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Role of FOXP3 in Immunohistochemical Expression in Preeclampsia

Malames M. Faisal[1], Hesham M. Harb[1], Fatma S. Hafez[2], Mohamed T. Elsaid[1*]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[2]Department of Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
Email: *dr.mohamedtalaat15@gmail.com

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Abstract

Background: FoxP3 gene variants have been linked to endometriosis, infertility, and autoimmune illnesses, according to numerous researches. Maternal sensitivity to the PE gene and the genetic variations of FoxP3 has not been thoroughly investigated. **Objective:** Investigation of the immune-histochemical expression of FoxP3 in placental tissue of PE patients. **Methods:** A total of 26 pre-eclamptic women as a case and 26 ethnically matched healthy pregnant women as a control group aged between 18 and 40 years old of different gravidity and parity referred to the labor ward for delivery either by vaginal delivery or cesarean section was enrolled to investigate the immunohistochemical expression of FOXP3 in placental tissue of PE patients. **Results:** Lower expression of FOXP3 IHC was statistically significant and noted in the group of preeclampsia compared to the healthy control group. Lower gestational age at delivery and a higher percentage of cesarean section were statistically significant and noted in the group of preeclampsia compared to the healthy control group. **Conclusion:** In comparison to the healthy control group, preeclampsia patients had statistically significantly lower FOXP3 IHC expression, and FOXP3 polymorphism was associated with the development of PE. Our findings can serve as a guide for statistical analyses and functional investigations that are more in-depth.

Keywords

FOXP3, Immunohistochemical Expression, Preeclampsia

1. Introduction

Preeclampsia (PE), which is characterised by a worldwide prevalence of 2% - 8% and contributes significantly to maternal and perinatal morbidity and mortality, is a serious pregnancy complication [1].

Uncertainties still exist regarding the aetio-pathogenesis of PE. According to some theories, inadequate implantation may result in infertility, whereas improper placentation may cause foetal development restriction. Pre-eclampsia, however, is caused by placental malfunction, which affects the mother's system and progresses the condition from a preclinical to a clinical stage before manifesting in the mother [2].

Preeclampsia has a complex nature and is impacted by a variety of elements, including fetal/paternal genetic risk factors and environmental risk factors. Despite significant research efforts, the aetiology and pathogenesis of preeclampsia remain unknown [3].

Many data point to the importance of immunological variables in the development of PE. It appears that the pathophysiology of preeclampsia is heavily influenced by an abnormal and prolonged maternal systemic inflammatory response to pregnancy with cytokine-mediated endothelial damage [4]

FoxP3, a member of the family of fork head transcription factors expressed by the X chromosome, is a master regulatory gene necessary for Treg formation and activity. FoxP3 is required for the transformation of naive T-cells into Tregs, which is thought to be necessary for the maintenance of a successful pregnancy [5].

Only a few researches have examined Foxp3's function in human beings up to this point. It has been shown in mouse models that Foxp3 inactivation causes a lack of Tregs, and it is noteworthy that organ-specific Foxp3 is a key regulator for the growth and operation of Tregs. Foxp3 deficiency may affect Tregs' ability to regulate growth, therefore [6].

Foxp3 gene polymorphisms have been linked to autoimmune illnesses such as autoimmune thyroiditis, allergic rhinitis, type I diabetes, and systemic lupus erythematosus in a number of prior studies [7].

According to Zhou Jianjun *et al.*, Foxp3 mRNA expression was lowered in pre-eclampsia and Th1 type immunity predominated [8].

The decreased expression of Foxp3 in preeclampsia shows how the pathophysiology of preeclampsia may be influenced by immunologic tolerance between the mother and foetus and the reduction of Treg levels. Several geographically diverse regions' published literature on the FoxP3 variance led to a rather contentious result [9].

2. Methods

This case-control study was conducted in antenatal care wards and delivery room of Department of Obstetrics and Gynecology at the Faculty of Medicine, Ain Shams University from June 1st, 2021 to August 15th, 2022. A total of 26 pre-eclamptic women and 26 ethnically matched healthy pregnant women as a control group aged between 18 and 40 years old of different gravidity and parity referred to labor ward for delivery either by vaginal delivery or cesarean section were enrolled to investigate the immunohistochemical expression of FOXP3 in placental tissue of PE patients.

2.1. Inclusion Criteria

26 PE patients and 26 ethnically matched healthy pregnant women as control group aged from 18 to 40 years old were enrolled.

2.2. Exclusion Criteria

Subjects with chronic hypertension, diabetes, kidney diseases, chronic diseases as autoimmune diseases, collagen vascular disease, hemoglobinopathy, thrombophilia, chorioamnionitis, history of endometriosis, coagulation disorders, urinary tract infections, periodontal disease, history of cardiovascular disease, preterm delivery, premature rupture of membranes, multiple gestation, preterm delivery foetal infection, stillbirth & foetal anomalies or chronic aspirin use were excluded.

2.3. Ethical Considerations

All included patients gave an informed written consent after explanation in accordance to the local ethical committee regulation. Data were analyzed and processed anonymously.

2.4. Study Procedures

The International Society for the Study of Hypertension in Pregnancy (ISSHP) defined preeclampsia (PE) as hypertension with systolic blood pressure = 140 mmHg and diastolic blood pressure = 90 mmHg after 20 weeks of gestation, along with proteinuria = 300 mg in a 24-hour collection and/or = 1+ on dipstick testing that was not associated with urinary tract infection or ruptured membranes. Women in the control group (26 women) had pregnancies free of gestational hypertension and proteinuria, were normotensive, delivered healthy newborns at term (more than 37 weeks gestation), and had no obstetric or medical complications like chronic hypertension, diabetes, renal insufficiency, congenital anomalies, intrauterine growth restriction (IUGR), or foetal death.

2.5. Immunohistochemistry

The placenta was divided into small blocks, flushed with phosphate buffered saline multiple times to eliminate extra blood, preserved in neutral buffered formalin for 12 - 16 hours, and then embedded in paraffin.

An avidin-biotin solution was used for the immunohistochemistry (peroxidase method).

Sections (5 m) were cut from the tissue blocks that had been paraffin-embedded and put on APES-coated slides.

Each specimen underwent histological examination after being H&E stained.

Human Foxp3-specific antibodies were used to stain the sections (Abcam, Cambridge, UK).

The endogenous peroxidase was stopped by employing 3% hydrogen peroxide after each paraffin section was deparaffinized, followed by antigen retrieval with

epitope retrieval solution (10 mmol citrate buffer, pH 6.0) in a preheated water bath at 98˚C for 10 minutes.

Sections were then treated with the diluted (1 - 200 in PBS, Biolegend, USA) mouse antihuman FOXP3 antibody for an overnight period at 4˚C in a humid environment.

The sections were then developed with 3,30-diaminobenzidine (Zymed, USA) for 5 minutes, counterstained with hematoxylin, and treated for 30 minutes with streptavidin conjugated horseradish peroxidase at room temperature.

The primary antibody's suitable serum was used as the negative control.

For each immunohistochemistry stain, both positive and negative controls were employed.

Using a Leica DM 4000 B microscope, pictures were taken (Solms, Germany)

2.6. Immunohistochemically Staining for FOXP3

52 instances with accessible tissue were stained with immunohistochemistry using paraffin blocks (IHC). FOXP3 was used to automate IHC staining on 4 m formalin-fixed and paraffin-embedded sections. Blocks of paraffin that included placenta tissue that had been formalin fixed were cut into sections that were 4 m thick. The main antibody, mouse monoclonal FOXP3 (2A11G9), was used to stain the slides utilising the fully automated Benchmark Staining System (Ventana Medical Systems).

Primary antibody

A mouse monoclonal antibody (IgG) was used as the primary defence against human-derived, truncated recombinant FOXP3.

Universal Kit

A detection system with ultra-vision was employed. HRP/DAB, polyvalent antigen (Ready-to-use) was used. A Multilink biotinylated secondary antibody, a streptavidin coupled to horseradish peroxidase, and a DAB chromogen/substrate were employed in this labelled streptavidin-biotin immunoenzymatic antigen detection technique to show the antigen in the cells or tissues.

Reagents supplied in this Kit are

Ultra V Block: one vial 110 ml.

Biotinylated Goat Anti-polyvalent Plus: one vial 110 ml.

Streptavidin Peroxidase Plus: one vial 110 ml.

DAB Plus Substrate: one vial 15 ml.

DAB Plus Chromogen: one vial 2 ml.

Other regents used

Xylene, ethanol, absolute alcohol.

Distilled water.

Antigen retrieval solutions.

Harris haematoxylin.

HIER Citrate Buffer (PH 6.5).

Phosphate buffer solution (PBS)

Control slides

Positive control

Sections from a healthy lymph node were produced, and stained. Cells were deemed positive when they exhibited nuclear FOXP3 staining. The components of the tissue that stain positively serve as proof that the instrument and antibody were employed as intended.

Negative control

Sections from the same tissues were processed in the same immunostaining procedure with the omission of the primary antibody.

Immunohistochemical procedure

For immunohistochemical staining, sections of 4 - 5 μm thickness were cut on positively charged slides and left for 30 minutes at 60˚C in the oven to allow good adherence of the tissues to the slides.

Deparaffinization and rehydration

Paraffin embedded tissues were deparaffinized in two changes of xylene 5 minutes each and rehydrated through graded alcohol series (absolute, 90%, 70%, 50%) (2 minutes each) then water two minutes.

Pretreatment

Tissue sections were treated in a ready to use antigen retrieval (Citra PH6) by insertion in a microwave oven for 3 - 5 minutes until boiling, then ceasing and adding distilled water, that was prepared for another 3 minutes, then sections were left to cool for 20 minutes.

Serum blocking

Two drops of protein blocking serum were added for 10 minutes without rinsing. Peroxidase blocking solution was applied for 30 minutes at room temperature.

Addition of primary antibody

The concentrated primary antibody was reconstituted in 100 ul of sterile water, centrifuged to remove any insoluble material, and diluted to the dilution of 1:100. Two drops of diluted preparation were added to each section and incubated in moist chamber overnight at 4˚C followed by rinsing with PBS.

Addition of the link

Two drops of prediluted biotinylated secondary antibody were added to each sectionfor 30 minutes.This antibody serves as a link between the primary antibody and the label. Then, rinsing with PBS was done.

Addition of the label

Adding 2 drops of the horseradish peroxidase conjugated streptavidin followed by incubation for 30 minutes. Then, rinsing with PBS was done.

Addition of substrate/chromogen (DAB) mixture

Adding 2 drops and incubating for 10 minutes followed by rinsing with distilled water (This substrate was prepared immediately before use). Counterstaining with Harris Haematoxylin & mounting in Canada balsam followed.

2.7. Evaluation of Immunohistochemical Expression of FOXP3

Scoring of Foxp3 immunostaining was done without knowing the group of the section. At least, ten fields per subject were examined in each section and scored

as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. See Figures 1-7.

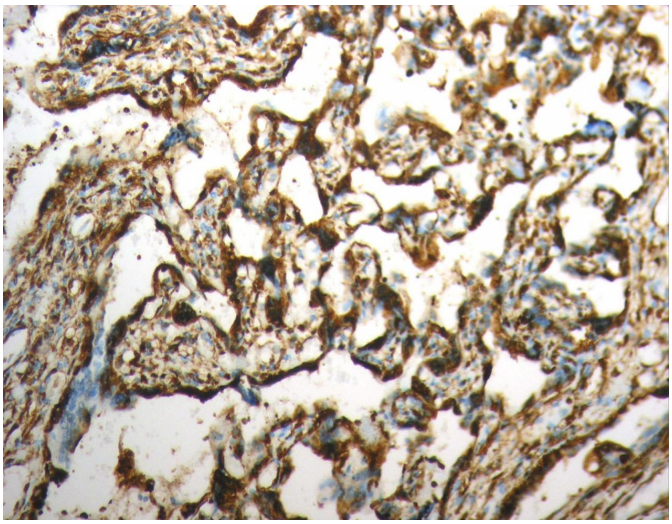

Figure 1. Strong immunohistochemical expression of FOXP3 in PE case (×200).

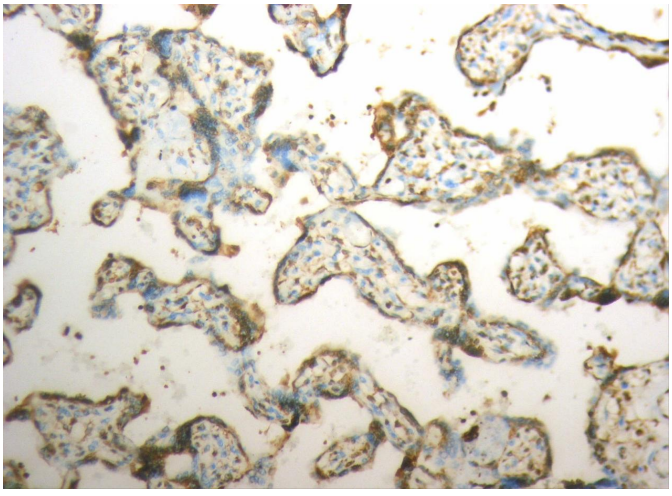

Figure 2. Moderate immunohistochemical expression of FOXP3 in PE case (×200).

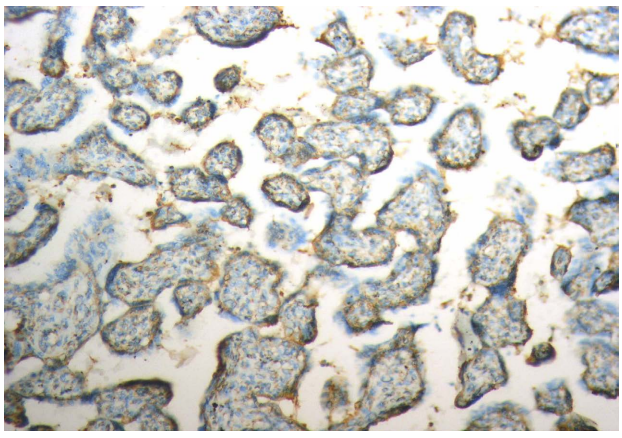

Figure 3. Weak immunohistochemical expression of FOXP3 in PE case (×200).

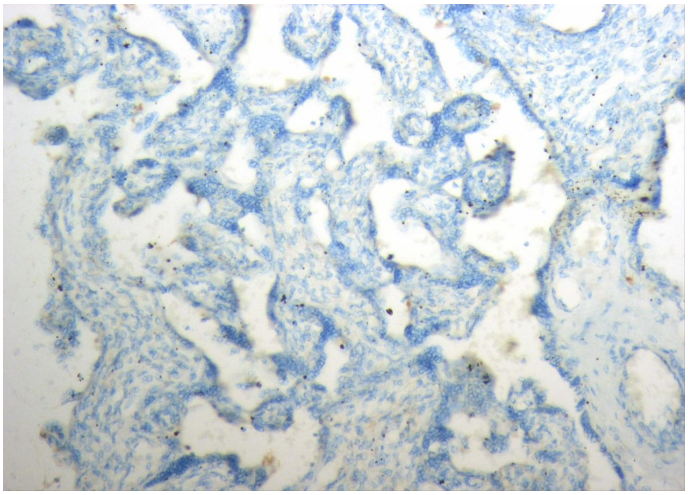

Figure 4. Negative immunohistochemical expression of FOXP3 in PE case (×200).

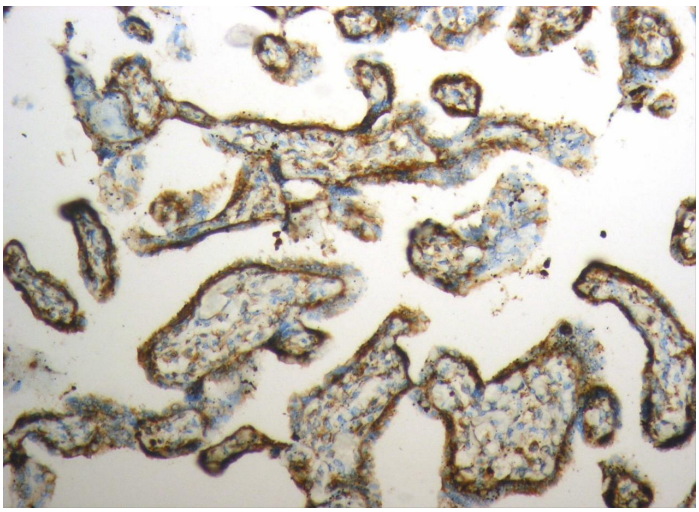

Figure 5. Strong immunohistochemical expression of FOXP3 in normal control (×200).

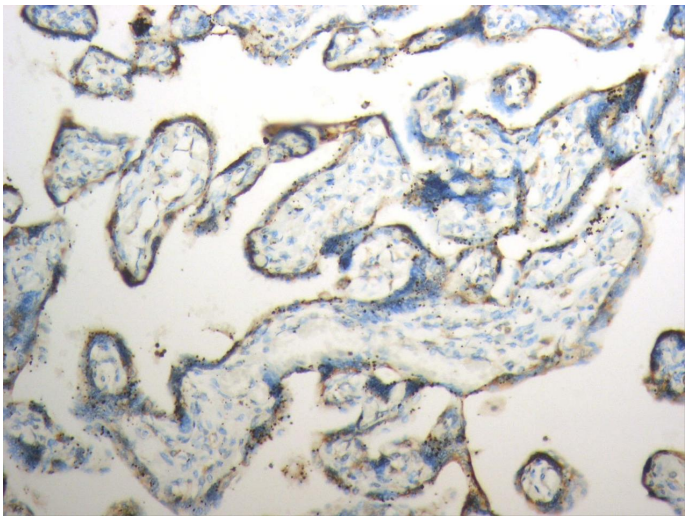

Figure 6. Moderate immunohistochemical expression of FOXP3 in normal control (×200).

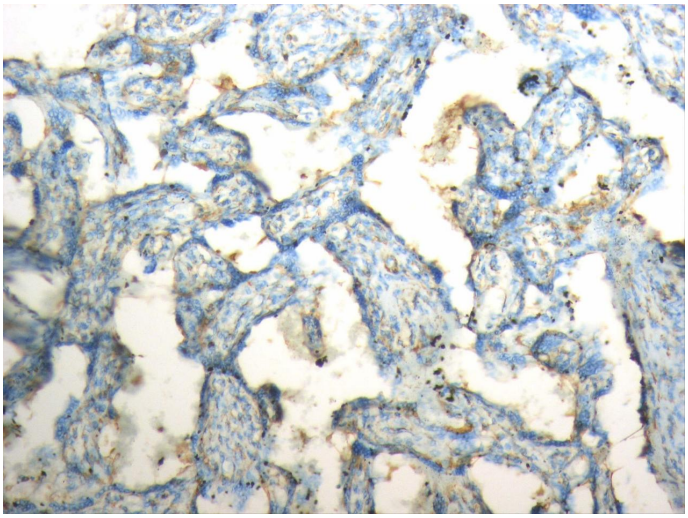

Figure 7. Weak immunohistochemical expression of FOXP3 in normal control (×200).

3. Statistical Analysis

Recorded data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were presented as mean± standard deviation and ranges. Also qualitative variables were presented as number and percentages. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk Test.

4. Results

The current study showed that there was no significant difference between study groups regarding maternal age [27.77 ± 6.50 versus 27.58 ± 5.38 years] ($p = 0.91$) (Table 1).

The current study showed that there were no significant differences between study groups regarding parity and past history of abortion, cesarean sections and preeclampsia ($p = 0.33$, 0.11, 0.73 and 0.49) respectively (Table 2).

The current study showed that gestational age at delivery was statistically significant lower in group of preeclampsia compared with control group 36.92 ± 1.32vesrus 38.96 ± 1.37 weeks ($p < 0.001$) (Table 3).

The current study showed that systolic and diastolic blood pressures were statistically significant higher in group of preeclampsia compared with control group ($p < 0.001$) (Table 4).

The current study showed that percentage of cesarean section was statistically significant higher in group of preeclampsia compared with control group 18 (69.2%) versus 9 (34.6%) ($p = 0.01$) (Table 5).

Among cases with preeclampsia, in present study showed that albumin in urine was negative in 5 cases (19.2%), +1 in 12 cases (46.2%), +2 in 4 cases (15.4%) and +3 in 5 cases (19.2%) (Table 6).

Regarding Immunohistochemical expression of foxp3 in current study there was statistically significant correlation between cases and control group with

lower expression in cases group $p < 0.001$ as strong expression was detected in 7.7% of cases versus 57.7% of control group, moderate expression was detected

Table 1. Age distribution.

	Cases (N = 26)		Controls (N = 26)		t*	p value
	Mean	SD	Mean	SD		
Age (years)	27.77	6.50	27.58	5.38	0.12	0.91

*Student t test.

Table 2. Obstetric history.

		Cases (N = 26)		Controls (N = 26)		χ^{2*}	p value
		N	%	N	%		
Parity	0	4	15.4%	0	0.0%	5.82 FE	0.33
	1	3	11.5%	2	7.7%		
	2	4	15.4%	8	30.8%		
	3	5	19.2%	7	26.9%		
	4 or more	2	7.7%	2	7.7%		
	PG	8	30.8%	7	26.9%		
History of abortion	No	22	84.6%	26	100.0%	4.33 FE	0.11
	Yes	4	15.4%	0	0.0%		
History of CS	No	21	80.8%	20	76.9%	0.12	0.73
	Yes	5	19.2%	6	23.1%		
History of PE in previous pregnancy	No	24	92.3%	26	100.0%	2.08 FE	0.49
	Yes	2	7.7%	0	0.0%		

*Chi square test (FE: Fisher exact).

Table 3. GA at delivery.

	Cases (N = 26)		Controls (N = 26)		t*	p value
	Mean	SD	Mean	SD		
GA at delivery (weeks)	36.92	1.32	38.96	1.37	5.45	<0.001

*Student t test.

Table 4. Blood pressure.

	Cases (N = 26)		Controls (N = 26)		t*	p value
	Mean	SD	Mean	SD		
SBP	160.96	12.49	115.15	6.25	16.72	<0.001
DBP	104.81	10.72	73.46	8.10	11.90	<0.001

*Student t test.

Table 5. Route of delivery.

		Cases (N = 26)		Controls (N = 26)		χ^{2*}	p value
		N	%	N	%		
Delivery route	NVD	8	30.8%	17	65.4%	6.24	**0.01**
	LSCS	18	69.2%	9	34.6%		

*Chi square test (FE: Fisher exact).

Table 6. Amount of albumin in urine among cases.

		N	%
Albumin in urine	Nil	5	19.2%
	1	12	46.2%
	2	4	15.4%
	3	5	19.2%

Table 7. FOXP3 IHC expression.

		Cases (N = 26)		Controls (N = 26)		χ^{2*}	p value
		N	%	N	%		
FOXP3 IHC	Negative	6	23.1%	0	0.0%	20.25 FE	**<0.001**
	Weak	10	38.5%	3	11.5%		
	Moderate	8	30.8%	8	30.8%		
	Strong	2	7.7%	15	57.7%		

*Chi square test (FE: Fisher exact).

in 30.8% of cases versus 30.3% of control group, weak expression was detected in 38.5% of cases versus 11.5% of the control group, the negative expression was detected in 23.1% of cases versus 0% of control group (Table 7).

5. Discussion

In order to identify Tregs in the deciduas, Meister *et al.* (2022) performed immunohistochemical labelling of FoxP3 on 32 PE and 34 control placentas. Tregs are crucial participants in processes mediating immunological tolerance. They concurred with us and noted that there were considerably fewer FoxP3-positive cells in PE placentas (1.07±1.203, ranging from 0 to 3.67) compared to control placentas (1.80±1.497, ranging from 0 to 6.33) (p = 0.046) [10].

Chen *et al.* (2015) investigated the association between preeclampsia and Foxp3-924 (rs2232365) polymorphisms as well as changes in Foxp3 expression in the deciduas in preeclampsia patients. They agreed with us and stated that compared to healthy pregnant women, preeclampsia patients have significantly lower levels of Foxp3 expression in their placental tissue. This suggests that the decreased Foxp3 expression reduces the immunosuppressive function and re-

sults in an imbalance in maternal-fetal immune tolerance, which in turn causes preeclampsia. Foxp3 positive expression rates in the deciduas were substantially lower than those in the control group (86.67%, P 0.05) and were 51.52% in cases of mild preeclampsia and 28.57% in cases of severe preeclampsia. Foxp3-924G/G, G/A, and A/A genotype frequencies in preeclampsia patients were 0.1346, 0.4615, and 0.4038, respectively, and Foxp3-924A and Foxp3-924G frequencies were 0.6346 and 0.3654, respectively [11].

The possible impact of Foxp3 polymorphism on preeclampsia (PE) susceptibility was assessed by Chen *et al.* in 2013. According to their findings, PE may develop because PE patients have reduced immune suppression functions. In comparison to the normal control group (63.33%), the positive rate of Foxp3 expression in PE (26.67%) was significantly lower. In comparison to controls, PE patients had considerably lower frequencies of the Foxp3-6054 TT genotype. Foxp3-3279 genotypes between PE and control, as well as for the mutant allele, showed no discernible variation.

A relative maternal immune tolerance of the foetus is necessary for normal pregnancy.

PE develops from a pre-clinical stage to a clinical stage due to placental malfunction, which alters the maternal system and causes a maternal presentation of the disease.

In a healthy pregnancy, the foetus frequently diminishes the immune system's ability to fight off infection and increases its protective effects, but immune system modifications brought on by PE have negative side effects including greater foetal rejection and diminished immune protection.

Our case-control study's findings corroborate those of Gholami *et al.* (2017) and suggest that PE and reduced IHC expression of FOXP3 may be related.

Poor FOXP3 expression may result in lower Treg cell suppression, which eventually contributes to the development of PE.

Other pregnancy problems such as recurrent spontaneous abortion, unexplained infertility, and inability to implant are also linked to the downregulation of FOXP3.

Chen *et al.* (2015) sought to identify alterations in Foxp3 expression in the deciduas in preeclampsia patients and explore the relationship between preeclampsia and Foxp3-IHC polymorphisms. They reported that the IHC expression level of Foxp3 in the placental tissue of preeclampsia patients was significantly lower than that in normal pregnant women, indicating that decreased Foxp3 expression reduces the immunosuppressive function and causes an imbalance of immune tolerance between maternal and foetal to cause preeclampsia [11].

Lastly, Metz *et al.* (2012) dissented from us and came to the opposite conclusion, concluding that preeclampsia is not related to FOXP3 gene IHC, which has been linked to other autoimmune illnesses. They wanted to know if preeclampsia was linked to IHC in the FOXP3 gene. 120 preeclamptic women were compared to 120 healthy, normotensive controls in a case-control study. The FOXP3

gene's genetic variations (single nucleotide polymorphisms and microsatellites) were examined. Studies of the FOXP3 gene in various autoimmune diseases were taken into consideration while choosing polymorphisms. Between cases and controls, there were no variations in the genotypes or allele frequencies of the single nucleotide polymorphisms. Compared to controls, patients had a lower frequency of the FOXP3 GT microsatellite allele at 266 bp (1.0% vs. 5.2%). However, after repeated comparisons were taken into account, this was no longer significant.

6. Conclusion

In comparison to the healthy control group, preeclampsia patients had statistically significantly lower FOXP3 IHC expression, and FOXP3 polymorphism was associated with the development of PE. Our findings can serve as a roadmap for functional investigations and more in-depth statistical analyses. Preeclampsia patients had lower gestational ages at birth and a higher proportion of caesarean sections than the healthy control group, which was statistically significant. When it came to maternal age, parity, history of prior abortions, caesarean sections, and preeclampsia, there was no discernible difference across study groups.

7. Limitation of Study

There are some limitations of the study. Although the sample size of our study was large enough to draw statistically significant conclusions, the population does not represent the entire Egyptian population because regional variation plays crucial role in preeclampsia. The nature of the study was very difficult.

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Ethical Approval

The study was approved by the Institutional Ethics Committee.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Mol, B.W., Roberts, C.T., Thangaratinam, S., Magee, L.A., De Groot, C.J. and Hofmeyr, G.J. (2016) Pre-Eeclampsia. *The Lancet*, **387**, 999-1011. https://doi.org/10.1016/S0140-6736(15)00070-7

[2] Jahan, P., Sreenivasagari, R., Goudi, D., Komaravalli, P.L. and Ishaq, M. (2013) Role of Foxp3 Gene in Maternal Susceptibility to Pre-Eclampsia—A Study from South India. *Scandinavian Journal of Immunology,* **77**, 104-108. https://doi.org/10.1111/j.1365-3083.2012.02760.x

[3] Phipps, E.A., Thadhani, R., Benzing, T. and Karumanchi, S.A. (2019) Pre-Eclampsia: Pathogenesis, Novel Diagnostics and Therapies. *Nature Reviews Nephrology*, **15**, 275-289. https://doi.org/10.1038/s41581-019-0119-6

[4] Norouzian, M., Rahimzadeh, M., Rajaee, M., Arabpour, F. and Naderi, N. (2016) FoxP3 Gene Promoter Polymorphism Affects Susceptibility to Preeclampsia. *Human Immunology*, **77**, 1232-1238. https://doi.org/10.1016/j.humimm.2016.09.001

[5] Ghosh, S., Roy-Chowdhuri, S., Kang, K., Im, S.H. and Rudra, D. (2018) The Transcription Factor Foxp1 Preserves Integrity of an Active Foxp3 Locus in Extrathymic Treg Cells. *Nature Communications*, **9**, Article No. 4473. https://doi.org/10.1038/s41467-018-07018-y

[6] Yang, J., Wei, P., Barbi, J., Huang, Q., Yang, E., Bai, Y., Nie, J., Gao, Y., Tao, J., Lu, Y. and Xie, C. (2020) The Deubiquitinase USP44 Promotes Treg Function during Inflammation by Preventing FOXP3 Degradation. *EMBO Reports*, **21**, e50308. https://doi.org/10.15252/embr.202050308

[7] Konopacki, C., Pritykin, Y., Rubtsov, Y., Leslie, C.S. and Rudensky, A.Y. (2019) Transcription Factor Foxp1 Regulates Foxp3 Chromatin Binding and Coordinates Regulatory T Cell Function. *Nature Immunology*, **20**, 232-242. https://doi.org/10.1038/s41590-018-0291-z

[8] Jianjun, Z., Yali, H., Zhiqun, W., Mingming, Z. and Xia, Z. (2010) Imbalance of T-Cell Transcription Factors Contributes to the Th1 Type Immunity Predominant in Pre-Eclampsia. *American Journal of Reproductive Immunology*, **63**, 38-45. https://doi.org/10.1111/j.1600-0897.2009.00763.x

[9] Metz, T.D., Nelson, L.M., Stoddard, G.J. and Silver, R.M. (2012) FOXP3 Gene Polymorphisms in Preeclampsia. *American Journal of Obstetrics and Gynecology*, **206**, e1-165.e6 https://doi.org/10.1016/j.ajog.2011.09.005

[10] Brown, M., Magee, L., Kenny, L., *et al.* (2018) International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. *Hypertension*, **72**, 24-43. https://doi.org/10.1161/HYPERTENSIONAHA.117.10803

[11] Chen, X., Gan, T., Liao, Z., *et al.* (2013) *Foxp3* (−/ATT) Polymorphism Contributes to the Susceptibility of Preeclampsia. *PLOS ONE*, **8**, e59696. https://doi.org/10.1371/journal.pone.0059696