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Original Article

Immunolocalization of FOXP3, JAK1 and STAT5 in preeclamptic, intrauterine growth restricted and gestational diabetic human placentas

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ABSTRACT

Objective: The aim of the study to show the relation of T cells in placental villous fragments with FOXP3,JAK1 and STAT5 receptors in different conditions such as GDM, PE and IUGR placental tissues.

Materials and Methods: Specimens of 10 diabetic placentas, 10 preeclamptic, 10 intrauterine growth restricted placentas and 10 control placentas were collected by systematic uniform random sampling. Immunohistochemical detections of FOXP3, JAK1 and STAT5 were performed in histological sections for each group's placental tissue. The H-score value was derived for each specimen by calculating the sum of the percentage of syncytiotrophoblast and syncytial nodes in placenta and intervillus area. They were categorized by intensity of staining, multiplied by its respective score.

Results: FOXP3, JAK1 and STAT5 immunoreactivity comparisons are shown in four groups of placentas. FOXP3 immunoreactions significantly increase in GDM group. JAK1 and STAT5 immunoreactions significantly decrease in PE group. STAT5 immunoreactivity was detected crucially increase in GDM group.

Conclusion: The results showed that in different conditions such as PE, GDM and IUGR, T cells in placental villous fragments have relation with FOXP3,JAK1 and STAT5 receptors and that FOXP3 can inactivate the PE and IUGR in the placental tissue. We have also confirmed as other studies that JAK-STAT pathway plays important role in PE, IUGR and GDM placental tissue.

Keywords: preeclampsia; intrauterin growth restriction; gestational diabetes mellitus; FOXP3, JAK1 and STAT5 receptors

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Introduction

Preeclampsia (PE) is known as a multisystemic illness and globally is the cause of several complications of pregnancy with a relatively high incidence. It is associated with substantial maternal/perinatal morbidity and mortality and may impair placental perfusion, function of endothelial cells and appropriate trophoblastic infiltration of maternal spiral arteries [1–3]. The reasons for these prominent pathological alterations still are unclear. Nevertheless, an overt maternal inflammatory process together with cytokine-mediated endothelial damage during pregnancy underlies pathogenesis of PE [4].

Fetal growth is dependent on the coordination of genetic and epigenetic determinants in conjunction with maternal, fetal, and placental factors [5]. Intrauterine growth restriction (IUGR) is described as the failure of achievement of the growth potential potentiated by these aforementioned issues. IUGR presents as a variable syndrome characterized with suboptimal growth and development and it has a diverse etiology such as syndromes, infections, metabolic factors and placental disorders. The precise mechanism of isolated placental dysfunction in IUGR is still obscure.

Lack of endovascular trophoblastic infiltration of myometrial segments of the spiral arterioles attributed to decreased maternal immune tolerance is accused for placental maldevelopment [6].

Carbohydrate intolerance which could evolve on or in the course of pregnancy is known as gestational diabetes mellitus (GDM). This is seen mostly on 2–9 % of all pregnancies in developed Western countries and is a common metabolic problem [7]. In the fetal period, fetusus could be affected by the outcome of maternal hyperglycaemia which is related with many side effects such as overweight and neonatal hypoglycaemia. Problems for the mother could amplify the risk of preeclampsia [8].

These women who are subjected to GDM are also under risk for developing type 2 diabetes mellitus in five to fifteen years after giving birth [9] and the babies born from these mothers do have the potential of attaining type 2 diabetes, later in their lifes [10].

In ordinary pregnancies, physiological changes are taken into consideration by resistance to insulin and immune response inflammation. The physiopathology is not exactly known but it is known that GDM is a chronic inflammatory process [11] and there is an augumented humoral immune response [12].

Impaired functions of the placenta is often responsible for the pregnancy complications by not to provide the needs of the developed fetus.

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For that reason, an appropriately functioning placenta is important for pregnancy outcome. Definitely, the placenta is crucial for fetal growth since it enhances exchange of nutrients and gas between mother and fetus. Throughout implantation, the cytotrophoblasts of the placenta undergo maturation and infiltrate into the uterine tissue where they transform the uterine spiral arteries to vessels with low resistance and high capacity [13, 14]. This modification permits an enhanced exchange of nutrients and oxygen [13, 14]. In various pregnancy related pathologies, remodeling of trophoblasts is insufficient and the placenta is unable to maintain the requirements of the maturing fetus [15]. Given the role of FOXP3-JAK1-STAT5 in immunologic processes, our aim was to investigate the immunolocalization of FOXP3-JAK1-STAT5 in human placentas of pregnants with preeclampsia, IUGR and GDM.

FOXP3-JAK-STAT

Normal pregnancy depends on the appropriate relationship between maternal immune system and immune suppression linked with regulatory T (Treg) cells. This immune suppression occurs by restriction of antigen-specific immune responses [16]. Treg cells are characterized with the expression of forkhead family transcription factor forkhead box p3 (Foxp3). Tregs represent a subgroup of immunosuppressive T-cells and Foxp3 gene is necessary for appropriate functioning of Tregs [17].
Janus kinase 1 (JAK1), is a member of an associate family of protein-tyrosine kinases (PTK) [18].
The interaction between JAK1 and TYK2 as well as JAK1 and JAK2 pathways may be involved in the connection of interferon receptor mechanisms [19]. These kinases are supposed to pair cytokine ligand binding to tyrosine phosphorylation of signaling proteins and a family of transcription factors termed as signal transducers and transcription activators, or STATs [20].
The protein encoded by this gene belongs to the STAT family of transcription factors [21]. Members of STAT family form dimers and serve as activators of transcription [22]. This protein mediates the responses of various cell ligands, like IL2, IL3, IL7 GM-CSF, erythropoietin, thrombopoietin, and different growth hormones [23]. STAT phosphorylation in terms of STAT5 over STAT3 and Foxp3 demethylation among the iTregs were notewothy [24]. This STAT5-dependent Treg differentiation pathway competes with IL-6 signaling, that in turn deteriorates STAT5-FOXP3 molecular interactions [25].

Material and methods

Subjects

All subjects were conscripted from the Department of Obstetrics and Gynecology, Izmir Tepecik Training and Research Hospital, Izmir, Turkey during Oct. 2011 and June 2012. The study subjects included women who consequently developed PE (n = 10), IUGR (n = 10), GDM (n = 10) and healthy pregnant women (n = 10) as controls. Ordinary pregnancy women were randomly selected from the concurrent women who were normotensive, deprived of proteinuria throughout the pregnancy, and delivering a healthy baby at term (>37 weeks of gestation) with no medical or obstetric complications, for instance chronic hypertension, renal insufficiency, diabetes, intrauterine growth restriction (IUGR), congenital anomalies or fetal death. PE was defined as hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg) and proteinuria (≥ 300 mg in a 24-hr urine collection and / or ≥ 1+ on measuring stick testing) after 20 weeks of gestation [26]. Severe PE was defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg; severe proteinuria (urinary protein excretion ≥2.0 g per 24-hr and/or ≥2+ on measuring stick testing); evidence of pulmonary edema; seizures; oliguria (<500 mL/day); thrombocytopenia (platelet count < 100.000/dL); and severe central nervous system symptoms, such as altered mental status, headaches, unclear vision, or blindness. IUGR was defined as estimated fetal weight less than the 10th % for gestational age [27]. For some cases doppler ultrasound advanced for given growth percentile. Diminished fetal arterial and venous doppler flows in key vascular beds were observed in some cases. The diagnosis of gestational diabetes was made between 24 and 28 weeks of gestation by a positive 2-h 75 g oral glucose tolerance test (OGTT) with the following criteria: a fasting plasma glucose ≥ 5.1 mmol/l (92 mg/dl), or a 1-h plasma glucose level of ≥ 10.0 mmol/l (180 mg/dl), or a 2-h plasma glucose of ≥ 8.5 mmol/l (153 mg/dl) [28].

Exclusion criteria included most important congenital anomalies; prior PE, IUGR and GDM, alcohol drinking, taking drugs, smoking and previous medical conditions such as chronic hypertension, renal disease or autoimmune disease. The Ethics Committee at Izmir Tepecik Training and Research Hospital approved the use of the clinical information and the collection of samples for the study. Written informed consent was obtained from all joined subjects. Sample demographics are shown in Table 1.

Human Placental Tissues

Placentas were collected soon after delivered either by C-section or vaginally. For each group, ten samples were examined.

Histochemical staining

Placental tissue was first washed with PBS (phosphate buffered saline) for remove blood. For light microscobic analysis fixed with 10 % formalin solution for 24-48 hours. After fixation, placental tissue samples dehydrated in ethanol, cleared in xylene and embedded in paraffin. Then 5 µm. sections were stained with hematoxylin and eosin (H&E). Two area from placenta as central and peripheral were analyzed under a light microscop. Basic structures as syncytiotrophoblasts, villous fields and syncytial nodes were compared between two groups. The examiner was blinded to the treatment of each group. Histochemical process was repeated 3 times.

Immunohistochemical staining

Embedded paraffin tissue sections 5 µm. thick were mounted on polylysine-coated slides, deparaffinized, rehydrated and then incubated in Trypsin solution (00-3008, Invitrogen®). After two washes with PBS, slides were then incubated with 0.3 % hydrogen peroxide in methanol for 30 min. to quench endogenous peroxidase activity. After washing with PBS, tissues were incubated with blocking serum (85-9043, Histostain Plus Bulk Kit, Invitrogen®) at room temperature for 1 h. Then, a primary antibody diluted in blocking serum [anti-FOXP3 mouse monoclonal antibody (SC-65988 Santa-Cruz, USA), anti-JAK1 rabbit polyclonal antibody (SC-277 Santa-Cruz, USA), anti-STAT5 rabbit polyclonal antibody (SC-836 Santa-Cruz, USA); 1:100 dilution)] was added to the slides and incubated at 4°C overnight in a humidified chamber. After washing 5 min. in PBS, tissue sections were incubated for 30 min. with 3 µg./ml. biotinylated antibody (anti-rabbit or anti-mouse). Subsequently, slides were washed with PBS and incubated with avidin-biotin complex reagent containing horseradish peroxidase (85-9043, Histostain Plus Bulk Kit, Invitrogen®) for 30 min. Slides were washed with PBS for 5 min. and color development was achieved using DAB substrate. The tissue sections were counterstained with hematoxylin.

Data presentation and statistical analysis

Immunohistochemically staining was jointly scored on all cases, using a semi quantitative system that is based on the H index (McCarty et al., 1985).The intensity of immunoreactivity evaluated as low (+), mild (++), moderate (+++), strong (++++) and very strong (+++++).

Table 1. Characteristics of patients

	Control (n=10)	PE (n=10)	IUGR (n=10)	GDM (n=10)	P value
Age (years)	30.8 ± 4.6	30.2 ± 2.7	28.6 ± 9.5	32.5 ± 4.8	0.543
Gestational age (weeks)	38 ± 1.2§	34.4 ± 2.0	37.4 ± 3.0	39.0 ± 1.3%	<**0.001**
USG (weeks)	37.1 ± 1.0†§	33.6 ± 2.0	32.8 ± 2.7¶	38.0 ± 0.9%	<**0.001**
Birth weight (gram)	3506 ± 155†§	2380 ± 369	2310 ± 609¶	3676 ± 246%	<**0.001**
1-min APGAR score	8 (7-8)	7 (7-8)	7 (4-8)	8 (7-8)	0.225
5-min APGAR score	7 (7-8)	8 (8-8)	7.5 (7-8)	7.5 (7-8.25)	0.179
Gender (female/male)	(4/6)	(5/5)	(6/4)	(4/6)	>0.05
SBP (mm/Hg)	109.0 ± 7.3§	149 ± 25.1	112.0 ± 7.8k	112.0 ± 7.8%	**<0.001**
DBP (mm/Hg)	64 ± 5.1§	96 ± 12.6	69 ± 7.3 k	71 ± 7.3%	**<0.001**
Insulin(positive)	0/10	0/10	0/10	3/10	**0.012***

Data are presented as mean ± SD. except for 1-min APGAR score and 5-min APGAR score that are presented as median (interquartile range)

*Abbreviations: SBP: systolic blood pressure; DBP: diastolic blood pressure*P = 0.012 GDM vs others †P < 0.001 Control vs IUGR*

§P < 0.001 Control vs preeclampsia ¶P < 0.001 IUGR vs GDM, kP < 0.001 IUGR vs preeclampsia, %P < 0.001 GDM vs preeclampsia

The H-score value was derived for each specimen by calculating the sum of the percentage of syncytiotrophoblast and syncytial nodes in placenta and intervillus area. They were categorized by intensity of staining, multiplied by its respective score, by means of using the formula: H-Score=∑ Pi (i+1), where, i = intensity of staining with a value of minimal (+), mild (++), moderate (+++), strong (++++) very strong (+++++) with Pi is the percentage of stained syncytiotrophoblast and syncytial nodes for each intensity, varying from 0% to 100%. For each slide, four different fields were evaluated microscopically at 100 × magnification. H-score evaluation was performed by at least tree investigators (Inan S., Ekizceli G., Dilber Y.) independently, blinded to the source of the samples as well as to each other's results and the average score was then utilized.

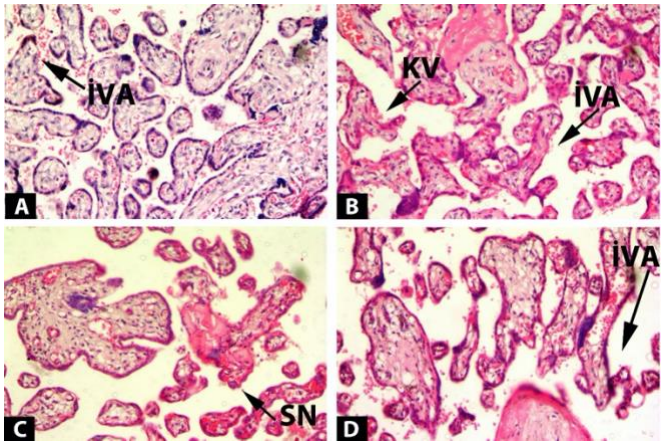
Statistical analysis was performed using SPSS 15.0 software (SPSS, Inc., Chicago, IL, USA). The Kruskal-Wallis non-parametric test was used to compare the results among all four treatment groups, while the one way Anova test was used for pairwise comparison of the groups. P<0.05 was considered to indicate a statistically significant difference. The Bonferroni correction was applied for the post-hoc analysis results, where P<0.005 was regarded as a statistically significant difference for the two group comparisons.

Results

Histopathological Findings

Under light microscopic examination of H&E stained control placentas, villous structures were observed with central connective tissues core covered with trophoblastic cell layers. Every villous tissue has fetal capillaries and villi were separated by intervillous spaces filled with maternal blood in control group (Fig 1A). Specific histopathological alterations were detected in PE placentas. Distribution of the nuclei in the syncytiotrophoblasts displayed some changes such as formation of clusters, particularly where the syncytial layer exhibits sprouts towards the intervillous spaces (Fig 1B). Long and slender syncytial strands cross the intervillous spaces connecting villi to each other and give the villous tree an appearance of pseudo labyrinthine in PE group (Fig 1B). Sectioned syncytial strands or sprouts were identified due to the absence of villous core in IUGR group (Fig 1C). The villous stroma of connective tissue displays a substantial cellularity and fibrillar content with an amplified affinity for staining with collagen for the entire villous core in GDM group. Fetal capillaries seem to disappear in the majority of villi; nevertheless, fetal capillaries are still evident in GDM group (Fig 1D).

Figure 1. Histologic analysis of the placenta tissue samples



H&E (X100) sections histologic view from image A, group 1 also control normal placenta intervillous space architecture (arrow); image B, group 2 show severe chorionic villous and intervillous space(arrow); image C, ın the group 3 show significantly syncytial strands organization(arrow), image D, ın the group 4, increase intervillous space in placenta tissue.

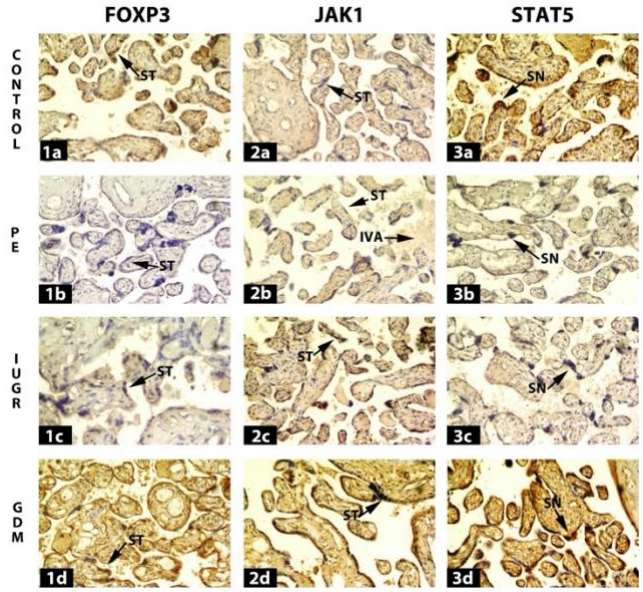
A remarkable point was increased deposition of intervillous fibrinoid in PE and IUGR placenta samples compared with control group. Ischemia was remarkable in villous areas of

PE, IUGR and GDM groups. Moreover, syncytial nodes were increased in PE and IUGR groups. Immaturely ischemic areas were encountered in villi of GDM group. Both PE and IUGR placenta samples exhibited significantly lower mean placental weights, thicknesses and surface areas compared with control group and the biggest placentas were noted in GDM group (Figure 1).

Immunohistochemical Evaluations

Immunoreactivities of anti-FOXP3, anti-JAK1 and anti-STAT5 primary antibodies were positive in the cellular compartments of the placental villous tree, mainly in villous trophoblasts and stromal endothelial cells in control, PE, IUGR and GDM placentas. Moderate cytoplasmic FOXP3 immunoreactivity was seen in control (2.57±0.53) placenta samples (Fig 2-1a). While minimal FOXP3 immunoreactivity was observed in PE (1.40±0.52) (Fig 2-1b); minimal to moderate immunoreactivity was seen in IUGR (1.86±0.38) placenta samples (Fig 2-1c). A strong staining pattern was observed in the GDM (3.71±0.49) placenta samples (Fig 2-1d).

Figure 2.Immunohistochemistry for the FOXP3/JAK1/STAT5 expression cell (x100)



This arrow shows ST (syncytiotrophoblast), SN (syncytial strands), IVA (intervillous space). The arrow shows the level of FOXP3 immunoreactions minimal SN (syncytial strands) of PE group (Fig. 2-1b). Also, a strong increase was detected for IVA immunoreactivity in GDM group. JAK1 immunoreaction significantly decreases in PE (Fig. 2-2d), while increases in syncytial strands of GDM group (Fig. 2-2d). STAT5 immunoreactivitiy increase very strong in syncytial strands of GDM group (Fig. 2-3d). In PE respectively IUGR (Fig 2-3b-3c) placenta samples, minimal STAT5 immunoreaction was detected.

When compared all groups, FOXP3 immunoreactions were observed on syncytiotrophoblast and syncytial nodes in placental tissues. The results of the immunohistochemical analysis of FOXP3 protein in placentas, FOXP3 immunoreactivity was detected markedly decrease in PE and IUGR (P<0.01) when compared to the immunoreactivity in Control group (Table 2).

JAK1 immunoreactivity was observed in moderate degree in the control group (2.86±0.50) (Fig 2-2a) different from other groups minimal immunoreactions were observed in PE (1.30±0.48) (Fig 2-2b). The gradually increase in IUGR (3.29±0.49) (Fig 2-2c) and strong immunoreactivity in GDM group (3.71±0.49) (Fig 2-2d) placenta samples. The results of the immunohistochemical analysis of JAK1 protein in placentas, only PE group placentas showed that the immunoreactivity of JAK1 markedly increased (p<0.01) compared to the immunoreactivity in control group (Table 3). STAT5 immunoreactivity was observed moderate immunoreactions in control (3.86±0.44) (Fig 2-3a), in Group PE (2.10±0.57) (Fig 2-3b) and same immunoreactions IUGR (2.71±0.49) (Fig 2-3c) placenta samples.

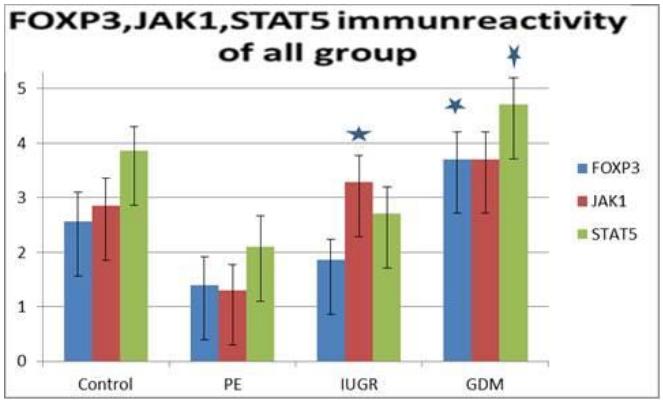
Table 2. H- score values of FOXP-3, JAK-1 and STAT-5 in placental tissues Mean±SD

Type of Placental Tissue	FOXP-3	JAK-1	STAT-5
Normal Term Placenta (Control)	++/+++ 2,57±0,53	++ 2,86±0,50	+++ 3,86±0,44
Pre-Eclampsia(PE)	+ 1,40±0,52	+ 1,30±0,48	++ 2,10±0,57
Intra Uterine Growth Restriction (IUGR)	++ 1,86±0,38	++/+++ 3,29±0,49	++/+++ 2,71±0,49
Gestational Diabetes Mellitus (GDM)	+++ 3,71±0,49	+++ 3,71±0,49	+++/++++ 4,71±0,49

PE and IUGR with comp Control, p<0.01 (FOXP3), IUGR and GDM with comp Control p<0.01 (JAK1), GDM with comp Control, p<0.01 (STAT5)

Moderate to strong immunoreactivities were observed in GDM group (4.71±0.49) (Fig 2-3d) placenta samples. The results of the immunohistochemical analysis of STAT5 protein in placentas, it was observed that markedly increased immunoreactivity of STAT5 in GDM compared to the immunoreactivity in PE,IUGR and control group placenta samples (P<0.01) (Table 2).

Table 3. FOXP3, JAK1 and STAT5 immunoreactivity comparisons are shown in four groups of placentas.



Control group, PE group, IUGR group, GDM group about (mean±SD). FOXP3, JAK1 and STAT5 immunoreactivity was detected very strong in Group GDM. JAK1 immunoreactivity was detected minimal in PE group. STAT5 immunoreactivity was detected same value PE and IUGR Group.

Discussion

Standard pregnancy calls for a comparative immune tolerance of the mother for the fetus. Reduced immune tolerance may lead to placental dysfunction attributed to inadequate trophoblastic infiltration in PE and IUGR. It has been proposed that PE and IUGR may be partially facilitated by the immune system. Regulatory T cells are responsible for immune suppression and it occurs by restriction of antigen-specific immune response [29]. Treg cells (Tregs) are characterized with the expression of Foxp3, a gene that is located in the short arm of X chromosome (Xp11.23) [30]. Diminished numbers of circulating Tregs were detected in complicated pregnancies such as recurrent pregnancy loss and PE [29]. The exact function of Foxp3 gene in humans has not been fully understood [31]. In mouse models, it has been shown that inactivation of Foxp3 may cause an impairment of Tregs function and development [32]. A link between Foxp3 gene polymorphisms and autoimmune diseases has been postulated in several publications [33-36].

Diminished expression of Foxp3 in PE and IUGR remind that the reduction of Tregs may be related with an imbalance of maternofetal immune function.

Another study has yielded that the expression of Foxp3 was similar between PE and control group placentas and Foxp3 expression in PE group was significantly higher compared to controls. The rates of positivity for Foxp3 expression in the decidua were found to be 16.67% and 66.67%, respectively for PE and control groups. These results demonstrate that Foxp3 expression was notably lower in PE than control. In another study; number of Tregs was significantly higher in patients with insulin-dependent GDM [37, 38].

In preeclampsia patients, dysregulation of immune-associated signaling pathways linked with atypical sialic acid modification, may cause shallow infiltration of trophoblasts and insufficient vascular remodeling in uterus. These changes are associated with inefficient JAK1 expression in PE patients [39]. Therefore, retardation of growth modifies specific targets in the JAK/STAT signalling pathway, with changed JAK2 and STAT3 mechanisms that may contribute to an increased risk of cardiovascular disease in the growth restricted males [40].

In the course of normal pregnancy, the fetus is protected from rejection by means of attenuated immune response and amplified protective function of immune tolerence [41]. It has been shown that STAT3, STAT2 and STAT5B were expressed at significantly lower levels in IUGR placenta compared with gestation matched controls [42].

The level of expression for Foxp3-positive cell is less in PE patients compared to that of controls with normal pregnancy. Placental expression of Foxp3 can be an aspect involved in maternal immune tolerance against embryo antigen [37]. However, the exact functions of Foxp3, JAK1 and STAT5 in placentas with preeclampsia, IUGR and GDM are still vague. Studies focusing on functional aspects are warranted to gather knowledge on underlying mechanism of those disorders.

To conclude, our results indicated that T cells in placental villous fragments may be related with FOXP3, JAK1 and STAT5 receptors and FOXP3 may be inactivated in the placental tissue in various conditions such as PE, GDM and IUGR. We have confirmed that JAK-STAT pathway may have a critical role in PE, IUGR and GDM. However, further trials are needed to enhance underlying mechanisms and interactions between Treg cells, Fox-P3 and JAK-STAT pathways in PE, GDM and IUGR placental tissues.

Disclosure

Authors have no potential conflicts of interest to disclose.

References

[1] Wang LL, Cao XW (2010) Patients with preeclampsia in peripheral blood and umbilical CD+4CD+25 of Foxp3+of Treg levels. Shandong Medical Journal 50 (26): 10.

[2] Cao WP, Qian QJ, Jian W (2010) Changes and significance of the peripheral blood CD4+ CD25+Foxp3+regulatory T cell in gestational hypertension patients. Pathophysiology 26 (7): 1425–1427.

[3] Park O, Grishina I, Leung PS, Gershwin ME, Prindiville T (2005) Analysis of the Foxp3/scurfin gene in Crohn's disease.Ann N Y Acad Sci 1051: 218–28.

[4] Chen X, Gan T, Liao Z, Chen S, Xiao J. Foxp3 (-/ATT) polymorphism contributes to the susceptibility of preeclampsia. PLoS One. 2013;8(4):e59696.

[5] Gardosi J, Chang A,et al. (1992). Customised antenatal growth charts. Lancet339, 283–287

[6] Scifres CM, Nelson DM. Intrauterine growth restriction, human placental development and trophoblast cell death. J Physiol. 2009 Jul 15;587(Pt 14):3453-8.

[7] Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffreis WS, Robinson JS for the Australian Carbohydrate Intolerance Study in Pregnant Women (ACHOIS) Trial Group. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 2005;352:2477–86.

[8] The HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991–2002.

[9] Kim C, Newton KM, Knopp RH. Gestational diabetes and the incidence of type 2 diabetes: a systemic review. Diabetes Care 2002;25:1862–8.

[10] Tam WH, Ma RC, Yang X et al. Glucose intolerance and cardiometabolic risk in adolescents exposed to maternal gestational diabetes: a 15 year follow-up study. Diabetes Care 2010; 33:1382–4.

[11] Richardson AC, Carpenter MW. Inflammatory mediators in gestational diabetes mellitus. Obstet Gynecol Clin North Am 2007; 34:213–24.

[12] Steinborn A, Saran G, Schneider A, Fersis N, Sohn C, Schmitt E. The presence of gestational diabetes is associated with increased detection of anti-HLA-class II antibodies in the maternal circulation. Am J Reprod Immunol 2006;56:124–34.

[13] Arroyo JA, Winn VD: Vasculogenesis and angiogenesis in the IUGR placenta. Semin Perinatol 2008; 32:172–177

[14] Zhou Y, Damsky CH, Fisher SJ: Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? J Clin Invest 1997; 99:2152–2164.

[15] Saito S, Nakashima A: A review of the mechanism for poor placentation in early-onset preeclampsia: the role of autophagy in trophoblast invasion and vascular remodeling. J Reprod Immunol 2014; 101–102:80–88.

[16] Wu Z, You Z, Zhang C, Li Z, Su X, et al (2012) Association between functional polymorphisms of Foxp3 gene and the occurrence of unexplained recurrent spontaneous abortion in a Chinese Han population. Clin Dev Immunol 2012: 896458.

[17] Park O, Grishina I, Leung PS, Gershwin ME, Prindiville T (2005) Analysis of the Foxp3/scurfin gene in Crohn's disease.Ann N Y Acad Sci 1051: 218–28.

[18] A F Wilks, A G Harpur, R R Kurban, S J Ralph, G Zürcher, and A Ziemiecki . Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol Cell Biol. 1991 Apr; 11(4): 2057–2065

[19] Müller M, Briscoe J, Laxton C, Guschin D, Ziemiecki A, Silvennoinen O, Harpur AG, Barbieri G, Witthuhn BA, Schindler C, et al. The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and -gamma signal transduction. Nature. 1993 Nov 11;366(6451):129-35.

[20] Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene. 2002 Feb 20;285(1-2):1-24.

[21] Leek JP, Hamlin PJ, Bell SM, Lench NJ (1997). "Assignment of the STAT6 gene (STAT6) to human chromosome band 12q13 by in situ hybridization". Cytogenetics and Cell Genetics 79 (3-4): 208–9

[22] Liu, L., McBride, K. M., and Reich, N. C. (2005b). STAT3 nuclear import is independent of tyrosine phosphorylation and mediated by importin-alpha3. Proc. Natl. Acad. Sci. USA 102, 8150–8155

[23] Putoczki TL, Thiem S, Loving A, Busuttil RA, Wilson NJ, Ziegler PK et al. Interleukin-11 is the dominant IL-6 family cytokine during gastrointestinal tumorigenesis and can be targeted therapeutically. Cancer Cell 2013; 24: 257–271.