

The comparison of total bile acid concentration and alcohol dehydrogenase activity as markers of intrahepatic cholestasis of pregnancy

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Introduction: 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 bile acids concentration and other liver function tests. ICP is connected with increased risk of fetal mortality but is unfortunately detected quite late. Therefore, it is important to recognize the disease in its early stages. 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 for routine investigation from 80 pregnancies with ICP in the second or third trimester of pregnancy and from 80 healthy pregnant women at the same time of pregnancy. For measurement of class I activity, we used the spectrofluorometric methods. The total ADH activity was measured by the photometric method. **Results:** The analysis of results shows a statistically significant increase in the activity of ADH I and ADH total (about 60% and 41.3%, respectively). Activity of ADH I correlated well with aminotransferases (alanine ALT and aspartate AST) and total bile acids (TBA) concentration. The total ADH activity was also positively correlated with ALT, AST and total bile acids. **Conclusion:** We can state that the activity of class I alcohol dehydrogenase isoenzyme in the sera of patients with ICP is increased and seems to be a good indicator of liver cells destruction during this disease and is comparable with the value of other markers.

Keywords: total bile acids; alcohol dehydrogenase, intrahepatic cholestasis of pregnancy

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Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ -GT, γ -glutamyltransferase; ICP, intrahepatic cholestasis of pregnancy; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide reduced form; OC, obstetric cholestasis; p-NDMA, p-nitrosodimethylaniline; TBA, Total bile acid

INTRODUCTION

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 Bolivian, Chilean and Scandinavian. The etiology of ICP is poorly understood and is thought to be multifactorial, both genetic and environmental factors contribute to ICP

pathogenesis (Floreani *et al.*, 2013). Intrahepatic cholestasis of pregnancy is associated with increased risks of perinatal complications, including spontaneous preterm labor, fetal respiratory distress syndrome or sudden intrauterine death (Floreani & Gervasi, 2016). The clinical manifestations of ICP include cutaneous pruritus, formation of jaundice and abnormal hepatic function (Günaydin *et al.*, 2017). ICP is usually detected quite late, therefore it is necessary to search for a new intrahepatic cholestasis of pregnancy markers that would enable the diagnosis of ICP in the earlier stages of the disease. Presently, the diagnosis of ICP is based on only one manifestation – pruritus and is further supported by the raised levels of concentration of total bile acids (TBA) and/or liver enzymes (Pataya *et al.*, 2017). 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 (Jelski *et al.*, 2008; Chrostek & Szmitkowski, 1999). 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 (Jelski *et al.*, 2018a; Jelski *et al.*, 2018b; Jelski *et al.*, 2018c).

In this study we investigated the effect of ICP on the serum activity of alcohol dehydrogenase. 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).

MATERIAL AND METHODS

Material

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

Serum samples were taken for routine biochemical investigations from 80 pregnancies (age range 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 and hepatic viral infection type A, B

or C. Tested group was compared with 80 healthy pregnant women (age range 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 of 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 and others (Jelski *et al.*, 2014). The assays were performed in a reaction mixture containing a substrate (150 µL of 300 µM), serum (60 µL), nicotinamide adenine dinucleotide reduced form (NADH) (100 µL of 1 mM) and 0.1 M of sodium phosphate buffer pH 7.6 (2.69 mL) in conditions previously described (Jelski *et al.*, 2009a).

Determination of total ADH activity

The reduction of NDMA was monitored at 440 nm on a Shimadzu UV/VIS 1202 spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany) (Jelski *et al.*, 2009b). Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline (NDMA) as substrate. The reaction mixture (2 mL) contained 0.1 mL of serum and 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.

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

Statistical analysis was performed employing the χ^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 by 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 (by 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 the 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 phosphatase and γ-glutamyltransferase did not correlate with ADH I.

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) (Williamson & Geenes, 2014). It has been reported that inflammation is responsible for hepatocyte damage, dysfunction of biliary transport system, increased toxicity of bile acids and altered apoptosis (Webb *et al.*, 2014). Inflammation seems to be a risk factor for etiological pathology in the occurrence of intrahepatic cholestasis of pregnancy. Similarly, inflammation-related hepatic cell degradation products are probably responsible for the injury of 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 (Ozyuncu *et al.*, 2019). The pathogenesis of ICP is under investigation. ICP laboratory diagnostics are also underway. A lack of consensus in the diagnostic criteria contribute so the differences in 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

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

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

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 p<0.01*	r=0.487 p<0.01*	r=0.534 p<0.01*	r=0.153 p=0.132	r=0.066 p=0.261	r=0.727 p<0.01*
ADH Total	–	r=0.392 p<0.01*	r=0.426 p<0.01*	r=0.106 p=0.173	r=0.024 p=0.308	r=0.638 p<0.01*

r is the correlation coefficient. *Linear dependence

sign of chronic liver disease. Elevated serum total bile acid levels (≥ 10 $\mu\text{mol/l}$) 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 activities, can cause hepatocyte injury. Thus, increased bile acid levels in hepatocytes may account for some of the liver damage in cholestatic liver diseases (Voile, 2017). 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 bile acids (Glantz *et al.*, 2004). No typical diagnostic biomarker, other than the TBA marker, 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 (Muresan *et al.*, 2008; Reinhart *et al.*, 2000). 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 regulating gene expression as well as cell signaling on phenomena such as cell growth and death. They are regulators of hepatic homeostasis, modulators of liver regeneration and markers of liver injury (Maceyka & Spiegel, 2014). Mikucka and others (Mikucka *et al.*, 2020) reported that sphingolipids can potentially become screening markers as well as markers for monitoring the treatment of ICP in pregnant women. However, their study does not suggest that the use of sphingolipids can substitute TBA as earlier or better markers of ICP. Preliminary studies by Zou and others (Zou *et al.*, 2018) indicated the usefulness of microRNA as ICP biomarkers. MicroRNAs (miRNAs) are small, single-stranded non-coding RNAs (18–24 nt in length) that affect various biological processes including cell proliferation, metabolism, and tissue patterning during development (Zhang *et al.*, 2016). 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 (Zou *et al.*, 2018).

In the present study, we found that 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 have shown an increase of ADH I (by 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 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 the 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 strongly correlated with cytosolic enzymes (aminotransferases).

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.

Authors' Potential Conflict of Interest

No Conflicts of Interest

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