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WIPI-2 protein expression increases in the placentas of patients with preeclampsia

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Abstract

Objective: This study aimed to investigate WD-repeat protein interacting with phosphoinositides-2 (WIPI-2) protein expression in the placentas of preeclamptic patients by immunohistochemical method.

Methods: Placentas of 25 healthy normotensive women and 25 women with preeclampsia were enrolled in this study. Placental samples were fixed in zinc-formalin and further processed for paraffin wax tissue embedding. Demographic and laboratory parameters of patients were recorded. WIPI-2 immune activity was analyzed in the placental section with immunohistochemistry.

Results: There was a high number of placental lesions such as vascular congestion, fibrinoid accumulation, hemorrhage, and villous degeneration in preeclamptic placentas compared to normotensive placentas. WIPI-2 immune reaction was significantly increased in preeclamptic placentas when compared to normotensive placentas. WIPI-2 expression was predominantly observed in the placental villi.

Conclusion: Autophagy is a process that occurs in normal placentation. However, dysfunction in the autophagic mechanism is observed in the placentas of preeclamptic patients and WIPI-2 expression could be used to show autophagy.

Keywords: Preeclampsia, WIPI-2 expression, endothelial dysfunction, hypoxia, autophagy

Introduction

Preeclampsia is a pregnancy complication characterized by new-onset hypertension and proteinuria after the 20th week of pregnancy or postpartum in a normotensive patient. Preeclampsia can also be seen in the form of hypertension (with and without proteinuria) accompanying end-organ dysfunction. It is globally one of the main causes of maternal mortality. The only recognized treatment for preeclampsia is delivery of the placenta, which frequently ends in the premature delivery of the fetus, exposing the infant to the immediate risks of prematurity and additional risks for metabolic disorders and chronic diseases at any age of the child.[1-3]

Preeclampsia has a complex pathophysiology and several theories have been proposed. One is the vascular endothelial dysfunction related to spiral artery modeling that led to placental hypoxia, an increase in oxidative stress, and maternal systemic inflammation.[4-6] Autophagy is a cellular process that normally takes place in placentation. It is critical for fetal development and cellular homeostasis.[7] However, many diseases can reverse this phenomenon by interfering autophagy mechanism. Recent studies showed that immune response increases the rate of autophagy. Inhibition of autophagy reduced the preeclamptic symptoms (blood pressure, proteinuria) in a preeclampsia animal model.[8,9]

WD-repeat protein interacting with phosphoinositides-2 (WIPI-2) is a protein that is highly expressed in cancer cells. Several studies showed that its expression increases in other human tissues. Hypoxia causes upre-

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gulation of WIPI-2.[10] Placental hypoxia and WIPI-2 relation may be a clue for the pathogenesis of preeclampsia. The present study investigated the expression levels of WIPI-2 protein by an immunohistochemical technique that may a role in the pathogenesis of preeclampsia.

Methods

Ethical approval was taken from Dicle University Medical Faculty, Ethics Committee for, Non-interventional Studies (Date: 06/01/2023, No:09). 25 normotensive women and 25 preeclamptic women without any systemic disorders were enrolled. Preeclampsia was diagnosed based on the ACOG criteria.[11] Demographic and laboratory parameters were recorded for each patient. Blood samples were collected at admission to the hospital before labor and further analyzed for laboratory parameters. All patients were informed and accepted to participate in the study. An informed consent form was signed by all participants.

The exclusion criteria included the presence of eclampsia, HELLP syndrome, fetal congenital abnormalities, multiple pregnancies, premature rupture of amniotic membranes, intrauterine fetal demise, maternal infections, and co-existing maternal systemic diseases, including collagen vascular disorder, diabetes mellitus, and malignant tumor.

Histological tissue processing

Placentas were acquired from the Obstetrics Department of Dicle University Faculty of Medicine. Placental samples for histologic analysis were excised and further analyzed for histological evaluation. Samples were fixed in zinc-formalin and dehydrated through grading alcohol series, immersed in xylene, and incubated in paraffin wax. 5 µm sections were cut from paraffin blocks and stained for immune staining of WIPI-2.

Immunohistochemical examination

Placental sections were dewaxed, hydrated in grading alcohol series, and washed in distilled water. Slides were allowed to react with 3% hydrogen peroxide (H2O2) to block endogen peroxidase activity. After washing in PBS, sections were immersed in a blocking solution. Without washing, the previous solution was drained and sections were incubated with WIPI2 (catalog no: A38452, AFG Scientific, Northbrook, US) overnight at + 4°C. Sections were biotinylated and then reacted with streptavidin peroxidase for 15 minutes. After PBS washing, diaminobenzidine (DAB) chromogen was used as a chromogen to observe color change. The reactions were stopped with PBS solution and sections were counter-stained with hematoxylin dye. Slides were imaged with Zeiss Imager A2 light microscope. All images were processed and quantified using ImageJ software.

ImageJ analysis

The staining intensity of WIPI2 expression was measured by Image J software (version 1.53, http://imagej.nih.gov/ij). Measurement was calculated by the method of Crowe et al..[12] The graphical illustration of Table 2 is shown in Figure 1.

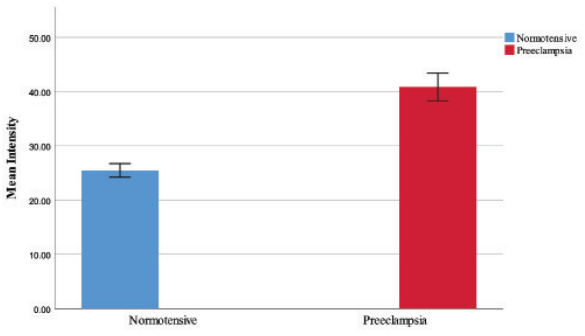

Fig 1. Mean intensity of WIPI-2 signal (expression)

Quantification was recorded by analyzing ten fields from each specimen per group. In specimens, the brown color stands for the positive expression of the antibody of interest while the blue color represents a negative expression of the antibody of interest. Signal intensity (expression) from a field was calculated by dividing the intensity of the antibody of interest to the whole area of the specimen. A value for staining area/whole area was calculated for each specimen from ten fields. An average value was measured for groups and analyzed for semi-quantitative immunohistochemistry scoring.

Statistical analysis

Statistical analysis was done using the IBM SPSS 25.0 software (IBM, Armonk, New York, US). The data were recorded as mean ± standard deviation. Statistical distribution was evaluated with the Shapiro-Wilk test. Binary group comparisons were done with the Mann-Whitney U test or Student's t-test. Significance was considered for p-values <0.05. The number of patients for each group was calculated by G Power analysis (version 3.1). Cohen's criteria were defined according to the study of Alviggi et al..[13]

Results

The demographic, clinical characteristics and laboratory values of the participants are shown in Table 1.

Histological analysis

Hematoxylin eosin staining of placental sections was

presented in Figure 2. The placental sections of normotensive patients showed normal histological appearance with no pathology (Figures 2a and 2b). Tertiary villi with fetal capillaries and trophoblast cells were evident. Sections of preeclampsia showed hemorrhage in the intervillous space with leukocyte infiltration, fibrinoid accumulation, and degenerated villi (Figures 2c and 2d).

Parameters	Normotensive group (n=25)	Preeclampsia group (n=25)	Significance (p-value)
Maternal age, years	28.3 ± 4.5	31.3 ± 5.2	0.104
Gravida, n	2.5 ± 1.1	2.3 ± 1.2	0.067
Parity, n	1.4 ± 1.1	1.0 ± 0.9	0.054
Systolic blood pressure, mmHg	110.1 ± 8.3	162.2 ± 15.3	**<0.001**
Diastolic blood pressure, mmHg	65.2 ± 6.2	99.8 ± 10.29	**<0.001**
24-hour proteinuria output	167.3 ± 65.2	1259 ± 325.6	**<0.001**
Alanine aminotransferase, U/L	15.3 ± 5.7	30.8 ± 12.7	0.383
Aspartate transaminase, U/L	23.7 ± 8.3	34.6 ± 11.2	0.173
Platelet count, 10^9/L	283.4 ± 76.2	165.8 ± 69.8	**<0.01**
Lactate dehydrogenase, U/L	307.3 ± 85.3	332.4 ± 98.3	0.412
Gestational week at birth	38.3 ± 1.4	35.4 ± 2.7	**0.030**

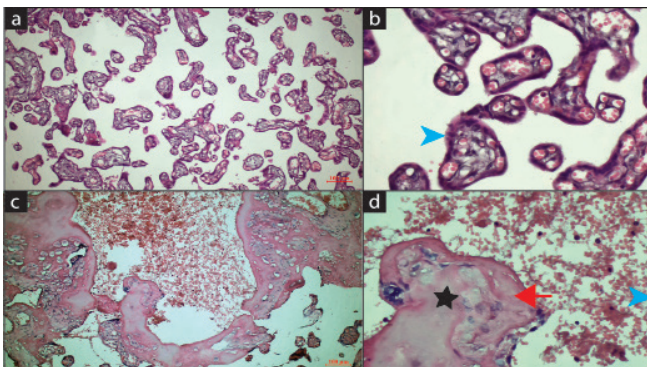

Fig 2. Hematoxylin-eosin staining of placental sections. a-b) Histologically normal villi (blue arrow) and capillaries (red arrow) in placentas of normotensive patients; c-d) Fibrin deposition (black star), intervillous hemorrhage with leukocytes (blue arrow), edema (red arrow) in preeclamptic placentas.

Figure 3 shows the immune reaction in the placentas of normotensive and preeclamptic patients. WIPI-2 expression was redundantly negative in the placenta of normotensive patients. The expression was negative in trophoblast cells (Figures 2a and 2b). Preeclamptic placentas reacted with WIPI-2 and expression was intense in trophoblast cells and villous connective tissue (Figures 3c and 3d).

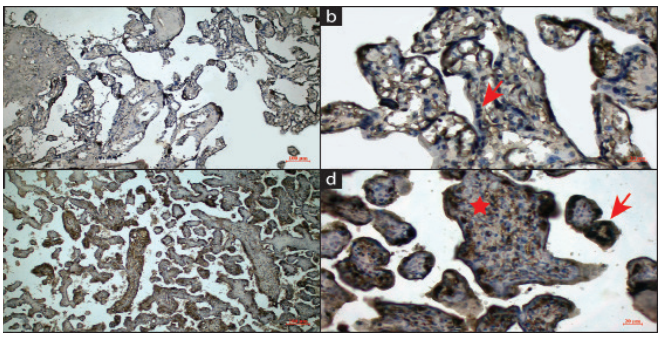

Fig 3. The immune activity of WIPI-2 protein in placental sections. a-b) Negative expression in villi (blue arrow) in placentas of normotensive patients; c-d) Intense positive WIPI-2 expression in villi (red arrow) and in villous connective tissue (red star) in preeclamptic placentas

Discussion

Preeclampsia is a multisystemic disease that occurs after the 20th week of pregnancy and is characterized by hypertension and proteinuria. 90% of the cases end with a healthy pregnancy process, but 10% of them pose a high risk for perinatal morbidity and mortality.[14-16] Preeclamptic placentas showed high placental pathology. A review and meta-analysis study showed that lesions in placental villi and vascular structures in preeclamptic patients were 4-7 fold higher than in normotensive patients.[17] Ojha et al. found that the number of syncytial knots, areas of fibrinoid necrosis, hyalinization, and calcification were increased in the placentas of preeclamptic patients.[18] The findings of this study were consistent with previous studies. We showed that placental pathologies were increased in the preeclamptic group (Figure 1). These findings support the idea of abnormalities during placentation in preeclamptic patients.

Abnormal placentation and maternal vascular dysfunction are accepted theories in the pathogenesis of preeclampsia and the latter causes placental hypoxic cells. Ischemia induces the expression of many genes which may be involved in the pathogenesis of preeclampsia.[19,20] Autophagy is regulated by more than 40 genes that are highly conserved in all living organisms. Half of these genes have been preserved in human beings. Autophagy begins with the formation of an autophagosome that delivers material to be degraded to the lysosome. WIPI-2, the mammalian

homolog of the Yeast ATG18 gene, plays an important role in autophagy.[21] Placental autophagy activation is considered a normal process in a normal pregnancy, but it is known that this situation is impaired in preeclampsia. In a study, it was reported that reoxygenation of primary human trophoblast cells caused a decrease in the number of autophagosomes and autolysosomes ultrastructurally.[22] Clarkson-Townsend et al. investigated the transcripts of some genes in mouse placenta and stated that WIPI-2 expression decreased.[23] This study showed that WIPI-2 immune expression was relatively high in preeclamptic placentas compared to placentas of normotensive patients (Figures 2 and 3). The immunohistochemical findings of this study are consistent with previous studies related to preeclampsia. Autophagy is a usual process but it seems that this mechanism is deteriorated in preeclampsia. Semi-quantitative analysis by Image J also confirmed our immunohistochemical findings (Table 2).

	Normotensive group (n=25)	Preeclampsia group (n=25)	Significance (p-value)
WIPI-2 signal	25.49 ± 3.08	40.85 ± 6.15	**<0.001**

Autophagy is a usual process but it seems that this mechanism is deteriorated in preeclampsia. This study showed that WIPI-2, one of the regulators of autophagy genes, increased. However, the upregulation of WIPI-2 in the cells doesn't directly indicate autophagy is promoted.[24] The formation of autophagosomes depends on autophagy-related proteins and other WIPI proteins (WIPI-1, 3, and 4).[25] Zhao et al. found that the beta isoform of protein kinase C (inhibitor of autophagy) was downregulated in preeclamptic patients, showing that the autophagy mechanism is still unclear and still an area of interest.[26] Furthermore, autophagy activation requires immune activation during preeclampsia which recruits complex molecular interactions in preeclampsia.[27,28] This study investigated WIPI-2 expression immunohistochemically and showed that WIPI-2 (one of the autophagy regulators) level was upregulated. More studies are needed to elucidate the molecular mechanism of autophagy in preeclamptic patients with more experimental methods.

Conclusion

Placentation is the formation of the placenta and many cellular processes are involved in this event. Autophagy is another cellular process that takes place in placentation. However, in preeclamptic placentas, dysfunction of the autopathic process with vascular lesions could lead to the development of preeclampsia. This study showed that WIPI-2 expression could be used as a marker for the impairment of the autophagy mechanism in preeclamptic placentas.

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