ORIGINAL ARTICLE

Evaluation of serum Glutathione-S Transferase-Alpha as a Biomarker of Intrahepatic Cholestasis of Pregnancy

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Abstract

Objective: To evaluate if serum Glutathione-S Transferase-Alpha (GSTA) can be used as a biomarker of intrahepatic cholestasis of pregnancy (IHCP)

Methods: 45 pregnant women with IHCP and 45 age and weight matched pregnant women as controls were enrolled and liver function tests including serum GSTA were measured. All cases and controls were followed for their maternal and foetal outcomes till delivery. Statistical analysis included unpaired t test, Mann-Whitney test and chi square test.

Results: IHCP group showed higher mean serum GSTA concentrations compared to controls (85.6 ± 42.7 mcg/l vs. 40.6 ± 6.8 mcg/l; p <0.001, CI 95%). A positive correlation was observed between serum GSTA and other markers of IHCP like serum bilirubin (r=0.346; p<0.001), AST (r=0.708; p<0.001), ALT (r=0.656; p<0.001) and bile acids (r= 0.491; p<0.001).Cut off value for GSTA of 47 mcg/l demonstrated good sensitivity (97.8%),specificity (88.9%), positive predictive value (89.8%) and accuracy (93.3%) to diagnose IHCP.

Conclusion: Serum GSTA may be a new promising, diagnostic tool with good accuracy compared to the routine markers for diagnosing IHCP.

Introduction

Intrahepatic cholestasis of pregnancy (IHCP), which is also known as obstetric cholestasis, is a pregnancy specific liver disease associated with increased rate of adverse maternal and foetal outcome. After viral hepatitis, IHCP is a frequent cause of liver dysfunction in pregnancy and reported incidence in India is between 1.2–1.5%.[1] This is a unique disorder of pregnancy characterised by mild to severe pruritus with disturbed liver function test and is not associated with any dermatological lesions other than excoriations due to severe itching. Pruritus is more severe in the evening and at night with a predilection to involve the soles and palms. It occurs in second and third trimester of pregnancy, although it is rarely noted in first trimester. Severity of the disease increases as pregnancy advances and shows complete resolution after delivery with high recurrence rate in subsequent pregnancies.

The diagnosis of IHCP is based on pruritus in pregnancy with deranged liver function tests in absence of other pathological conditions. However pruritus in pregnancy can be a common symptom, and may be the only presenting feature in intrahepatic cholestasis of pregnancy. No reliable test currently exists that can discriminate between those women destined to develop IHCP and those with the benign condition of pruritus gravidarum.

The etiology of IHCP is complex and not fully understood but it is likely to result from stasis due to reproductive hormones, estrogen, progesterone and their metabolites in genetically susceptible women. Mechanism by which the fetal complications occur is also not very clear.

Though it is accompanied with clinical jaundice in approximately 10%-15% pregnant women but serum bilirubin level is rarely above 5 mg/dl and has limited value in diagnosis or follow up if raised. Serum ALT and AST may also be elevated. Serum bile acid measurement is considered to be the most suitable biochemical marker for both diagnosis and monitoring of IHCP but there no consensus on whether a rise in serum bile acid precedes the onset of symptom and similarly, there is no agreement on whether serum bile acid should be measured in the fasting or post prandial state, frequency for monitoring in IHCP also not been defined. As bile acid test is costly (approximately Rs.1000-1500 per test), time consuming and not easily available, there is a need of sensitive, low cost screening marker. Most laboratories have a turn over time of 3-4 days for S. bile acid level result, making management decision based solely on bile acid levels difficult.

Serum glutathione s transferase alpha (GSTA) is a liver enzyme of phase II detoxification. GSTA is a potentially useful candidate for the early identification of hepatocellular damage in IHCP before serum ALT/AST levels increase, because it is rapidly released into the circulation after acute liver damage. GST alpha is specifically found in higher concentration in the liver and the levels in the plasma are known to rise exclusively in hepatic disease.[2] Therefore, GSTA may provide a more sensitive and specific evaluation of hepatic integrity in IHCP.[2]

Objective of this study was to evaluate if serum Glutathione S Transferase Alpha (GSTA) can be used as a biomarker in women with IHCP and to compare fetomaternal outcome of women with IHCP and healthy controls.

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Material and Method

This prospective, observational, case control study was conducted in the Department of Obstetrics and Gynecology, in collaboration with Department of Biochemistry. In this study pregnant women diagnosed as IHCP were taken as cases. Diagnosis of IHCP was made by history of pruritus, altered liver function tests, negative viral markers and ultrasound upper abdomen showing no other liver pathology. Age and weight matched healthy women with singleton pregnancy without any medical complications were enrolled as controls. Women with dermatitis of pregnancy, acute viral hepatitis, obstructive jaundice, acute fatty liver, HELLP syndrome were excluded from the study.

After taking approval from Ethics Committee of Human Research, convenient sample size of 45 pregnant women with IHCP and 45 healthy pregnant women were recruited for the study. After obtaining an informed consent, complete history and thorough clinical examination were done. Blood examination included complete blood count, liver function test including serum bilirubin, ALT, AST, alkaline phosphatase (ALP), prothrombin time, INR were done. Viral markers including HBsAg, HCV, HAV, HEV and USG upper abdomen for evaluating hepatobiliary system were done to rule out other cause of cholestasis. Serum bile acids report was noted whenever available as it is an expensive test and is not available in our institute. Serum Glutathione s transferase was measured for all recruited patients. GSTA was measured with double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) QAYEE-BIO. Samples were stored at -20'c to avoid loss of activity and contamination.

Statistical analysis was performed by the SPSS program for Windows, version 17. Continuous variable are presented as mean ± SD, median and categorical variables are presented as absolute numbers and percentage. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired t test, whereas the Mann-Whitney U test was used for those variables that were not normally distributed. Categorical variables were analysed using either chi square test or Fisher's exact test. P value of <0.05 was considered as statistically significant.

Results

Mean age of women with IHCP and healthy control group was 25.3±4.8 years and 24.4±3.4years respectively (p < 0.2). Mean weight of cases and controls was 56.2±5.9 Kg and 55.6±5.3 Kg respectively (p<0.5). In IHCP group there were 5 (11.1%) women who had history of intrahepatic cholestasis of pregnancy in previous pregnancy while no history of intrahepatic cholestasis of pregnancy was noted in controls (p<0.05).

Liver function tests are depicted in Table1. Seven women in the IHCP group had jaundice and the highest serum bilirubin was 1.9 mg/dl. All parameters including total bilirubin, AST, ALT, SAP and GSTA were significantly higher in IHCP group as compared to the controls. Serum bile acid (SBA) could be measured in 35/45 women of the IHCP group and was found to be high (≥10 µmol/L) in all subjects. Range of SBA varied between 10-25 µmol/L with a mean of 15+4.5µmol/L.

Maternal outcome of IHCP and control group is depicted in Table 2. In the study group 36 (80%) patient were induced (P<0.001) while only 7(15%) patients were induced the in the control group (P<0.001). All women were treated as per standard protocol of the department. Women with IHCP were kept under strict fetomaternal surveillance with daily fetal movement count, weekly biophysical profile and biochemical testing. They were given ursodeoxycholic acid tablets, injection Vitamin-K intramuscularly for 3 days and were induced at 37-38 weeks or earlier in severe cases to prevent adverse perinatal outcome. Women in the control group were admitted for anaemia (16), previous LSCS (11), safe confinement (8), false labour pains (10) and were treated as per their complaints.

There were 7 (15.6%) preterm deliveries in IHCP group while only one (2.2%) preterm delivery was noted in controls (p< 0.058). Fourteen women (31.1%) delivered by LSCS in IHCP group. The indication for LSCS was meconium stained liquor in 10(71%), foetal distress in 3(21%), breech in 1(7%). Three (6.7%) forceps deliveries were observed in IHCP group while no assisted deliveries occurred in control group.

Fetal outcome is shown in Table 3. In IHCP group significantly more adverse events noted were meconium stained liquor (MSL), foetal distress, low APGAR, admission to special care baby unit postpartum, intrauterine deaths (IUD), preterm delivery.

Discussion

Etiopathogenesis of IHCP includes

Table1: Liver function tests in IHCP and control group

	Cases			Controls			P value
	Mean ± SD	Range	Median	Mean ± SD	Range	Median	
T.BIL (mg/dl)	0.77 ± 0.30	0.30 - 1.90	0.70	0.45 ± 0.22	0.10 - 1.0	0.40	<0.001
AST (u/l)	168.53 ± 75.88	82 – 455	148	21.38 ± 10.58	10 – 56	18.00	<0.001
ALT (u/l)	196.76 ± 110.68	94 – 627	161	26.42 ± 14.33	10 – 80	23.00	<0.001
SAP (u/l)	371.09 ± 115.55	178 – 665	348	143.78 ± 39.41	88 – 266	134.00	<0.001
GSTA mcg/l	85.58 ± 42.67	37.750 – 238.05	75.25	40.59 ± 6.759	27.66 ± 57.15	41.41	<0.001

Table 2: Maternal outcome of IHCP and control group

	Cases (n=45)		Controls (n=45)		P value
	Frequency	%	Frequency	%	
Preterm	7	15.6%	1	2.2%	0.058
Term	38	84.4%	44	97.8%	0.058
Induced	36	80.0%	7	15.6%	<0.001
Vaginal	32	71.1%	44	97.8%	<0.001
LSCS	14	31.1%	0	0.0%	<0.001
Other complications	3	6.7%	2	4.4%	1.000

Table 3: Fetal outcome of IHCP and control group

Parameter	Cases (n=45)		Controls (n=45)		P value
MSL	10	22.2%	0	0.0%	<0.001
APGAR at 1 min	7.18 ± 1.79		8.07 ± 0.33		0.001
APGAR at 5 min	8.20 ± 1.97		9.00 ± 0.00		0.008
Baby transferred to mother	34	75.6%	44	97.8%	0.004
Baby transferred to nursery	8	17.8%	0	0.0%	0.006
NICU admission	1	2.2%	0	0.0%	1.000
IUD	2	4.4%	0	0.0%	0.494

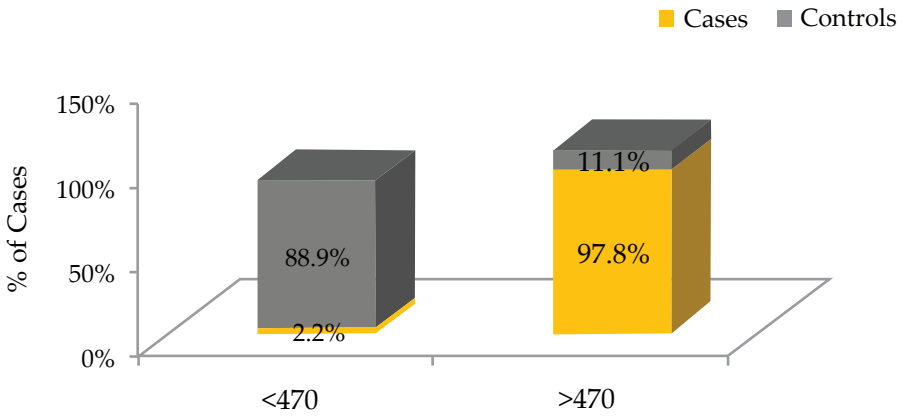

Fig. 1: Correlation of serum GSTA cut-off 47pg/dl value with cases and controls

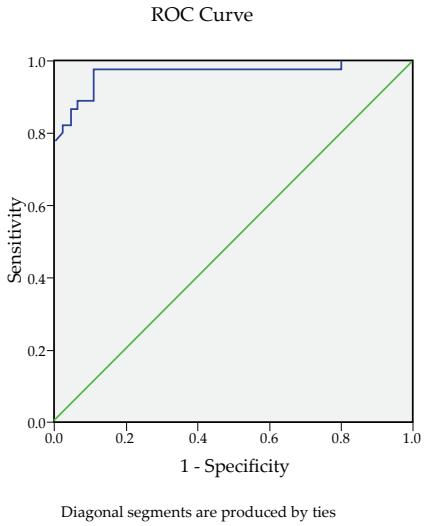

Fig. 2: Area under the receiver operating characteristics curve for GSTA for diagnosing of IHCP

genetic, hormonal and environmental factors.[3] It is associated with adverse fetal and maternal outcome. It has been observed that high level of bile acids in IHCP are associated with meconium stained liquor due to stimulation of the colon or may cause fetal lung injury or may be responsible for fetal heart rate abnormalities resulting in increased perinatal morbidity and mortality.[4-7] It may also lead to long term effects on health of the child, including altered hepatobiliary function even after birth and susceptibility to increased adiposity and metabolic disease.[8,9] Mothers with IHCP are at a higher risk of developing gestational diabetes,[10] gallstone formation and cholecystitis.[2]

Mean age of cases and controls were matched. There was no significant correlation of IHCP with age or weight of the patient. There were 5 (11.1%) women with history of IHCP in previous pregnancies. Of these, one woman revealed history of IHCP in 3 previous consecutive pregnancies with 2 full-term intrauterine deaths.

There was a significant difference noted in total bilirubin between cases and control groups (P<0.001) respectively. The transaminase enzymes are located within hepatocytes and thus raised serum levels in any condition indicates hepatocellular damage. In this study AST (P<0.001) and ALT (P<0.001) levels were raised significantly in the study group compared to controls but ALT level was raised more than AST.

The IHCP group showed significantly higher serum GSTA concentrations compared to controls (85.6 ± 42.7 vs. 40.6 ± 6.8; p <0.001, CI 95%). GST is a phase II detoxification enzyme which metabolizes chemotherapeutic agents, carcinogens, environmental pollutants and other harmful compounds and has four subtypes. GSTA are found in high concentration in the liver cytosol while aminotransferase and gamma-glutamyl transferase are located in periportal hepatocytes and are released in lower concentration to detect mild hepatic damage. A significant positive correlation was observed between serum GSTA and other markers of IHCP like serum bilirubin (r=0.346; 0<0.001), AST (r=0.708; 0<0.001), ALT (r=0.656; 0<0.001) and SBA (r=0.491; p<0.001). Dann et al[2] and Joutsiniemi T. et al[11] also observed that concentrations of GSTA was significantly elevated in patients with IHCP compared with women with pruritus gravidarum and normal pregnant controls and recommended it's use for diagnosis of IHCP.

In the present study, a cut off value for GSTA of 47 mcg/l demonstrated good sensitivity (97.8%), specificity (88.9%), positive predictive value (89.8%) and accuracy (93.3%) to diagnose IHCP. Cost of each test was approximately Rs 200/- using ELISA KIT and did not require sophisticated equipment. Figure 1 shows correlation of serum GSTA cut-off of 47 mcg/l value with cases and controls. Area under the receiver operating characteristic curve for serum GSTA measurements in diagnosis of intrahepatic cholestasis is depicted in Figure 2. The area under the curve was 0.968 with 95% CI (0.930-1.006).

To conclude, GSTA was significantly higher in women with IHCP with respect to controls. Cut-off value of 47 mcg/l distinguished women with IHCP and controls. Significant positive correlation was observed between serum GSTA levels with other liver function parameters like total bilirubin, ALT, AST and serum bile acid. According to the results, GSTA may be a promising, simple, low cost diagnostic test for IHCP with high sensitivity and specificity.

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