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Abnormal levels of serum N-terminal propeptide of collagen type IV (PIVNP) in women one year after preeclampsia

Asparuh G. NIKOLOV[1,*], Nikola K. POPOVSKI [2]

[1]Department of Preclinical and Clinical Sciences, Faculty of Pharmacy, Medical University, Pleven, Bulgaria
[2]Department of Obstetrics and Gynecology, Medical University Hospital, Pleven, Bulgaria

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Abstract

Proteins of the extracellular matrix (ECM) play an important role in normal pregnancy and preeclampsia (PE). Type IV collagen (COL4) is a major ECM structural element, uniquely presented in basement membranes. The N-terminal propeptide of collagen type IV (PIVNP) is a marker of COL4 synthesis. In the present study, we determined serum PIVNP levels in women one year after PE and tested whether PIVNP is related to hypertension development after preeclamptic pregnancy. The current research examined 32 women one year after PE (mean age 25.3±6.3 years) and a control group of 20 women one year after normal pregnancy (mean age 25.6±5.6 years). The enzyme-linked immunosorbent assay (ELISA) was used to determine concentrations of PIVNP. We found that at 1 year after delivery, 38.46% of women who suffered PE had developed arterial hypertension (AH), and 5.77% had developed diabetes mellitus. Women who had normal pregnancies developed neither AH nor diabetes mellitus 1 year after delivery. The distribution of women who had developed AH after preeclamptic pregnancy was as follows: Grade I AH-7; Grade II AH-4; and Grade III AH- 2. Serum PIVNP levels in women one year after PE were statistically significantly lower than in women one year after normal pregnancy: 0.26 (0.1÷0.65) vs. 0.45 ng/ml (0.3÷0.6) (KW= 5.342; p=0.02). PIVNP showed a correlation with creatinine (r= -0.26; p=0.05). Hypertensive women one year after PE showed the lowest levels of serum PIVNP.Our data showed decreased levels of PIVNP in the sera of women one year after PE. This finding demonstrated altered COL4 turnover after preeclamptic pregnancy. The diminished COL4 synthesis might play an important role in persistent vascular wall injury and dysfunction postpartum. We suggest that PIVNP might be involved in the pathogenic mechanisms determining the development of hypertension postpartum.

Keywords: arterial hypertension, collagen IV turnover, extracellular matrix, history of preeclampsia, N-terminal propeptide of collagen type IV

1. Introduction

Preeclampsia (PE) is a critical complication of pregnancy that develops after the 20th gestational week. It is described by the occurrence of new-onset hypertension (140/90 mmHg) and either proteinuria (0.3g in a 24-hour urine sample) or end-organ dysfunction (1). It is an important cause of maternal and perinatal morbidity and mortality (2,3). Patients with PE have an increased long-term risk of developing cardiovascular disease (CVD) (4). There is growing evidence that arterial hypertension (AH) is associated with decreased degradation of connective tissue proteins (5,6). However, little is known about the changes in the composition of the extracellular matrix (ECM), particularly Type IV collagen (COL4) after preeclampsia.

ECM is considered pivotal for maintaining tissue structure and modulating of cell differentiation (7). An important factor in the development of vascular wall alterations is the degradation of the extracellular matrix‘s major protein, collagen (8). COL4 is uniquely present in basement membranes of arteries and represents their predominant structural component (9,10). The uterine wall consists mainly of COL1 and COL3 (11). On the other hand, COL4 is a major component of arterial vasculature (12).

ECM plays a crucial role in normal pregnancy. It has been reported that ECM might modulate trophoblast invasion and contribute to the remodeling of the decidua at the maternal-fetal interface (13). Therefore, it can be concluded that the extracellular matrix of the uterus, placenta, and vasculature breaks down and remodels during physiological pregnancy (14,15). During preeclampsia, the uterine and spiral arteries' ECM metabolism has been found to be altered. Collagen metabolism is shifted, and the delicate balance between synthesis and degradation is disturbed (16). These processes are characterized by impaired collagen turnover, which might affect the remodeling of the uterine ECM and spiral arteries. Arterial vessels' collagen structure has been shown to be disturbed in women with PE. It is, therefore, possible that COL4 turnover is also affected. Hence, markers of COL4 turnover could be found in the sera of patients with PE.

Very few studies have investigated COL4 turnover in preeclampsia. Despite that, a consensus exists that marker of collagen degradation cannot be used as preeclampsia predictor biomarkers (17). Scientific efforts should focus on collagen

*Correspondence: a_nicoloff@yahoo.com

synthesis markers- the collagen propeptides. In turn, the N-terminal propeptide of collagen type IV (PIVNP) has been reported as an important indicator of COL4 synthesis (18-19). Interestingly, changes in indicators of collagen synthesis and particularly PIVNP in preeclamptic women 1 year after delivery have not been explored yet. We studied PE patients one year later, because previous investigations focused on this pregnancy complication (20-21) have reported current time frame to be suitable for assessment of changes in serum markers and clinical parameters like blood pressure. This time point (1 year after delivery) is neither too early in the healing phase of the illness, nor too later in the postpartum period and allows alterations to be detected.

This study aimed to: (1) determine circulating PIVNP levels in the sera of women one year after preeclampsia and normal pregnancy; and (2) investigate the possible role of PIVNP in the development of hypertension in previously preeclamptic women.

2. Material and methods

2.1. Study design

The currentresearch was a longitudinal study.

2.2. Subjects

The current study included women who had preeclampsia symptoms 1 year ago and met the ACOG (2019) criteria: PE was defined as complication of pregnancy after 20 weeks of gestation,described by the occurrence of new-onset hypertension (140/90 mmHg) and either proteinuria (0.3g in a 24-hour urine sample) or end-organ dysfunction (1). All subjects signed and informed consent form to take part in the investigation and maintained a regular diet and exercise routine throughout the research. Women who had diabetes mellitus, renal and heart disease, signs of chorioamnionitis, or the presence of a fetus with a chromosomal abnormality were excluded. All patients were residing in the Clinic of Obstetrics and Gynecology, University Hospital "Georgi Stranski" Pleven one year ago.

The present study was part of a university scientific project under the national Program “Young Scientists and Postdoctoral Students-2“, approved by the Ethics Committee of the Medical University of Pleven (Protocol N70/2023). All participants signed informed consent. The study procedures were consistent with all guidelines for ethical standards of the responsible committee on human experimentation, along with the Helsinki Declaration of 1975, as revised in 2000. A sample of subjects was taken from February to March 2023 for the purposes of the present investigation. The study group consisted of 32 women one year after preeclampsia (mean age 25.3±6.3 years) and a control group of 20 women one year after normotensive pregnancy (mean age 25.6±5.6 years).

2.3. ELISA

For the purpose of the current investigation, an enzyme-linked immunosorbent assay (ELISA) was applied to determine PIVNP levels in serum samples. The following ELISA kit was used (RJ-HUFI02977 Human N-terminal Propeptide of Collagen Alpha-1 (IV) Chain / PIVNP ELISA Kit, AssayGenie, Dublin, Ireland), according to the manufacturer’s instructions.

2.4. Blood pressure

The arterial blood pressure was assessed by a standard aneroid sphygmomanometer, to the nearest 2 mmHg, in the dominant arm after at least 10 minutes of rest in the supine position. Blood pressure measuring was determined by the Riester blood pressure measuring tool, Type Precisa® N, 64 mm aluminum, single-tube, cotton hook cuff, adult, No. 1362–104.

2.5. Statistical analyses

In order to analyze the research data, following computer programs were used: Excel (Microsoft Corporation, Redmond, WA), SPSS, and Statgraphics Plus (Manugistics, Rockville, MD) for Windows. The level of significance was determined as ($p<0.05$). Stnd. Skewness and Stnd. Kurtosis tests were used to check the normality of distribution and equality of variances. To discover significant differences between groups, the Student's t-test and ANOVA with mean±SD was used in cases with normal distribution (LSD, Tukey HSD, Scheffe, Bonferroni, Newman-Keuls, Duncan). $\chi 2$ and Kruskal-Wallis H test with median (M) value were used in cases with a different normal distribution, together with the first and third quartiles Q1 and Q3 (twenty-fifth and seventy-fifth percentiles P25 and 75P). A Pearson type of correlation was used.

3. Results

Clinical data of women who had preeclampsia and women who had normotensive pregnancy at 1 year after delivery are presented in Table 1.

We found that at 1 year after delivery, 38.46% of women who suffered PE had developed arterial hypertension, and 5.77% had developed diabetes mellitus. Women who had normal pregnancies developed neither hypertension nor diabetes mellitus 1 year after delivery. The distribution of women who had developed AH after preeclamptic pregnancy was as follows: Grade I AH- 7; Grade II AH-4; and Grade III AH- 2 women (Fig. 1). Serum PIVNP levels in women one year after PE were statistically significantly lower than in women one year after normal pregnancy: 0.26 (0.1÷0.65) vs. 0.45 ng/ml (0.3÷0.6) (KW= 5.342; p=0.02) (Table 2) (Fig. 2). Hypertensive women one year after PE showed lower levels of PIVNP than normotensive women one year after PE, but not significantly: 0.22 (0.1÷0.4) vs. 0.25 ng/ml (0.1÷0.8) ($p>0.05$). PIVNP showed a correlation with creatinine ($r= -0.26$; $p=0.05$).

Table 1. Clinical data of women one year after preeclampsia and one year after normal pregnancy

	Women one year after normal pregnancy	Women one year after preeclampsia	P
Age	25.6±5.6	25.3±6.3	p>0.05
BMI	26.9±4.38	30.5±6.9*	P=0.03*
SBP (mmHg)	115±7.6	135.6±15.6*	p<0.001*
DBP (mmHg)	75±7.07	86.9±9.22*	p<0.001*
Past history ofPE	0/20	7/32	
Family history of AH	1/20	14/32	
AH before pregnancy	0/20	5/32	
PP	40.8±6.9	58.2±16.8*	P=0.001*
MAP	88.5±8.75	121.7±13.4*	P=0.001*
Urea	2.96±0.78	3.75±1.63*	p=0.01*
Creatinine	75.78±14.45	73.33±15.33	p>0.05
Uric acid	205.6±40.2	326.8±105.93*	P=0.001*
TCL	3.86±0.94	4.97±1.27*	P=0.001*
LDL	2.48±0.62	3.27±1.23*	P=0.01*
HDL	1.92±0.48	1.44±0.76*	P=0.02*
TG	1.35±0.37	2.12±1.01	P=0.002
AH GRADE I	0/20	7/32	
AH GRADE II	0/20	4/32	
AH GRADE III	0/20	2/32	
T2DM	0/20	3/32	
Number	(n=20)	(n=32)	

BMI- body mass index; SBP- systolic blood pressure; DBP- diastolic blood pressure; PE-preeclampsia; AH-arterial hypertension; T2DM- type 2 diabetes mellitus; PP- pulse pressure; MAP- mean arterial pressure; HDL- high density lipoprotein cholesterol; LDL- low density lipoprotein cholesterol; TCL- total cholesterol; TG- tryglicerides. Data are shown as the mean±SD; *p<0.05

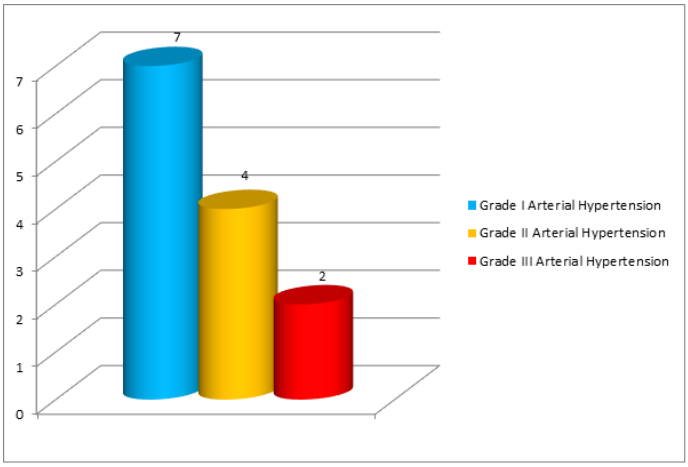

Fig. 1. Distribution of women who had developed hypertension one after preeclamptic pregnancy

7 women developed Grade I AH, 4 women developed Grade II AH and 2 women developed Grade III AH after preeclamptic pregnancy.

Table 2. Serum levels of PIVNP in women one year after preeclampsia and one year after normal pregnancy

	Women one year after normal pregnancy	Women one year after preeclampsia	P
PIVNP (ng/ml)	0.45 (0.3÷0.6)	0.26 (0.1÷0.65)	0.02

PIVNP- Human N-terminal propeptide of Collagen IV; Data are expressed as median (interquartile range)

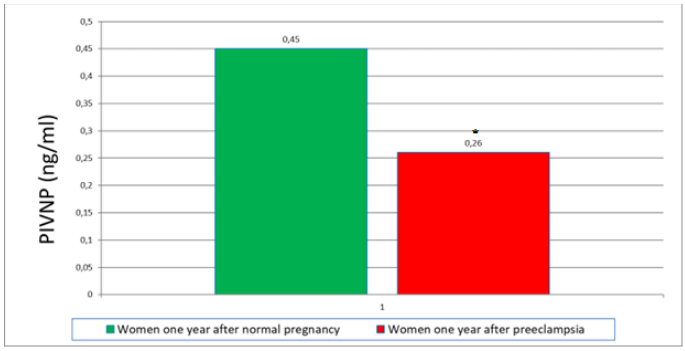

Fig. 2. Serum PIVNP levels in in women one year after preeclampsia and one year after normal pregnancy determined by ELISA

Serum PIVNP levels in women one year after PE were statistically significantly lower than in women one year after normal pregnancy 0.26 (0.1÷0.65) vs. 0.45 ng/ml (0.3÷0.6) (p=0.02). Values were presented as mean±SD; *P<0.05 compared with women one year after normal pregnancy.

4. Discussion

The primary component of basement membranes is COL4. It also supports the underlying endothelial cells. Therefore, COL4 is essential for the endothelial cells' function. Endothelial cells lie on and adhere to the basement membrane, a thin sheet of extracellular matrix (22–26). COL4 interactions with endothelial cells are important for maintaining endothelial cell function. "COL4 is also essential for cell adhesion and cell-matrix communication and plays an important role in maintaining endothelial cell function" (27). Abnormal changes in COL4 are likely to shift its interactions with endothelial cells and basement membrane components. These processes can lead to impaired basement membrane structure and endothelial cell dysfunction.

COL4 is only found in basement membranes, where it is their main structural constituent. In fact, the main component of the basement membrane is the network forming COL4, which comprises 50% of the basement membrane. Hence, COL4 is the most abundant collagen in basement membranes and is considered to be responsible for their mechanical stability (28). ECM remodeling plays an important role in the development and progression of AH. By means of vascular wall structural and functional alterations, ECM is involved in the pathological process of hypertension. AH is associated with

increased connective tissue proteins' degradation, loss of elasticity, elevated rigidity of the arterial wall, and an abnormal ratio of collagen to elastin. Interestingly, there is limited data in the literature about the ECM changes, and particularly COL4, after preeclampsia

One of the first examinations of COL4 in pregnancy was performed by Ogawa H. et al. (1994) when they localized COL3 and COL4 in the placenta by immunohistochemistry and measured by radioimmunoassay other collagen-related substances such as the amnio-terminal peptide of type III procollagen (P-III-P) and type IV collagen 7S domain (7S) in the serum of pregnant women. The authors used immunohistochemical techniques and studied the localization of COL3 and COL4 in normal and toxemic placentas. A special focus of interest were maternal serum levels of COL3 procollagen peptide and COL4 collagen 7S domain (7S) in non-pregnant women, normal term women, and cases of toxemia in pregnancy. "Immunohistochemical studies revealed that type III collagen exists in the connective tissues composing the villous core and type IV collagen in the basement membranes of trophoblast cells and fetal vascular elements. Even in normal-appearing toxemic placenta, the amount of type III and IV collagen appeared to be increased compared with that in normal-term placenta, but the amount of COL3 and COL4 appeared to be decreased in the necrotized chorionic villi of severe toxemia" (29). The results provided evidence that collagen-related substance levels in toxemic pregnancy were much higher than those in normal term pregnancy. The authors concluded that their data "support type III procollagen and 7S in maternal serum flow from the necrotized chorionic villi into the intervillous space and that these measurements are significant indicators of placental damage caused by toxemia in pregnancy" (29).

In the same year, Furuhashi et al. (1994) investigated serum COL4 and laminin levels in preeclampsia. The authors measured COL4 levels in the maternal serum of preeclamptic and normal pregnant women by radioimmunoassay. They found significantly higher serum COL4 levels in patients with PE than those in the normal pregnant group. Of note, in the preliminary investigations, when studying the changes in COL4 levels during gestation, they found a significant difference in the COL4 levels between the early gestational week and the late gestational week. These findings indicated that the serum COL4 levels of all pregnant groups were significantly higher than those of the non-pregnant controls. Moreover, the maternal serum COL4 levels of preeclamptic groups were significantly higher than those of the normal pregnant group. A correlation was found among the maternal serum COL4 levels in each period of gestation. The authors concluded that "COL4 may have an important role in the maintenance of pregnancy. These results suggest that there is early damage to endothelial cells in preeclampsia" (30).

In another study, Oefner et al. (2015) investigated COL4 at the fetal-maternal interface. Immunohistochemistry has been used by the researchers to examine the distribution of COL1, COL3, COL4, and COL6 in the endometrium and decidua during the menstrual cycle and the first trimester of pregnancy. Quantitative polymerase chain reaction and protein localization by immunohistochemistry were used to determine the expression of COL4 alpha chains during the reproductive cycle. In turn, of the COL4 structure of the placenta was examined using transmission electron microscopy. As for the expression of COL4 alpha chain NC1 domains and collagen receptors, it was localized by immunohistochemistry. The researchers found "a novel expression pattern of col-IV in the mesenchyme of placental villi as a three-dimensional network. NC1 domains of col-IV alpha chains are known to regulate tumor cell migration, and the selective expression of these domains in decidua basalis compared to decidua parietalis was determined" (31). The authors concluded that COL4 is expressed in novel forms in the placenta. These results show that COL4 is not merely a structural protein providing tissue integrity but also plays an integral role in invasive trophoblast cell behavior at the site of implantation.

As mentioned above, ECM proteins play an important role in normal pregnancy and preeclampsia. Because it is very important to find characteristics of COL4 metabolism after preeclamptic pregnancy and its role in hypertension development, we studied COL4 turnover postpartum via measuring PIVNP, a biomarker of COL4 synthesis. The present research found that at 1 year after delivery, 38.46% of women who suffered PE developed arterial hypertension (AH), and 5.77% developed diabetes mellitus. Women who had normal pregnancies developed neither hypertension nor diabetes mellitus 1 year after delivery. Our data showed decreased levels of PIVNP in women one year after PE. This can be partially explained by the current knowledge that arterial hypertension is connected with diminished degradation of connective tissue proteins. In light of these understandings, we propose decreased production of COL4 postpartum, which may contribute to COL4 alteration in the basement membranes and impaired structure of arterial walls. This could be one of the pathways favoring the development of AH after delivery. The lowest levels of serum PIVNP in hypertensive women one year after PE also provide arguments supporting that hypothesis. Our findings demonstrated altered COL4 turnover after preeclamptic pregnancy. The diminished COL4 synthesis might play an important role in persistent vascular wall damage postpartum.

The current evidence demonstrated, for the first time, decreased serum PIVNP levels and altered COL4 turnover in women who had PE. Such abnormal changes, expressed by diminished COL4 synthesis, can contribute to the processes favoring persistent vascular wall damage after preeclamptic pregnancy. We suggest that PIVNP might be involved in the mechanisms determining the development of hypertension

postpartum. Larger studies are warranted to clarify PIVNP's role in the ongoing vascular injury/dysfunction after preeclampsia and the pathogenic pathways of AH manifestation in previously PE women.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.N., N.P., Design: A.N., N.P., Data Collection or Processing: N.P., Analysis or Interpretation: A.N., N.P., Literature Search: N.P., Writing: A.N., N.P.

Ethical Statement

The Project was approved by the Ethics Committee of Medical University Pleven with protocal N70/2023.

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