including cell proliferation, metabolism, and tissue patterning during development [23]. The expression levels of three miRNAs (miR-371a- 5p, miR-6865-5p, and miR-1182) were significantly increased in ICP patients and may serve as noninvasive biomarkers of ICP [22].

The ADH profile of ICP patients has not previously been investigated. In present study, we found that the total ADH activity changed in the serum in the course of ICP. The cause for the increase of total alcohol dehydrogenase is an elevation of class I ADH. We showed an increase of ADH I (about 60%) activity in the sera of women with intrahepatic cholestasis of pregnancy. In our study we found that baseline serum total bile acids concentration (one of the major markers of ICP) increased in women with ICP. Additionally, TBA was positively correlated with ADH I and ADH activity. In the our study, we also demonstrated that the serum ADH isoenzyme activity during ICP was similar to the activity of aminotransferases (2-times elevation for alanine and 1,5-times for aspartate aminotransferase). The total ADH and class I isoenzyme activities did not correlate with alkaline phosphatase and γ-glutamyltransferase, which are typical enzymatic markers of cholestasis. It is commonly accepted that ALP and γ-GT are membrane bound enzymes and their increase in the sera of patients indicates dysfunction of hepatocyte membranes and enzyme synthesis in parenchymal cells in the course of cholestasis. The rise of aminotransferases always indicates liver cell injury. In the present study, the ADH activity did not correlate with membrane-bound enzymes but was strongly correlated with cytosolic enzymes (aminotransferases).

Conclusions

The serum activity of class I alcohol dehydrogenase isoenzyme and total ADH is comparable to the specific biochemical markers of liver cell damage in the women with intrahepatic cholestasis of pregnancy (especially concentrations of total bile acids and activities of alanine and aspartate aminotransferases). This is a preliminary work on the application of alcohol dehydrogenase as early biomarkers of intrahepatic cholestasis of pregnancy

Abbreviations

ADH, alcohol dehydrogenase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; ICP, intrahepatic cholestasis of pregnancy; miRNAs, microRNAs; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide reduced form; NDMA, p-nitrosodimethylaniline; OC, obstetric cholestasis; TBA, total bile acids;

Declarations

Ethics Approval

The research protocol was approved by the Human Care Committee of the Medical University in Bialystok, Poland (Approval Nr R-I-002/434/2017). All patients gave their informed consent for the examination.

Consent for publications

The authors have read and approved the final manuscript and consent to its publication.

Availability of supporting data

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

Joanna Piechota - general concept, editing the thesis and collecting material for research

Wojciech Jelski - general concept, compiling results and editing the thesis

Karolina Orywal - compiling results and collecting material for research

Barbara Mroczko - general concept, supervision

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