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Original Article

Histopathological Changes in Placenta and Correlation with Fetal Outcome in Cholestasis of Pregnancy

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Abstract

Introduction: Intrahepatic cholestasis of pregnancy (IHCP) is associated with an increased risk of adverse fetal outcome, for example, prematurity, meconium-stained amniotic fluid, perinatal hypoxia, and sudden intrauterine fetal death. The exact mechanism of cholestasis-induced fetal complications is not yet fully understood. The aim of the present study was to evaluate and compare the histopathological changes in the placentas of patients with IHCP and healthy pregnant women, and to see the correlation with adverse fetal outcomes with these histopathological changes of IHCP.

Material and Methods: The effect of IHCP on the placental microstructure was investigated using placental tissue from patients with IHCP treated with ursodeoxycholic acid (UDCA) and from healthy pregnancies. Seven placental histopathological features were analyzed: Increased calcification, increased syncytial knots, trophoblastic cell proliferation, fibrinoid necrosis, perivascular fibrinoid deposition, terminal villous capillarization, and features of chorioamnionitis.

Results: There were significant differences in placental histopathology of increased syncytial knots, fibrinoid necrosis, perivascular fibrinoid deposition, and terminal villous capillarization in IHCP patients treated with UDCA and in patients with uncomplicated pregnancy. However, in the control group, the rate of neonatal intensive care unit admission was significantly associated with trophoblastic cell proliferation ($P = 0.009$).

Conclusions: Various histopathological features suggestive of placental inflammation are seen more in cases as compared to controls.

Key words: Histopathology, Intrahepatic cholestasis of pregnancy, Placenta, Ursodeoxycholic acid

INTRODUCTION

Intrahepatic cholestasis of pregnancy (IHCP) is the most common liver disorder occurring in the late second and early third trimester of pregnancy. The worldwide prevalence of IHCP is approximately 0.3–5.6% of pregnancy.[1,2] The incidence of IHCP is 2–8.2% in India.[3] The etiology of IHCP is complex, multifactorial, and not fully understood. Genetic, hormonal, environmental, and dietary factors are proposed.[4,5]

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It is characterized by pruritus, especially during the night, with increased serum bile acid (BA) levels of >10 μmol/L and abnormal liver function tests (raised AST and ALT usually below 250U/L).[6] The symptoms and biochemical abnormalities of IHCP rapidly resolve after delivery.

Cholestasis of pregnancy is associated with an increased risk of fetal complications: Perinatal hypoxia, prematurity, meconium-stained amniotic fluid, and sudden intrauterine fetal death.[7] Serum BA level is considered to be the most sensitive and specific marker for the diagnosis of IHCP and has an important role in the etiology of fetal complications.[8] The increased risk of adverse perinatal outcomes is associated with high bile acid concentration (> 40 mmol/L).[9,10] The exact mechanism of cholestasis-induced fetal sequelae is not yet fully understood. In normal pregnancy, fetal bile acid is transferred across the placenta into maternal circulation and excretion by the maternal

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liver. However, in patients with cholestasis of pregnancy, the high concentration of bile acids in maternal serum, the bile acid gradient across the placenta is reversed, which leads to impaired clearance of fetal bile acids. Raised bile acid can cause vasoconstriction of the chorionic vessels and damage the placental structure leading to impaired fetal-maternal transport of nutrients and oxygen to the fetus.

So far, very few studies with small sample sizes have been done to see the effects of IHCP on the placental structure. The understanding of placental pathological changes among pregnancies complicated by IHCP may help better understanding of the pathophysiology of this disease. The aim of present study was to evaluate the histopathological changes of placentas of patients with IHCP and healthy pregnant women to see if cholestasis affects placental microstructure.

MATERIALS AND METHODS

We included 30 patients with IHCP and 30 women as controls with physiological pregnancy for this prospective case–control study, during the duration from November 2018 to July 2020. Ethical clearance was obtained from the institutional ethical clearance committee for human research. We included singleton pregnancy at 3rd trimester, >28 weeks POG, and maternal age between 18 and 34 years. The diagnosis of IHCP was confirmed after excluding other liver diseases on the basis of unexplained itching along with increased bile acid (>10 mmol/L) and abnormal liver function tests. We also excluded pregnant patients with an anomalous fetus, any other obstetrics complications, known case of any chronic medical, or surgical illness such as diabetes, hypertension, thyroid dysfunction, renal, and hepatobiliary disease of any cause other than IHCP. The patients with physiological pregnancy matched for age and gestational age with cases were included in the control group. All patients gave written informed consent. On the basis of serum bile acid level (TBA-total bile acid level), IHCP cases were classified into mild group of cases (TBA 10-40μmol/L) and severe group of cases (TBA >40 μmol/L).

Controls having TBA level <10 μmol/L were included in the study and TBA level >10 μmol/L were not enrolled in the study.

All cases were followed till 37 weeks. The pregnancy was terminated by elective induction of labor or cesarean section. However, in the control group, the patients were followed up till delivery either by induction or spontaneous labor. Any obstetric problems such as preterm labor, pre-eclampsia, gestational diabetes mellitus, abruption placenta, meconium-stained amniotic fluid, PPROM, fetal distress, or intrauterine demise were managed as per standard hospital protocol and details were recorded in case pro forma. Maternal outcomes were noted as delivery details (preterm/term delivery/spontaneous labor/induced labor/vaginal/instrumental/cesarean delivery). Both cases and control were followed till discharge and complications if any were noted. Fetal/neonatal outcomes were noted in

Table 1: Comparison of obstetric profile in mild and severe category of IHCP cases

S. No.	Obstetrical profile	Mild IHCP (*n*=22)	Severe IHCP (*n*=8)	*P*-value
1	Gravida			
	Primigravida	9 (40.9%)	3 (37.5%)	1.000
	2nd gravida	8 (36.36%)	3 (37.5%)	
	3rd gravida or more	5 (22.72%)	2 (25%)	
2	Period of gestation at time of delivery			
	<32 weeks	2 (9.09%)	0 (0%)	0.573
	32–34 weeks	3 (13.63%)	0 (0%)	
	35–37 weeks	5 (22.72%)	4 (50%)	
	38–40 weeks	10 (45.45%)	4 (50%)	
	>41 weeks	2 (9.09%)	0 (0%)	

IHCP: Intrahepatic cholestasis of pregnancy

Table 2: Comparison and analysis of histopathological features of the placenta in cases and controls

S. No.	Placenta HPE grading	Cases (*n*=30)	Controls (*n*=30)	*P*-value
1	Increased calcification			
	Absent	13 (43.33%)	17 (56.67%)	0.3621
	+	6 (20%)	8 (26.66%)	
	++	4 (13.33%)	1 (3.33%)	
	Focal	7 (23.33%)	4 (13.33%)	
2	Increased syncytial knots			
	Absent	3 (10%)	2 (6.67%)	0.007*1
	+	16 (53.33%)	26 (86.67%)	
	++	11 (36.67%)	2 (6.67%)	
3	Trophoblastic cell proliferation			
	Absent	13 (43.33%)	20 (66.67%)	0.1191
	+	16 (53.33%)	10 (33.33%)	
	++	1 (3.33%)	0 (0%)	
4	Fibrinoid necrosis			
	Absent	3 (10%)	16 (53.33%)	<0.001*1
	+	15 (50%)	13 (43.33%)	
	++	11 (36.67%)	0 (0%)	
	Focal	1 (3.33%)	1 (3.33%)	
5	Perivascular fibrinoid deposition			
	Absent	9 (30%)	23 (76.67%)	<0.001*1
	+	19 (63.33%)	4 (13.33%)	
	Focal	2 (6.67%)	3 (10%)	
6	Terminal villous capillarization			
	Normal	8 (26.66%)	18 (60%)	0.020*2
	+	15 (50%)	6 (20%)	
	++	7 (23.33%)	6 (20%)	
7	Features of chorioamnionitis			
	Absent	25 (83.33%)	25 (83.33%)	1.0002
	+	5 (16.67%)	5 (16.67%)	

*P<0.05 is significant; [1]Fisher's exact test; [2]Pearson Chi-square test. HPE: Histopathological examination

both groups in terms of IUD, stillbirth fetal distress, MAS, birth weight, Apgar score, and neonatal intensive care unit (NICU) admission.

Collection of samples

At the time of delivery, placental tissue was taken in both the groups to study the histopathological examination (HPE). Two separate fresh tissues of size 2 × 2 × 2 cm were taken, having 10% formalin was sent for HPE of the placenta in the department of pathology, then the tissue was fixed with 4% formaldehyde, embedded by paraffin, and made into slices and the changes of the placenta were observed by the optical microscope using hematoxylin dyes.

RESULTS

Women in the case and the control group had a mean age of 25.2 and 24.83 years, respectively.

40.9% of patients were primigravida in the mild category, however, 37.5% of patients in the severe category were primigravida.

Gestational Age at Time of Delivery

46.67% of women from the cases and 63.33% of women from the control group delivered at 38–40 weeks. 45.45% ($n = 10$) in the mild category and 50% ($n = 4$) in the severe category were delivered between 38–40 weeks.

No significant statistical difference was found between the two groups in terms of parity ($P = 1.000$) and gestation age at the time of delivery and $P = 0.573$.

Placental Changes

On gross examination, we could not find any visible difference in size, color, texture, and weight of the placenta in both the groups [Figure 1a and b].

Histology

Histopathological analysis of the preparations ($n = 30$ ICP patients, $n = 30$ control patients) was performed by a pathologist who was not aware of gestational age and disease status.

Placental Histopathological Features

Grossly, there was not any visible difference in size, color, texture, and weight of the placenta in both the groups [Figure 1a and b]. The various histopathological features of the placenta such as increased calcification, increased syncytial knots, trophoblastic cell proliferation, fibrinoid necrosis, perivascular fibrinoid deposition, terminal villous capillarization, and features of chorioamnionitis were identified and analyzed in both the groups.

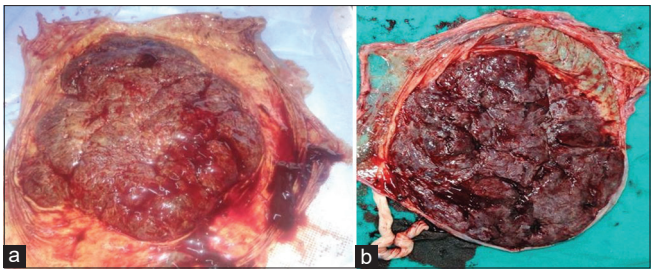

Figure 1: (a) Gross view of placenta of case. (b) Gross view of placenta of control

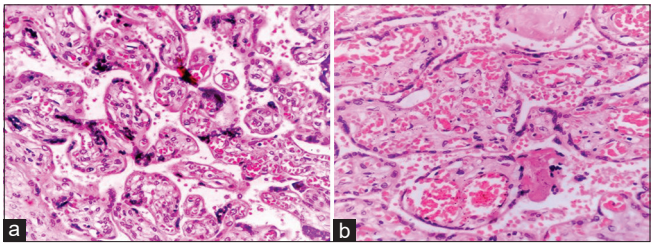

Figure 2: (a) Section shows an increased number of syncytial knots (H and E; ×390). (b) The section shows less number of syncytial knots (H and E; ×390)

The histopathological features of increased syncytial knots [Figure 2a and b], terminal villous capillarization, fibrinoid necrosis, and perivascular fibrinoid necrotic tissue deposition were significantly more in patients of the IHCP group (cases). However, trophoblastic cell proliferation and increased calcification were also more observed in the case group, but it was not statistically significant. Chorioamnionitis features were seen equally in both the groups and were found statistically insignificant.

The histopathological features were also compared in the mild and severe category of IHCP, but no statistical difference was found.

Maternal outcome was correlated with various histopathological findings of the placenta in cases and controls. We found a greater number of placental HPE changes in patients with maternal complications in the IHCP group in comparison to controls, but this difference was not statistically significant.

On comparison, we found that HPE features such as increased calcification, increased syncytial knots, fibrinoid necrosis, perivascular fibrinoid deposition, terminal villous capillarization, and features of chorioamnionitis were more in number in cases with adverse fetal outcome; however, *P*-value difference was not significant in the current study. However, in the control group, the rate of NICU admission was significantly associated with trophoblastic cell proliferation ($P = 0.009$). However, the rest of the fetal outcome parameters did not show any association with other HPE features.

Table 3: Comparison of placental histopathological features of the present study with previous similar studies

Parameter	Costoya *et al.*[16] 1980	Geenes *et al.*[14] 2011	Shemer *et al.*[13] 2012	Patel *et al.*[11] 2014	Ling *et al.*[15] 2014	Xie *et al.*[12] 2018	Present study, 2018-2020
Number of subjects	9	40	28	54	30	80	60
Groups	Controls (*n*=3) Anicteric IHCP (*n*=4) Icteric IHCP (*n*=2)	Controls (*n*=12) IHCP treated with UDCA (*n*=19) IHCP not treated with UDCA (*n*=9)	Controls (*n*=8) IHCP+UDCA (*n*=10) IHCP without UDCA (*n*=10)	Controls (*n*=30) Cases (*n*=24)	Controls (*n*=10) Mild IHCP (*n*=10) Severe IHCP (*n*=10)	Controls (*n*=50) Cases (*n*=30)	Controls (*n*=30) Cases (*n*=30) 1. Mild (*n*=22) 2. Severe (*n*=8)
Gross features of placenta	No difference	In Controls: Normal morphology.	No difference in volume of placenta ⬇Placental weights of UDCA treated IHCP; *P*<0.001	⬇ Placental weight; *P*=0.40	----	----	No difference
Histopathological features of placenta	In IHCP placenta: ⬇ in intervillous space, no significant association of fibrin deposition and ⬆ syncytial knots, cytotrophoblast proliferation (*P*<0.05), syncytial sprouts (*P*<0.05), loose, edematous and unduly abundant stoma of villi, thicker syncytiotrophoblast	In controls: Appropriate capillaries per villous (<10), infrequent syncytial knots, compact stroma In cases: Focal thickening of amniotic basement membrane, ⬇intervillous space, syncytial knots more in untreated IHCP (*P*=0.02), peri-villous fibrinoid deposition, chorionic villi: small for gestational age, dense fibrotic stroma, crowding of villi, congestion	In cases: ⬆surface area of terminal villi and capillaries (*P*<0.01), syncytial knots (*P*<0.01), ⬇ collagen; found more in UDCA treated IHCP (*P*<0.05), chorangiosis; 20% of IHCP placenta treated with UDCA, 60% of IHCP placenta not treated with UDCA and not seen in controls placenta.	17 HPE features. No statistically significant difference (*P*>0.05); Infarcts, VUE, edematous villi, immature intermediate villi, villous calcification, syncytial knots, perivascular fibrinoid deposits, necrotizing villitis, fetal stem vessel thrombosis, chorioamnionitis, funisitis, meconium staining, maternal atherosis, plasmacytic deciduitis, terminal villous capillarization, apoptosis of the chorion, fibrinous necrosis	In controls: Well organized vascular structure, consisting of large vessels. In cases: Fewer and smaller blood vessels in each villous indicating disturbed placental vascular formation and deficient vascular maturation.	In controls: Intact microvessels, complex nodules not obvious, normal trophoblasts, fibrinoid necrosis rarely seen In cases: No complete vascular structure, microvascular damage, complex nodules more obvious, trophoblastic cell proliferation, fibrinoid necrotic material deposition	7 HPE features calcification; 56.67%; *P*=0.362; syncytial knots; 90%; *P*=0.007; trophoblastic cell proliferation; 56.67%; fibrinoid necrosis; 90%; *P*<0.001; perivascular fibrinoid deposition; 70%; *P*<0.001; terminal villous capillarization; 73.33%; *P*=0.020, chorioamnionitis; 16.67%; *P*=1.000 No statistical difference in mild and severe IHCP.
Other features	----	BA exposed placental explant fragments: ⬆ apoptotic index, no effect on proliferative index, treated with UDCA: significant reduction in syncytial knots formation, no effect on apoptotic and proliferative index.	----	Treatment with UDCA decrease the VUE.	----	TBA levels positively correlated with damage of placenta (correlation coefficient 0.889; *P*<0.001)	Correlation of HPE features with maternal outcome; no difference was found. Correlation of HPE features with fetal outcome; no difference was found in the case group; significant difference was found between low Apgar score at 1 min with chorioamnionitis on HPE in control group.

(Contd...)

Table 3: (*Continued*)

Parameter	Costoya *et al.*[16] 1980	Geenes *et al.*[14] 2011	Shemer *et al.*[13] 2012	Patel *et al.*[11] 2014	Ling *et al.*[15] 2014	Xie *et al.*[12] 2018	Present study, 2018-2020
Maternal or fetal outcome	----	----	Vaginal delivery; 87% versus 80% versus 70%; $P>0.05$ 5 min Apgar; $P>0.05$	No difference in maternal, obstetric or fetal outcome in both the groups, ↓ birth weight; $P=0.46$, ↔1 min Apgar; $P=0.53$, ↔5 min Apgar; $P=0.74$	----	----	Results significant for adverse maternal outcome (P=0.015), fetal outcome (P=0.005), neonatal birth weight (P=0.001), care admission (P=0.001)

IHCP: Intrahepatic cholestasis of pregnancy, UDCA: Ursodeoxycholic acid, HPE: Histopathological examination

Similarly, we tried to find a correlation between the fetal outcomes in the mild and severe category of IHCP. We observed that in the mild category of cases, a significant association was found between Apgar score at the first minute of life with features of chorioamnionitis on HPE of the placenta ($P = 0.048$), whereas in the severe category of cases, a significant association was found between adverse fetal complications such as fetal distress and small for gestational age with trophoblastic cell proliferation. The rest of the parameters were not correlated significantly with features of placenta HPE [Tables 1-3].

DISCUSSION

Similar results were observed by Patel *et al.* in 2014, they took the placenta from 24 cases and 30 controls and compared the histopathological features in both the groups. They identified and reviewed various features (infarcts, villitis of unknown origin, increased villous calcification, increased syncytial knots, perivascular fibrinoid deposits, chorioamnionitis, fibrinous necrosis, terminal villous capillarization, apoptosis of the chorion, necrotizing villitis, and immature intermediate villi) but no significant difference was found. They also demonstrated the role of UDCA in the treatment of VUE.[11]

Similarly, Xie *et al.* also studied the placenta in IHCP and they took 30 cases and 50 controls and found various histopathological features. In contrast to features found in placenta from controls (normal trophoblast cell, not obvious complex nodules, and intact microvessels), placenta from the case group has features such as microvascular damage, complex nodules, trophoblastic cell proliferation, and fibrinoid necrotic tissue deposition. TBA levels were positively correlated with damage of the placenta with a correlation coefficient of 0.889 and $P < 0.001$.[12]

A prospective case–control study done by Shemer *et al.* found the increased surface area of terminal villi and capillaries ($P < 0.001$) and an abundant number of syncytial knots ($P < 0.001$) in the IHCP group placenta and it was found to be statistically significant.[13] Chorangiosis was seen in the placenta of the IHCP group and not seen in the control group placenta. Similarly, in the current study, we also found that syncytial knots were significantly abundant in the case placenta (P=0.007) but could not observe chorangiosis in any of the placenta.

Similar results of histopathological features of IHCP placenta were found in the study conducted by Geenes *et al.* published in 2011. These features were focally thickening of the amniotic basement membrane, small chorionic villi with congestion, crowding and dense

fibrotic stroma, reduced intervillous space, and abundant syncytial knots.[14]

Du *et al*. observed defective angiogenesis and they suggest disturbed placental vascular formation and deficient vascular maturation which were indicated by fewer and smaller blood vessels in each villous of the placenta of IHCP. However, the control group placenta exhibits a well-organized vascular structure consisting of large vessels.[15] However, we could not observe such HPE features in our study.

CONCLUSION

Various histopathological features suggestive of placental inflammation are seen more in cases as compared to controls. Some features such as abundant syncytial knots, fibrinoid necrosis, perivascular fibrinoid necrosis, and terminal villous capillarization are significantly found more in cases of the placenta as compared to the placenta from normal control. The difference is found in several HPE features and adverse fetal outcome in both the groups.

REFERENCES

1. Riely CA, Bacq Y. Intrahepatic cholestasis of pregnancy. Clin Liver Dis 2004;8:167-76.
2. Lee RH, Goodwin TM, Greenspoon J, Incerpi M. The prevalence of intrahepatic cholestasis of pregnancy in a primarily Latina Los Angeles population. J Perinatol 2006;26:527-32.
3. Padmaja M, Bhaskar P, Kumar GJ, Seetha R, Mahasweta C. A study of obstetric cholestasis. J Obstet Gynaecol India 2010;60:225-31.
4. Arrese M, Macias RI, Briz O, Perez MJ, Marin JJ. Molecular pathogenesis of intrahepatic cholestasis of pregnancy. Expert Rev Mol Med 2008;10:e9.
5. Arrese M, Reyes H. Intrahepatic cholestasis of pregnancy. A past and present riddle. Ann Hepatol 2006;5:202-5.
6. Zapata R. Intrahepatic Cholestasis of Pregnancy: Even Today a Puzzling Disease of Pregnancy. World Gastroenterology Association; 2018. Available from: http://www.worldgastroenterology.org/publications/e-wgn/e-wgn-expert-point-of-view-articles-collection/intrahepatic-cholestasis-of-pregnancy-even-today-a-puzzling-disease-of-pregnancy.2018 [Last accessed on 2023 Sep 25].
7. Geenes V, Chappell LC, Seed PT, Steer PJ, Knight M, Williamson C. Association of severe intrahepatic cholestasis of pregnancy with adverse pregnancy outcomes: A prospective population-based case-control study. Hepatology 2014;59:1482-91.
8. Walker IA, Nelson-Piercy C, Williamson C. Role of bile acid measurement in pregnancy. Ann Clin Biochem 2002;39:105-13.
9. Glantz A, Marschall HU, Mattsson LA. Intrahepatic cholestasis of pregnancy: Relationships between bile acid levels and fetal complication rates. Hepatology 2004;40:467-74.
10. Herrera CA, Manuck TA, Stoddard GJ, Varner MW, Esplin S, Clark EA, *et al*. Perinatal outcomes associated with intrahepatic cholestasis of pregnancy. J Matern Fetal Neonatal Med 2018;31:1913-20.
11. Patel S, Pinheiro M, Felix JC, Opper N, Ouzounian JG, Lee RH. A case-control review of placentas from patients with intrahepatic cholestasis of pregnancy. Fetal Pediatr Pathol 2014;33:210-5.
12. Xie F, Liu X, Xiao P, Huang Y, Chen Q, Zhou L. Bile acid effects on placenta damage in intrahepatic cholestasis of pregnancy. J Biosci Med 2018;6:42-52.
13. Shemer EW, Thorsell M, Östlund E, Blomgren B, Marschall HU. Stereological assessment of placental morphology in intrahepatic cholestasis of pregnancy. Placenta 2012;33:914-8.
14. Geenes VL, Lim YH, Bowman N, Tailor H, Dixon PH, Chambers J, *et al*. A placental phenotype for intrahepatic cholestasis of pregnancy. Placenta 2011;32:1026-32.
15. Du Q, Pan Y, Zhang Y, Zhang H, Zheng Y, Lu L, *et al*. Placental gene-expression profiles of intrahepatic cholestasis of pregnancy reveal involvement of multiple molecular pathways in blood vessel formation and inflammation. BMC Med Genomics 2014;7:42.
16. Costoya AL, Leontic EA, Rosenberg HG, Delgado MA. Morphological study of placental terminal villi in intrahepatic cholestasis of pregnancy: Histochemistry, light and electron microscopy. Placenta 1980;1:361-8.

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