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ORIGINAL ARTICLE

Diagnostic value of endoplasmic reticulum stress-induced proteins in intrahepatic cholestasis of pregnancy

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ABSTRACT

Objective: This study aimed to investigate the potential diagnostic value of endoplasmic reticulum (ER) stress-induced proteins, namely heat shock protein A5 (HSPA5) (also known as GRP78) and C/EBP homologous protein (CHOP), in maternal serum for identifying patients with intrahepatic cholestasis of pregnancy (ICP).

Material and Methods: A prospective case-control study was conducted with 37 pregnant women diagnosed with ICP and 36 healthy pregnant controls. Serum HSPA5 and CHOP levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits.

Results: While HSPA5 and CHOP levels were higher in the ICP group compared to the control group, the differences were not statistically significant (p-values of 0.164 and 0.310, respectively).

Conclusion: The findings of this investigation did not yield sufficient evidence to validate the use of serum HSPA5 and CHOP levels for diagnosing ICP. Further research with larger sample sizes and exploration of placental tissue levels are warranted.

Keywords: CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP), endoplasmic reticulum (ER), glucose-regulated protein 78 (GRP78, BIP, HSPA5), intrahepatic cholestasis of pregnancy (ICP).

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INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disease characterized by skin pruritus and elevated bile acids, leading to adverse perinatal outcomes.[1] Although there are management recommendations based on bile acid levels, there is a lack of consensus on the diagnostic criteria for ICP. Several factors such as genetic, hormonal, immunological, and environmental factors are proposed to be involved in the pathogenesis of ICP. However, the exact molecular mechanism is still unclear.[2] Emerging evidence suggests that endoplasmic reticulum (ER) stress may have a role in the development of ICP.[3,4]

The endoplasmic reticulum (ER) is a vital organelle in eukaryotic cells, playing a role in a number of functions including post-translational modification, protein folding, and oligomerization.[5] Under ER stress conditions such as ICP, the accumulation of misfolded proteins triggers the unfolded protein response, which can lead to apoptosis.[6] In the case of ICP, ER-induced apoptosis results in a decline in placental function.[7]

Glucose-regulated protein 78 (GRP78), also known as immunoglobulin heavy chain binding protein (BIP), is a chaperone protein in the heat shock protein 70 (HSP70) family, primarily located in the ER. It is also referred to as HSPA5.[8–11] HSPA5 plays a vital role in the physiological and pathological conditions of the ER. It is essential for protein folding, assembly, transport, calcium balance, and ER stress signaling.[12,13] In addition, overexpression of HSPA5 has been documented in various types of tumor, including lung, breast, stomach, prostate, and hepatocellular carcinoma.[14–18] HSPA5 and C/EBP homologous protein (CHOP) are key biomarkers associated with the ER stress response pathways.[19–25] Upregulation of these biomarkers indicates a cellular attempt to restore ER homeostasis or trigger apoptosis during ER stress.[26] Given the potential involvement of ER stress in the pathophysiology of ICP, the exploration of the diagnostic utility of HSPA5 and CHOP presents a promising avenue for addressing this disease.

Although several studies have investigated the potential diagnostic value of HSPA5 and CHOP markers in placental tissue for ICP, no studies have investigated them in maternal blood. Therefore, this study aims to explore the potential diagnostic value of HSPA5 and CHOP biomarkers in identifying patients with ICP.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of University of Health Sciences Türkiye, Zeynep Kamil Women and Children's Diseases Training and Research Hospital (Date: 05.05.2021, No: 105) in accordance with the principles set forth in the Declaration of Helsinki. Prior to their participation in the study, all pregnant women were informed of the nature of the study and provided written consent.

This prospective case-control study was conducted at the Department of Perinatology of a tertiary referral hospital between May 2021 and May 2022. The diagnosis of ICP was based on the presence of clinical symptoms (itchiness without skin changes) and abnormal laboratory test results, specifically an elevated concentration of serum fasting total bile acid (≥10.00 µmol/L) and a concentration of alanine aminotransferase (ALT)>33 U/L and aspartate aminotransferase (AST)>32 U/L.

The exclusion criteria were as follows: patients with incomplete data, congenital malformations, chromosomal abnormalities, systemic disease, multiple pregnancies, chronic or acute liver disease (Wilson's disease, cholecystitis, primary sclerosing cholangitis, primary biliary cirrhosis), and HELLP syndrome. The exclusion criteria included cirrhosis, alpha-1-antitrypsin deficiency, symptomatic cholelithiasis, cytomegalovirus, Epstein-Barr virus, autoimmune hepatitis, acute fatty liver of pregnancy, active or viral hepatitis, and HELLP syndrome.

The control group was composed of randomly selected pregnant women who did not have any chronic disease, had a singleton pregnancy, and made their regular obstetric visits in our outpatient polyclinic. These women had uneventful pregnancies and no obstetric disorders requiring preterm induction.

Sample size of the study was determined based on previously published research using the G*Power 3.1.[27] Statistical power (1–β) of 80% was deemed necessary at the 0.05 (α) significance level, and the two-way analysis of variance test was used for the medium effect size. The required sample size was calculated to be 31 subjects.

Following a physical examination and ultrasonographic evaluation, peripheral blood samples were collected via the ulnar vein from each pregnant woman for the analysis of CHOP and HSPA5 levels. The samples were centrifuged at 4000 rpm for 10 minutes, after which the serum was separated and stored at –80°C until assay.

Serum CHOP and HSPA5 concentrations were analyzed using the enzyme-linked immunosorbent assay (ELISA) method with commercial kits (Shanghai Sunred Biological Technology Co., Ltd., China, CHOP Catalog No: 201-12-5342; HSPA5 Catalog No: 201-12-3243) in accordance with the manufacturer's instructions. In the CHOP analysis, the intra-assay variation coefficient was<10%, and the inter-assay variation coefficient was<12%, with a sensitivity of 0.191 ng/mL. In the HSPA5 analysis, the intra-assay variation coefficient was<10%, and the inter-assay<12%, with a sensitivity of 0.048 ng/mL.

The measurements were taken automatically on the ELISA reader (ThermoScientific, Finland) using a computer program (ScanIt for Multiscan FC 2.5.1). The absorbancy of each well was determined at 450 nm. The concentration of samples and controls was calculated based on the standard curve. The results are reported as the CHOP and HSPA5 concentration (ng/mL) in the samples.

Statistical Analysis

Continuous variables were presented as mean±standard deviation or median (min–max), while categorical data were presented as numbers and percentages. To assess the normality of continuous variables, the Kolmogorov–Smirnov test was used. For variables that exhibited a normal distribution, the Student's t-test was applied. Conversely, for variables that exhibited a non-normal distribution, the Mann–Whitney U test was used. To compare categorical data, the Chi-square test and Fisher's exact test were employed. All analyses were conducted using IBM SPSS version 26.0 (IBM Corporation, Armonk, NY, USA), and statistical significance was set at p<0.05.

Table 1: Demographic and clinical characteristics of pregnant women with ICP and controls

	ICP (n=37)	Control (n=36)	p
Age (years)	30.59±4.40	29.11±6.79	0.271*
BMI (kg/m^2)	30.36±5.58	29.20±3.95	0.311*
GA at delivery (weeks)	35 (28–40)	37 (30–40)	**0.002****
Gravidity	2 (1–5)	2 (1–6)	0.424**
Parity	1 (0–5)	0.5 (0–4)	0.547**
Number of children	1 (0–5)	0 (0–4)	0.392**
History of cholestasis	4 (10.8)	0 (0)	0.115***
History of IUFD	1 (2.7)	0 (0)	1.000***
Progesterone use	2 (5.4)	1 (2.8)	1.000***
UDC treatment	26 (70.3)	0 (0.0)	**<0.001*****
Itching	32 (86.5)	1 (2.8)	**<0.001*****
Abdominal pain	1 (2.7)	1 (2.8)	1.000***
Nausea	2 (5.4)	1 (2.8)	1.000***
Loss of Appetite	5 (13.5)	0 (0.0)	**0.029*****
Steatorrhea	1 (2.7)	0 (0)	1.000***

*: Student's T Test; **: Mann-Whitney U Test; ***: Fisher's Exact Test. UDC: Ursodeoxycholic acid; BMI: Body mass index; GA: Gestational age; IUFD: intrauterine fetal death; ICP: Intrahepatic cholestasis of pregnancy. Data are expressed as median (Q1–Q3), mean±SD or number (percentage) where appropriate. A p value of <0.05 indicates a significant difference. Statistically significant p-values are in bold.

Table 2: Laboratory results of pregnant women with ICP and controls

	ICP (n=37)	Control (n=36)	p
HSPA5 (ng/mL)	2.28 (1.44–21.29)	2.13 (1.61–9.38)	0.164**
CHOP (ng/mL)	7.21 (3.78–55.14)	6.24 (3.50–17.68)	0.310**
AST	51 (8–340)	16 (6–34)	**<0.001****
ALT	76 (6–557)	9.5 (4–28)	**<0.001****
Hb	11.3 (8.3–185)	11.4 (8.7–14)	0.873**
PLT	222 (0.98–445)	205 (103–371)	0.287**
INR	0.96 (0.35–153)	0.98 (0.80–1.21)	0.579**
Bile acid (μmol/L)	22 (10.2–90)	–	–

: Mann-Whitney U Test; *: Fisher's Exact Test. ICP: Intrahepatic cholestasis of pregnancy; HSPA5: Heat shock protein A5; CHOP: C/-EBP homologous protein; AST: Aspartate aminotransferase; ALT: Alanine transaminase; Hb: Hemoglobine; PLT: Platelets; INR: International normalized ratio. Data are expressed as median (Q1–Q3), mean±SD or number (percentage) where appropriate. A p value of <0.05 indicates a significant difference. Statistically significant p-values are in bold.

RESULTS

The analysis included 37 pregnant women with ICP and 36 healthy pregnant women. The mean age of the pregnant women with ICP and controls was 30.59±4.40 and 29.11±6.79 years, respectively. There was no significant difference between the groups in terms of age, body mass index (BMI), gravidity, parity, number of children, family history of ICP, history of intrauterine fetal death (IUFD), or progesterone administration (p>0.05). A total of 26 (70.3%) of the ICP group was on ursodeoxycholic acid treatment, whereas none of the pregnant women in the control group received any medical treatment (p<0.001). Itching was observed in 32 (86.5%) patients in the ICP group and in 1 (2.8%) patient in the control group (p≤0.001). A loss of appetite was observed in 5 (13.5%) of the ICP group, whereas no such cases were identified in the control group (p=0.029). Significantly lower gestational weeks at delivery was observed in the ICP group compared to the controls (p=0.002). Demographic and clinical characteristics of pregnant women with ICP and controls are given in Table 1.

The HSPA5 and CHOP values were observed to be higher in the ICP group than in the control group. However, these differences did not reach statistical significance, with p-values of 0.164 and 0.310, respectively. Patients with ICP had significantly higher alanine transaminase (ALT) and aspartate transaminase (AST) values in biochemical tests than the control group (for all, p≤0.001). In terms of other laboratory parameters, there were no significant differences between the two groups. Laboratory results of pregnant women with ICP and controls are presented in Table 2.

DISCUSSION

Research has indicated that GRP78 and CHOP levels in placental tissue may be a valuable diagnostic tool for diseases characterised by elevated ER stress, including ICP.[27] In a similar vein, we hypothesised that if GRP78 and CHOP levels in placental tissue reflect increased ER stress, serum HSPA5 and CHOP measurement would also reflect increased ER stress and serve as diagnostic biomarkers for ICP. However, our findings indicated that while maternal serum HSPA5 and CHOP levels were higher in the patients with ICP compared to the healthy control group, the differences were not statistically significant. Further research is necessary to fully understand the diagnostic and prognostic significance of maternal serum ER stress biomarkers in ICP.

A recent study conducted by Yu et al.[27] explored the expression of HSPA5 and CHOP in placental tissue of ICP patients and reported significantly increased expression of these markers in comparison to healthy controls. Similarly, Wang et al.[7] also reported elevated levels of HSPA5 and CHOP in the placenta of ICP patients. In contrast, our study found no significant difference in the levels of HSPA5 and CHOP between the patients with ICP and the healthy control group. This discrepancy may be attributed to the fact that we examined these markers in maternal serum rather than in placental tissue.

Therefore, further research is needed to determine the association between HSPA5 and CHOP levels in ICP and their potential role as biomarkers for diagnosis.

Several limitations should be taken into consideration. Firstly, our study examined these markers in maternal blood serum, while other studies showing a connection assessed them in placental tissue. This raises the possibility that the markers may not be released into the bloodstream in detectable amounts. Secondly, the present study involved a relatively small group (37 with ICP, 36 healthy controls). Larger studies might yield more conclusive results. Lastly, the markers assessed in our study were not specific to the endoplasmic reticulum of the liver; they are also widely released in the lungs, breast, and stomach. Despite these limitations, our study was the first to examine the HSPA5 and CHOP markers in the maternal serum of ICP.

CONCLUSION

In conclusion, no robust evidence was found to support the use of HSPA5 and CHOP values in the diagnosis of intrahepatic cholestasis of pregnancy. However, this result may be attributed to the fact that the markers were examined in venous blood rather than placental tissue. It is recommended that further multicentric studies with larger sample sizes be conducted to establish the clinical value of serum HSPA5 and CHOP in the diagnosis of intrahepatic cholestasis.

Statement

Ethics Committee Approval: The University of Health Sciences, Turkey. Istanbul Zeynep Kamil Maternity and Children's Diseases Health Training and Research Center Ethics Committee granted approval for this study (date: 05.05.2021, number: 105).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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REFERENCES

1. Pillarisetty LS, Sharma A. Pregnancy Intrahepatic Cholestasis. In: StatPearls. StatPearls Publishing, Treasure Island (FL); 2025. Available at: https://www.ncbi.nlm.nih.gov/books/NBK551503/. Accessed May 13, 2025.
2. Pataia V, Dixon PH, Williamson C. Pregnancy and bile acid disorders. Am J Physiol Gastrointest Liver Physiol 2017;313:G1–6.
3. Li A, Song NJ, Riesenberg BP, Li Z. The emerging roles of endoplasmic reticulum stress in balancing immunity and tolerance in health and diseases: Mechanisms and opportunities. Front Immunol 2020;10:3154.
4. Liu X, Green RM. Endoplasmic reticulum stress and liver diseases. Liver Res 2019;3:55–64.
5. Schönthal AH. Endoplasmic reticulum stress: Its role in disease and novel prospects for therapy. Scientifica (Cairo) 2012;2012:857516.
6. Malhi H, Kaufman RJ. Endoplasmic reticulum stress in liver disease. J Hepatol 2011;54:795–809.
7. Wang HZ, Cai DC, Liao DD, Zhong M, Gao YF, Sheng C. Role of endoplasmic reticulum stress-induced apoptosis of trophoblasts in intrahepatic cholestasis during pregnancy. Nan Fang Yi Ke Da Xue Xue Bao [Article in Chinese] 2018;38:572–7.
8. Guzel E, Basar M, Ocak N, Arici A, Kayisli UA. Bidirectional interaction between unfolded-protein-response key protein HSPA5 and estrogen signaling in human endometrium. Biol Reprod 2011;85:121–7.
9. Lee AS. Glucose-regulated proteins in cancer: Molecular mechanisms and therapeutic potential. Nat Rev Cancer 2014;14:263–76.
10. Stetler RA, Gan Y, Zhang W, Liou AK, Gao Y, Cao G, et al. Heat shock proteins: Cellular and molecular mechanisms in the central nervous system. Prog Neurobiol 2010;92:184–211.
11. Ni M, Zhang Y, Lee AS. Beyond the endoplasmic reticulum: Atypical GRP78 in cell viability, signalling and therapeutic targeting. Biochem J 2011;434:181–8.
12. Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: Coordination of gene transcriptional and translational controls. Genes Dev 1999;13:1211–33.
13. Hebert-Schuster M, Rotta BE, Kirkpatrick B, Guibourdenche J, Cohen M. The interplay between glucose-regulated protein 78 (GRP78) and steroids in the reproductive system. Int J Mol Sci 2018;19:1842.
14. Uramoto H, Sugio K, Oyama T, Nakata S, Ono K, Yoshimastu T, et al. Expression of endoplasmic reticulum molecular chaperone Grp78 in human lung cancer and its clinical significance. Lung Cancer 2005;49:55–62.
15. Fernandez PM, Tabbara SO, Jacobs LK, Manning FC, Tsangaris TN, Schwartz AM, et al. Overexpression of the glucose-regulated stress gene GRP78 in malignant but not benign human breast lesions. Breast Cancer Res Treat 2000;59:15–26.
16. Zheng HC, Takahashi H, Li XH, Hara T, Masuda S, Guan YF, et al. Overexpression of GRP78 and GRP94 are markers for aggressive behavior and poor prognosis in gastric carcinomas. Hum Pathol 2008;39:1042–9.
17. Pootrakul L, Datar RH, Shi SR, Cai J, Hawes D, Groshen SG, Lee AS, Cote RJ. Expression of stress response protein Grp78 is associated with the development of castration-resistant prostate cancer. Clin Cancer Res 2006;12:5987–93.
18. Luk JM, Lam CT, Siu AF, Lam BY, Ng IO, Hu MY, et al. Proteomic profiling of hepatocellular carcinoma in Chinese cohort reveals heat-shock proteins (Hsp27, Hsp70, GRP78) up-regulation and their associated prognostic values. Proteomics 2006;6:1049–57.
19. Shao Q, Ren P, Li Y, Peng B, Dai L, Lei N, et al. Autoantibodies against glucose-regulated protein 78 as serological diagnostic biomarkers in hepatocellular carcinoma. Int J Oncol 2012;41:1061–7.
20. Duan SR, Wang JX, Wang J, Xu R, Zhao JK, Wang DS. Ischemia induces endoplasmic reticulum stress and cell apoptosis in human brain. Neurosci Lett 2010;475:132–5.
21. Khan MM, Yang WL, Wang P. Endoplasmic reticulum stress in sepsis. Shock 2015;44:294–304.
22. Guo B, Li Z. Endoplasmic reticulum stress in hepatic steatosis and inflammatory bowel diseases. Front Genet 2014;5:242.
23. Yin XL, Zhang W, Yang Y, Shen H. Increasing expression of (CCAAT

enhancer binding protein) homologous protein induced by endoplasmic reticulum stress in myocardium after cardiac arrest and resuscitation in rat. Resuscitation 2012;83:378–85.

24. Abhishek A, Benita S, Kumari M, Ganesan D, Paul E, Sasikumar P, et al. Molecular analysis of oxalate-induced endoplasmic reticulum stress mediated apoptosis in the pathogenesis of kidney stone disease. J Physiol Biochem 2017;73:561–73.
25. Zheng YZ, Cao ZG, Hu X, Shao ZM. The endoplasmic reticulum stress markers GRP78 and CHOP predict disease-free survival and responsiveness to chemotherapy in breast cancer. Breast Cancer Res Treat 2014;145:349–58.
26. Liu K, Zhao C, Adajar RC, DeZwaan-McCabe D, Rutkowski DT. A beneficial adaptive role for CHOP in driving cell fate selection during ER stress. EMBO Rep 2024;25:228–53.
27. Yu Y, Zhou CL, Yu TT, Han XJ, Shi HY, Wang HZ, et al. Effect of endoplasmic reticulum stress in trophocytes on the pathogenesis of intrahepatic cholestasis of pregnancy. Zhonghua Fu Chan Ke Za Zhi [Article in Chinese] 2017;52:392–7.