Original Research

Altered Expression of STAT3, RORγt, and IL-17A Proteins and Th17 Cell in Intrahepatic Cholestasis of Pregnancy

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Abstract

Background: Intrahepatic cholestasis of pregnancy (ICP) is a disorder specifically associated with pregnancy. Recent evidence suggests that the T helper 17 (Th17) cell population is related to a maternal and foetal immune imbalance associated with ICP. However, there has been insufficient attention paid to the potential roles of signal transducer and activator of transcription 3 (STAT3) and RAR-related orphan receptor gamma (RORγt) in modulations of Th17 cell in ICP. Accordingly, the purpose of our study was to investigate the alterations of Th17 cell in placenta and peripheral blood of patients with ICP and correlations between Th17 cell and STAT3, RORγt, interleukin (IL)-17A in ICP. **Methods**: Nine pregnant women with ICP and nine women with normal pregnancy served as the ICP and control groups, respectively. STAT3, RORγt, and IL-17A expression were examined by immunohistochemistry and western blotting in placental tissue. Flow cytometry was used to quantify Th17 cell in blood of peripheral circulation. We compared data between groups using Chi-square tests or paired *t* tests. Pearson or Spearman coefficients were used to measure correlations. **Results**: STAT3, RORγt, and IL-17A were mainly expressed in the trophoblasts of the two groups of patients. Comparatively to the control group, placental levels of STAT3, RORγt, and IL-17A proteins were significantly elevated in ICP group, as was maternal levels of Th17 cell in peripheral blood. Moreover, placental IL-17A protein level showed significantly positive relationships with placental STAT3 (r = 0.97, *p* = 2e-05) and RORγt (r = 0.91, *p* = 0.01) protein in control group, however, not in ICP group (STAT3, r = 0.5, *p* = 0.17; RORγt, r = 0.62, *p* = 0.07). **Conclusions**: Women with ICP showed an increase in Th17 cells in comparison to women with normal pregnancies. STAT3 and RORγt may increase Th17 cell proliferation and differentiation, appears to be altered in ICP. ICP may be adversely affected by excessive accumulation of Th17 cell.

Keywords: correlation; intrahepatic cholestasis of pregnancy; signal transducer and activator of transcription 3; T helper cell-17

1. Introduction

During pregnancy, intrahepatic cholestasis (ICP) causes excessive pruritus without a rash and elevations of total bile acids (TBAs) [1]. Incidences of ICP vary by geographic location and ethnicity, ranging from 0.2% to 2% [2]. After delivery, the mother's symptoms usually disappear, whereas foetal exposure to ICP results in perinatal and long-term complications, including premature delivery, neonatal respiratory distress syndrome, sudden intrauterine foetal demise, stillbirth, and metabolic syndrome later in life [3,4]. However, the aetiology of ICP remains unclear. Researchers recently found that the placenta's core genes are largely responsible for regulating immune function; expression of these genes is upregulated in mild ICP, and in severe cases the expression is further increased, which indicates immune system might play a major role in ICP [5]. Recently, the T helper 17 (Th17) cell in pregnant women has received attention. ICP patients' peripheral blood and placentas were reported to be significantly elevated in interleukin (IL)-17, a cytokine of Th17 cell [6,7].

There are both positive and negative impacts on the immune system caused by Th17 cell, a relatively new subset of CD4$^+$ T cells. It has been shown that excessive levels of Th17 cell and Th17-related cytokines are linked to disorders of pregnancy, including preeclampsia and repeated abortions, which could disrupt maternal and foetal immunity [8–10]. Many molecules play important roles in regulating Th17 cell differentiation and function. IL-17A is a proinflammatory cytokine and the major effector cytokine in Th17 cell. Additionally, as a Th17 signature transcriptional factor, retinoic acid-related orphan nuclear receptor gamma t (RORγt) is imperative for Th17 differentiation, which regulates the expression of key genes of Th17, like IL-17A and interleukin (IL)-23R [11]. Moreover, signal transducer and activator of transcription 3 (STAT3) is needed to differentiate Th17 under activation by interleukin (IL)-6 and transforming growth factor (TGF)-β [12]. STAT3 contributes to the differentiation of Th17 cell via multiple pathways, including by directly binding to the RORγt gene (*RORG*) to promote its expression [13], inhibiting forkhead

box protein (Foxp) 3 expression [12], directly activating *IL-17A* promoter [14]. Th17 cell differentiation involves three phases: the early phase, characterised by a transient induction of immune response pathways, including the expression of STAT3; the intermediate phase, marked by RORγt expression; and the late phase, characterised by the induction of signature cytokines of Th17 cell, including IL-17A [15]. Although several studies have examined IL-17's role in ICP, few researchers have paid attention to the roles of STAT3 and RORγt in the modulation of Th17 differentiation and function in ICP, and whether a consistent and predictable relationship exists among STAT3, RORγ, and Th17 cell in ICP and normal pregnancy. Therefore, the purpose of our study was to investigate the alterations of Th17 cell in placenta and peripheral blood of patients with ICP and to analyse their correlations with STAT3, RORγ, and IL-17.

2. Materials and Methods

Women were recruited from August 2020 to October 2020 at West China Second Hospital, affiliated with Sichuan University. Our study was approved by the hospital's ethics committee. Clinical information and samples were anonymized for statistical workup. Pregnancy healthcare profiles were created for all patients and they underwent routine pregnancy checks at our hospital. In the experimental and control groups were nine patients with singleton ICP and nine patients with normal-term singleton pregnancy,respectively. Delivery of all patients in both groups was by caesarean section. Based on Chinese Medical Association's "Guidelines for diagnosis and treatment of intrahepatic cholestasis of pregnancy (2015)", ICP was diagnosed.

Inclusion criteria for ICP group included the following: elevated TBA or aminotransferase; signs of prutitus or jaundice; no history of skin disease, gallstones, cholecystitis, liver cirrhosis, autoimmune diseases, acute and chronic infectious diseases, no history of infection with hepatitis virus, Epstein Barr virus, and cytomegalovirus; without any other obstetric complication. For the control group, the following criteria were used: normal liver or renal function; no history of gallstones, cholecystitis, liver cirrhosis, autoimmune diseases, acute and chronic infectious diseases, without history of infection with hepatitis virus, Epstein Barr virus, cytomegalovirus are not permitted; without any obstetrics complication.

Before the operation, 2 mL elbow venous blood was collected.Placental tissue was collected within 5 min of delivery. We incubated the paraffin-embedded placental sections with primary antibodies: anti-STAT3 (1:150), anti-IL-17A (1:200) (Proteintech Group, Chicago, IL, USA), and anti-RORγt (1:50) (Merck-Millipore, Bedford, MA, USA). Subsequently, these sections incubated with sheep anti-rabbit IgG (Biyuntian Biotechnology, Shanghai, China).

We homogenized placental tissues in ice-cold ly-sis buffer. Protein were subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (STAT3: 100 V, 90 min; RORγt: 100 V, 60 min; IL-17A: 40 V, 90 min). We blocked the membranes in Tris-buffered saline and incubated the membranes with the primary and secondary antibody in turn.

The whole blood was diluted using RPMI 1640 medium and incubated with stimulant and blocker in turn. The blood was then incubated for 5 h at 37 °C with 5% CO_2. Subsequently, the blood was incubated with APC-conjugated anti-human CD4 antibody (Biolegend, CA, USA), fixed with Fix Medium A (Fix & Perm kit, Invitrogen, CA, USA). After discarding the supernatant, the cells were permeabilised with Permeabilization Medium B (Fix & Perm kit, Invitrogen), incubated with FITC-conjugated anti-human IL-17A antibody (Biolegend).

Analysis of the data were conducted using IBM SPSS 23 software and R version 4.0.4 (IBM corp., Armonk, NY, USA). For the quantitative variables, the results were presented as the mean value ± standard deviation and the results were compared using *t*-tests for normally distributed variables or equivalent non-parametric tests otherwise. Chi-square tests was used to compare the data for categorical variables. For the correlation matrix, Pearson's parametric correlation test was utilized. Using Pearson correlation scatterplots, we examined the correlations among the variables within groups. Two-sided *p*-values < 0.05 were considered statistically significant in all tests.

3. Results

3.1 Clinical Data

Comparison of clinical and obstetric characteristics between groups is provided in Table 1 and the comparison of biochemical indicators is provided in Table 2. Regarding clinical and obstetric data, both groups showed some similar characteristics. However, significant differences in gestational days, foetal weights and ratio of placental weight and neonatal weight were noted in. ICP was associated with earlier gestational days, lower neonatal weights and higher ratio of placental weight and neonatal weight, which may reflect impaied placenta function. A few maternal serum biochemical parameters did not differ significantly between the two groups. However, there were much higher levels of maternal serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), TBA, direct bilirubin (DBIL), and alkaline phosphatase in the ICP group than in the control group (all $p < 0.05$).

3.2 STAT3, RORγt, and IL-17A Distribution in the Placenta

In the placenta, cytotrophoblasts and syncytiotrophoblasts were the main sites of STAT3, RORγt, and IL-17A expression, which were mainly distributed in the cytoplasm in both groups. However, STAT3 protein was ex-

Table 1. Clinical and obstetric characteristics of patients in the ICP and control groups.

Characteristic	Control (n = 9)	ICP (n = 9)	t	p
Age (years)	32.33 ± 4.12	31.00 ± 2.69	−0.81	0.43
Weight gain (kg)	12.78 ± 2.59	12.89 ± 4.49	0.06	0.95
Body mass index (cm/kg^2)	26.30 ± 1.93	25.86 ± 3.14	−0.36	0.72
Gravidity	2.22 ± 0.83	2.11 ± 0.93	−0.27	0.79
Parity	1.67 ± 0.50	1.44 ± 0.53	0.70	0.37
Gestational day (day)	274.89 ± 2.47	262.22 ± 8.48	−4.3	0.02*
Neonatal weight (g)	3216.67 ± 317.49	2922.67 ± 204.45	−1.71	0.03*
Umbilical artery S/D ratio	2.20 ± 0.37	2.45 ± 0.31	1.39	0.13
Placental weight (g)	560.78 ± 46.18	563.56 ± 62.66	0.11	0.92
Placental weight/neonatal weight	0.19 ± 0.01	0.17 ± 0.01	2.88	0.02*
10 min Apgar score	10 ± 0	10 ± 0		1.00
History of ICP	0% (0/9)	33.3% (3/9)		0.21
Preterm birth	0% (0/9)	33.3% (3/9)		0.21
Intrauterine foetal stress	0% (0/9)	1.1% (1/9)		1.00
Meconium-stained amniotic fluid	0% (0/9)	11.1% (1/9)		1.00
Neonatal intensive care unit	0% (0/9)	0% (0/9)		1.00
Perinatal death	0% (0/9)	0% (0/9)		1.00

Note: * Compared with those in the control group, gestational age, foetal weight and placental weight/neonatal weight were significantly decreased ($p < 0.05$) in ICP group.

Abbreviations: S/D, ratio of maximum systolic flow velocity (S) to end diastolic flow velocity (D) of umbilical artery.

Table 2. Biochemical indicators of the patients in the ICP and control groups.

Indicator	Control (n = 9)	ICP (n = 9)	t	p
Alanine aminotransferase (U/L)	14.67 ± 4.92	196.33 ± 179.68	3.03	0.01*
Aspartate aminotransferase (U/L)	17.89 ± 4.40	127.44 ± 115.05	2.86	0.02*
Total bile acid (μmol/L)	1.98 ± 0.91	43.32 ± 26.06	4.76	<0.001*
Total bilirubin (μmol/L)	9.84 ± 3.22	12.94 ± 5.13	1.54	0.14
Indirect bilirubin (μmol/L)	7.98 ± 2.67	7.89 ± 1.63	−0.09	0.93
Direct bilirubin (μmol/L)	1.86 ± 0.63	5.05 ± 3.74	2.53	0.03*
γ-glutamyltransferase (U/L)	14.67 ± 9.90	42.22 ± 37.45	2.13	0.06
Albumin (g/L)	38.27 ± 2.46	38.14 ± 1.31	−0.13	0.90
Globulin (g/L)	28.49 ± 3.91	28.60 ± 2.05	0.08	0.94
Alkaline phosphatase (U/L)	107.62 ± 24.89	198.00 ± 89.10	2.89	0.02*
Lactate dehydrogenase (U/L)	178.33 ± 35.55	200.44 ± 45.31	1.15	0.27
Fasting plasma glucose (mmol/L)	4.30 ± 0.61	4.42 ± 0.47	0.54	0.62
Prealbumin (mg/L)	226.78 ± 21.48	207.11 ± 45.30	−1.12	0.26

Note: * Compared with those in the control group, TBA, ALT, AST, and DBIL levels in the ICP group were significantly higher ($p < 0.05$).

pressed in both the cytoplasm and nucleus of trophoblasts with particularly prominent staining (Fig. 1).

3.3 STAT3, RORγt, and IL-17A Levels in the Placenta

Comparatively to the control group, placental levels of STAT3, RORγt, and IL-17A proteins were significantly elevated in ICP group ($p = 0.01$; $p = 0.01$; $p = 0.02$; Fig. 2).

3.4 CD4$^+$IL-17A$^+$ Lymphocyte (Th17 Cell) Levels in the Peripheral Blood

Flow cytometry showed that comparatively to the control group, the proportion of Th17 cell in the peripheral

blood was significantly higher in ICP group ($p = 0.01$; Fig. 3).

3.5 Correlations of STAT3, RORγt, Th17 Cell, and IL-17A

Data of maternal peripheral blood Th17 cell and placental STAT3, RORγt, and IL-17A protein levels were used to generate scatterplots to determine their correlations. There were significant positive correlations between placental IL-17A protein level and the level of Th17 cell in the blood in both the ICP ($r = 0.79$, $p = 0.01$) and control ($r = 0.8$, $p = 0.01$ groups. Moreover, strong positive correlations were noted between placental STAT3 and RORγt
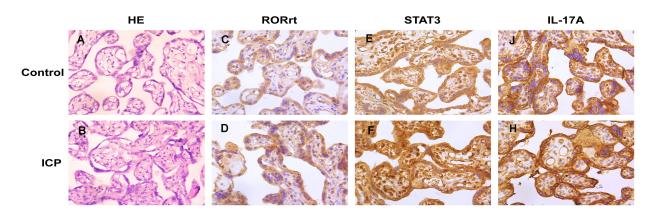
Fig. 1. STAT3, RORγt, and IL 17A protein distribution in the placenta of the two groups (nine samples per group). (A,B) Hematoxylin and eosin staining of the blank control group. (C,D) RORγt protein in the cytoplasm of placental trophoblasts in control and ICP groups. (E,F) STAT3 protein distributed in both the nucleus and cytoplasm of trophoblasts in the two groups. (G,H) IL-17A protein located in the cytoplasm of placental trophoblasts in both groups. All images were acquired following immunohistochemical analysis at 400× magnification.
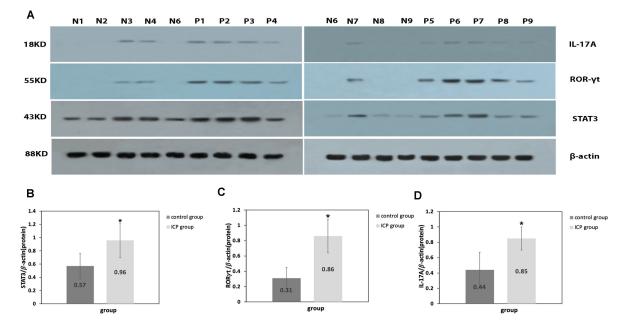

Fig. 2. Protein expression levels of IL-17A, RORγt, and STAT3 in the placentas of patients in the two groups (nine samples per group). (A) STAT3, RORγt, and IL-17A expression in the placentas from the two groups: 'N', control group and 'P', ICP group. (B–D) Quantitative analysis of IL-17A, RORγt, and STAT3 proteins expressed in the placentas of the ICP and control groups. Results are presented as the mean ± SD, *$p < 0.05$.

protein expression in both the ICP ($r = 0.89$, $p = 0.01$) and control ($r = 0.91$, $p = 7\text{e-}04$) groups. Additionally, placental IL-17A protein showed significantly positive relationships with placental STAT3 ($r = 0.97$, $p = 2\text{e-}05$) and RORγt ($r = 0.91$, $p = 0.01$) protein in control group; but, no correlation was observed in the ICP group (STAT3 $r = 0.5$, $p = 0.17$; RORγt $r = 0.62$, $p = 0.07$; Fig. 4).

3.6 Pearson Correlation Matrix

The correlation matrix was constructed by testing Pearson parametric correlations among maternal serum bio-chemical indices related to hepatic and biliary parameters, matrnal peripheral blood Th17 cell; and levels of placental STAT3, RORγt, and IL-17A proteins (Fig. 5). There are significant and positive relationships btween TBA ($r = 0.64$, $p < 0.05$), DBIL ($r = 0.48$, $p < 0.05$), AST ($r = 0.50$, $p < 0.05$) and placental IL-17A protein levels. Additionally, There are positive correlations between TBA ($r = 0.74$, $p < 0.05$), DBIL ($r = 0.64$, $p < 0.05$), AST ($r = 0.51$, $p < 0.05$), ALT ($r = 0.49$, $p < 0.05$) levels and placental RORγt protein levels. There are also positive correlations between TBA ($r = 0.66$, $p < 0.05$), DBIL ($r = 0.65$, $p < 0.05$), AST
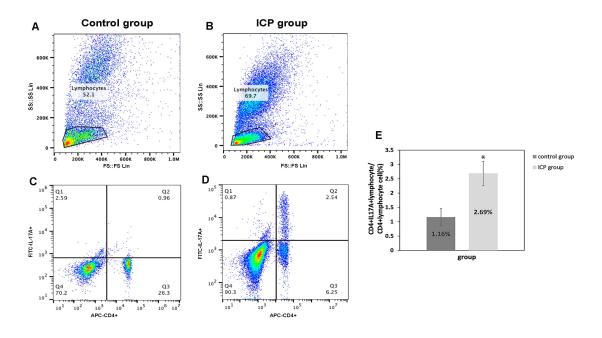
Fig. 3. The proportion of CD4$^+$IL-17A$^+$ lymphocyte (Th17 cell) in the two groups (nine samples per group). (A,B) Flow cytometry analysis of lymphocytes in the peripheral blood of patients in the two groups. (C,D) Proportion of CD4$^+$IL-17A$^+$ lymphocyte in the CD4$^+$ lymphocyte of the peripheral blood in the two groups. (E) Quantitation of the proportion of CD4$^+$IL-17A$^+$ lymphocyte in the peripheral blood CD4$^+$ lymphocyte. Results are presented as the mean $\pm$ SD, $*p < 0.05$.
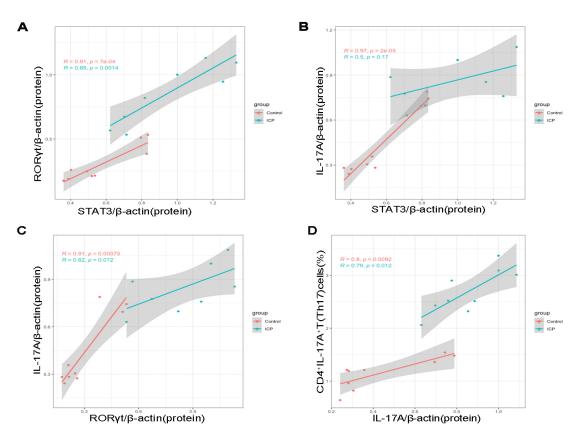

Fig. 4. Pearson correlation scatterplots of placental STAT3, RORγt, and IL-17A proteins and peripheral blood Th17 cell in the two groups (nine samples per group). The blue and orange dots on behalf of variables of the ICP and control groups, respectively. (A) Placental STAT3 and RORγt proteins. (B) Placental STAT3 and IL-17A proteins. (C) Placental RORγt and IL-17A proteins. (D) Placental IL-17A and peripheral blood Th17 cell. Correlations with $p < 0.05$ were considered significant.

(r = 0.54, $p < 0.05$), and ALT (r = 0.49, $p < 0.05$) and placental protein STAT3 levels. Furthermore, TBA (r = 0.72, $p < 0.05$), DBIL (r = 0.50, $p < 0.05$), and AST (r = 0.50, $p < 0.05$) levels were significantly positively correlated with the peripheral blood Th17 cell proportion.
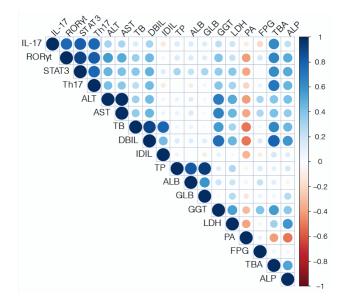

Fig. 5. Pearson correlation matrix of peripheral blood Th17 cell, and levels of placental STAT3, RORγt, and IL-17A proteins maternal serum biochemical indexes. The correlation matrix was constructed using the R package 'corrgram'. The colour-coded map displays the value of the Pearson correlation coefficient. Positive correlation was indicated as red and negative correlation was indicated as blue. The size and colour intensity of the circle are in proportion to the correlation coefficients. Correlations with $p < 0.05$ were regarded as significant.

4. Discussion

Our study highlights the alters of STAT3, RORγt and Th17 cell in ICP along with the correlations between Th17 cell and ICP. We found that the ICP group had substantially elevated placental STAT3, RORγt, and IL-17A protein expression levels, as well as a greater peripheral blood Th17 cell count, relative to those in the control group. Our finding of an increased count of Th17 cell is consistent with the finding of previous studies [7,16,17]. Additionally, placental IL-17A protein showed significantly positive relationships with placental STAT3 and RORγt expression in the control group; in contrast, the ICP group did not show such a correlation. This indicated that the differentiation and function of Th17 cell in ICP differed from those in normal pregnancy. Our results can thus provide novel insights into the correlations between Th17 cell and ICP.

As a key player that defends against external pathogens and mediates immune rejection in the host, Th17 cell are vital in mediating maternal-foetal immunity. Recent studies have shown that an increased Th17 cell count is associated with ICP as it disrupts the delicate immunity and tolerance balance at the maternal-foetal interface [6,7]; however, the mechanism underlying differentiation and functional regulation of Th17 cell in ICP remains unclear. We found that STAT3/RORγt/IL-17A proteins were mainly distributed in the trophoblast cells of the placenta, which is an important component of maternal-foetal immunity. Our study suggested that Th17 cell might be closely related to the maternal-foetal immune response. This result is consistent with those reported in previous studies [6,7].

STAT3 is indispensable for the differentiation of Th17 cell. Our study revealed that STAT3 protein was distributed in the nucleus and cytoplasm of trophoblasts. Shochet *et al*. [18] found that STAT3 promoted the invasion of trophoblast cell and facilitated embryo implantation in primary pregnancy. Additionally, Ye *et al*. [19] demonstrated that STAT3 is mostly located in trophoblasts and ICP participants have higher STAT3 levels compared to controls. Our study demonstrated increased expression levels of STAT3 protein in the placenta of the ICP group, which is consistent with earlier studies. STAT3 can regulate Th17's differentiation via directly binding to the RORγt encoding gene and promoting its expression [13]. Our study confirmed positive correlations between placental STAT3 and RORγt were presented in ICP and control groups.

RORγt is the lineage-specific transcription factor expressed in Th17 cell, which is required to initiate the Th17 differentiation and maintain Th17 cell increase [20]. RORγt$^{-/-}$ mice lack tissue-infiltrating Th17 cell, and autoimmune disease is alleviated in this model [21]. Here, we demonstrated there was higher level of placental RORγt protein in the ICP group compared to control group. Furthermore, placental IL-17A protein showed remarkably positive relationships with placental STAT3 and RORγt protein in control group, but not in ICP group. IL-17 is a specific effector of Th17. IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F are six fellows of IL-17 family [22]. Among them, the family prototype is IL-17A. IL-17F and IL-17A share high homology, and their coding genes are located in the same chromosome region; however, IL-17F has poor stability. A complex network of cytokines and transcription factors was required for Th17 cell differentiation and function. Du *et al*. [23] reported there were substantially increased levels of IL-6 but decreased levels of TGF-β in ICP patients serum. IL-6 and TGF-β can activate the STAT3 signalling pathway to promote Th17 cell differentiation, at the same time Th17 cell secrete IL-6 [24]. The increased proportion of Th17 cells via autoimmune regulation would inhibit the inflammatory responses [15]; hence, the effects of STAT3 and RORγt on the differentiation of Th17 cell are likely inhibited in ICP. However, since small number of patients in our study, these results may change if a larger patient cohort is analysed. Further study is still needed to validate this.

IL-17 is an important inflammatory mediator of immunity and several studies have focused on its role in ICP. Ren *et al*. [6,25] found that IL-17 levels in the decidual tissue and peripheral blood of ICP patients were significantly elevated compared with healthy pregnancy; they positively correlated with serum TBA. However, Kirbas *et al*. [17] found serum IL-17 level was significantly increased in ICP patients than normal pregnant patients, but no significant relationship between IL-17 and TBA was abserved. Based on these results, no definite conclusion could be reached on the correlation between IL-17 and TBA ICP patients. We found that placental IL-17A protein levels and peripheral blood Th17 cell levels were significantly higher in ICP group than control group. Our results are similar to previous studies [6,25]. Additionally, our study also revealed positive correlations among maternal serum TBA, DBIL, AST, and ALT and placental STAT3 and RORγt proteins in both the ICP and normal groups. Moreover, maternal serum TBA, DBIL, and AST levels positively related with placental IL-17A protein and the proportion of Th17 cell. TBA measurement is regarded as a gold-standard method for diagnosing and assessing ICP [26,27]. In the setting of ICP, liver injuries typically have a hepatocellular pattern, which is not surprising and thus AST and ALT are usually elevated in patients with ICP. However, there was no relationship between Th17 cell and maternal serum ALT. Another previous study [28] used maternal routine liver tests to reliably exclude ICP and showed that combined laboratory scores including AST, GGT, ALK, and TB, but not ALT, contribute to this prediction ability. This needs to be evaluated in further studies. In some cases, patients with ICP develop jaundice. ICP patients with jaundice are considered to have a more severe form of the condition and are more likely to have adverse pregnancy outcomes [29]; moreover, maternal DBIL levels may be another important predictor of adverse pregnancy outcomes. Our data indicated that Th17 cells might be a potential marker for ICP prediction and assessment.

Bile acid can induce the production of inflammatory mediators like IL-23 [30], and IL-17 can promote the these inflammatory mediators production, which in turn induces Th17 cell expansion through a positive feedback loop [31]. IL-17 could act on bile duct epithelial cells and promot e the Th17 cell differentiation and aggregation. Th17 cells around the bile duct, which may interfere with bile acid metabolism and induce ICP [30]. Moreover, the expansion in Th17 cell might further aggravate the inflammatory response at the maternal-foetal interface [32]. We found significant positive correlations between placental IL-17A protein and the proportion of Th17 in both the ICP and control groups. These results indicated the proportion of peripheral blood Th17 could reflect immune activity at the maternal-foetal interface.

Our study revealed ICP patients were associated with earlier gestational days, lower neonatal weights and higher ratio of placental weight and neonatal weight comparing with normal pregnancy. The difference in gestational days may affected the comparisons' accuracy of Th17 cell between normal and ICP groups. However, previous and recent studies both found Th17 cell count keep stable during all pregnancy [33,34]. Additionlly, the mean gestational days in both groups was over 37 weeks. So the gestational days difference may has little effect on Th17 cells in our study. The difference of ratio of placental weight and neonatal weight in two group may reflect impaied placenta function,which need further study.

Our study presents some limitations. We studied the expression levels of STAT3/RORγt /IL-17A in placenta only at the protein level and not at the mRNA level. The histological features of the placenta were not recorded when the placenta tissue were collected. In addition, all ICP patients in our study were treated with ursodeoxycholic acid. As reported by Lee *et al*. [35], ursodeoxycholic acid colud inhibit the function of Th17 cells.There was no high quliaty study that examined ursodeoxycholic acid effect on Th17 cell in ICP. In our study, we did not examine the alters in Th17 cell before and after treatment with ursodeoxycholic acid. In future studies, we plan to include a larger number of samples and observe the effect of ursodeoxycholic acid for Th17 cell in patients with ICP, simultaneously. Moreover, *in vitro* experiments will be carried out to verify the results.

5. Conclusions

In summary, our data indicated that with an increase of Th17 cells, the roles of STAT3 and RORγt in the differentiation and function of Th17 in ICP are likely inhibited and attenuated. Th17 might play a negative role in ICP by aggravating inflammatory responses and disrupting the immune balance at the maternal-foetal interface. Excessive accumulation of Th17 cells may be associated with adverse outcomes of ICP.

Author Contributions

YXW is responsible for research performing and manuscript writing; YYH is responsible for the design of the research study; QC is responsible for the data analysis; QHC and DS is responsible for the help and advice. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of West China Second Hospital, Sichuan University, Chengdu, China and the Ethics board number is 2020-67. All patients signed an informed consent form.

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Conflict of Interest

The authors declare no conflict of interest.

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