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Endogenous maternal serum preimplantation factor levels in early-onset preeclamptic pregnancies

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ABSTRACT

Objective: Preimplantation factor (PIF) is a new peptide with many potential functions. We aimed to compare the maternal serum PIF levels among early preeclamptic patients with the healthy controls at the same gestational age.

Patients and Methods: Thirty-nine early-onset preeclamptic (< 34 gestational weeks) patients and 45 healthy expecting women were included to our study. Patients with or suspicion of any chronical maternal disease, gestational diabetes, twin pregnancies, fetal or placental anomalies or any other obstetric complication have been excluded. Competitive ELISA has been used to analyze the PIF levels in the collected samples. Gestational age, maternal age, gravida, parity, fetal growth, BMI, maternal weight and height, plasma PIF levels have been collected/measured and analyzed in both groups.

Results: The primary outcome of our study was that PIF was significantly higher in study group than the healthy controls (100.36 ± 41.92 vs. 83.14 ± 51.27. p=0.016).

Conclusion: Preimplantation factor levels were statistically higher in the study group. PIF levels might have a role in the progression and pathogenesis of the preeclamptic patients. Further studies with larger groups have to be planned and performed to reveal the real relation between PIF and preeclampsia.

Keywords: Preimplantation factor, Preeclampsia, Maternal serum

1. INTRODUCTION

Preeclampsia (PE) is a unique disease which occurs during pregnancy. The systemic inflammatory response, the pathogenesis of which stems from the implantation period, causes various clinical symptoms in each patient at the later stages of pregnancy [1]. Although, it has been the subject of many studies, the pathophysiology of PE is still poorly understood. Placental blood flow and remodeling of spiral arteries, imbalance in angiogenic factors and anti-angiogenic state, immune factors and inflammation, low oxygen tension, oxidative stress in gene expression have been the main focus of ongoing studies for years [2].

The preimplantation factor (PIF) is a recently discovered 15 amino acid peptide (MVRIKPGSANKPSDD) released from healthy embryos, thought to play a role in implantation and decidualisation [3]. The role of PIF in implantation has been studied in different experimental and animal models in various studies, and its synthetic version has also been produced as the synthetic preimplantation factor (sPIF). After its introduction by Barnea, as a novel peptide secreted as early as the 2-cell stage of viable mammalian pregnancies [4], its potential therapeutic effects and the role of its endogenous secretion have been evaluated in pregnancy related/reproductive diseases such as recurrent pregnancy loss, PE and endometriosis [5-7].

The preimplantation factor has proven effects on regulating local and systemic immunity, embryo adhesion – decidualisation improvement and trophoblast invasion enhancement [8]. The immunomodularity effects of PIF and its synthetic analog also brought up the question that it can be utilized in the treatment of different autoimmune diseases other than pregnancy related diseases [9]. Muller et al., studied the analogous sPIF which may promote neuro-protection in rodent models of experimental

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autoimmune encephalomyelitis and prevent perinatal brain injury [10].

Based on the data that PIF targets Kv1.3β – cortisone and causes a similar effect to cortisone according to Dr. Eytan Barnea, its role in the receptivity of a semi-allegenous or-in cases of donor pregnancies – allegenous embryo is better understood. It has been also shown by the same study that sPIF admission potentialized embryo protection and development by preventing oxidative stress and protein misfolding in embryo cultures [11].

Due to its various effects on immune-receptivity, PIF is found in the maternal circulation of bovine models by using chemiluminescent PIF ELISA. It was detected shortly after artificial implantation, by day 20, and correlated 100% with live pregnancy outcomes. On the other hand, its absence correlated at 100% with a non-pregnant status. By using anti-PIF monoclonal antibodies, Barnea et al., found that endogenous PIF is expressed mostly in the trophoblastic layer of bovine placenta [12]. The study conducted by Moindjie et al., in 2014, strengthened the theory that PIF is also secreted in human first-trimester placentas, to a lesser extent till the third-trimester human placentas. Their further achievement was the observation that PIF is localized in the syncytiotrofoblasts and extravillous-trofoblasts as evidence of the effects of PIF on the human placenta endocrine function [13].

In a more recent study, Dos Santos et al., evaluated the effects of sPIF on the endometrial stromal cell function, and found that it significantly upregulates the mRNA expression of IGFBP-1 and connexin-43, and prolactin secretion, which is essential in the decidualisation of human receptivity and a favorable pregnancy outcome [14].

Consequently, as current data strongly demonstrates, endogenous PIF secretion plays an important role in human placentation. PIF affects various steps, such as its role against oxidative stress, its promotion of implantation and trophoblast invasion and modulation of immune response. As critical as these factors are for implantation, they shape and cause PE pathogenesis and evaluation [7].

Considering its functions, the maternal serum levels of endogenous PIF levels and its response to preeclampsia is still not defined. Besides the potential therapeutical effect of synthetic PIF, endogenous PIF levels might play a role in the diagnosis and evaluation of preeclamptic patients. That is why; we conducted a case-controlled observational study to compare the endogenous PIF levels of early preeclamptic patients and healthy controls at the same gestational age. Our aim is to measure the PIF levels and define –if there is any – clinical correlation with the PE manifestation.

2. PATIENTS and METHODS

This study started with the approval of the local Clinical Research Ethics Committee, decision number 54 dated 10.03.2017. The study was carried out between March 2017 and October 2017 in our tertiary perinatology clinic.

Patient Selection

Preeclampsia has two major clinical presentations, early-onset and late-onset PE [15]. Its early-onset presentation is thought to be more related with the placental implantation and immunologic maladaptation [16]. Considering PIF's effect on placental invasion and immune modulation, we included patients who were diagnosed with early-onset PE in this study for study population. Early onset PE is defined as the onset of maternal hypertension and proteinuria after 20 and before 34 weeks of gestation (systolic blood pressure ≥140 mm Hg; diastolic blood pressure ≥90 mm Hg; spot urine protein/creatinine ratio ≥0.3).

The control group was chosen from patients with healthy pregnancies. All control cases had negative diabetes screening, normal amniotic fluid index and estimated fetal weight appropriate for gestational age.

Pregnant women diagnosed with polyhydramnios and anhydramnios, pregnant women diagnosed with diabetes mellitus and systemic medical disease were excluded from the study. After collection of samples, control cases were followed up until delivery. Samples of patients with any other obstetrical complication (preterm birth, antepartum bleeding etc.) or lost in follow-up were excluded.

The study was conducted in a tertiary center. Most of the cases diagnosed with PE included in the study were referred from an external center. Betamethasone (Celestone™) treatment was started in an external center in some of these cases who applied to our hospital. Although, at the beginning of the study it was planned to collect blood samples at the time of the patient's admission to the hospital, as the study progressed, it was decided to collect blood samples after the second dose of antenatal steroid so that all blood was collected at a similar time. Only 2 patients' samples were taken after the first dose, because they had emergency caesarean section for placental abruption.

In the literature review, no scientific article was found mentioning maternal serum PIF levels. Also, data on maternal serum PIF values in normal healthy pregnant women could not be obtained from the kit supplier regarding the examination of PIF values in maternal serum. (Elabscience Biotechnology Co™). Although, this makes the study unique, it makes it difficult to evaluate the results. After receiving the results of PIF values in our control group in pregnant women who were not diagnosed with PE, we assumed these values as normal and calculated 30% difference (positive or negative) with 80% power and p values smaller than 0.05 significant, we found out a sample size of 33 patients for each group (Confidence interval CI: 95%, Power 80%).

Informed consent was obtained from all participants.

Sample Collection

In order to ensure standardization in the study group, blood was taken from the patients in two separate 2 mL citrate tubes, after the betamethasone (Celestone™) doses were applied. Only 3 patients were delivered by emergency caesarean section due to the seriousness of their clinical situation. Blood sampling

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had to be done after 1 dose of betamethasone. Blood samples of the control group patients were taken in the outpatient clinics of our hospital. The collected blood samples were centrifuged at 1000 RPM for 30 minutes in a cold centrifuge device (-9 °Celsius). Plasma samples were collected and placed in 2 separate Eppendorf tubes. The tubes were labeled with the patient's name and case number, and then stored at – 40 °Celsius. In the hourly monitoring chart of the freezer temperature, it was observed that the temperature did not rise above – 39 °Celsius during the entire storage period.

Competitive PIF ELISA

Competitive ELISA (Elabscience Biotechnology Co™ USA) method was used to evaluate the collected plasma samples. 1000pg/mL standard solution included in the kit was diluted 15 minutes before the procedure and prepared in 8 different concentrations. 50-µL microliter samples taken from plasma samples stored in Eppendorf tubes were placed in a microplate. Biotinylated antibody was diluted to 50 µL microliters and added to all plates. It was then put in an incubator at 37 °C for 45 minutes and watched for antigen-antibody conjugation. After that automatic washing was made three times, 100 µL microliters of horse radish peroxide (HRP) (Horse Radish Peroxidase, Elabscience Biotechnology Co™ USA) conjugate was diluted and pipetted. It was put in the incubator again at 37 °C for 30 minutes. Afterwards, automatic washing was performed 5 times. After adding 90 microliters of substrate, samples were placed in an incubator at 37 °C for about 15 minutes to facilitate coloration reaction. Following the addition of the stop solution (Elabscience Biotechnology Co™) to stop the reaction, a spectrophotometric reading was performed at 450 nm wavelength. Values calculated according to standard chart (Figure 1) were reported as ng/mL (Nano grams per milliliters).

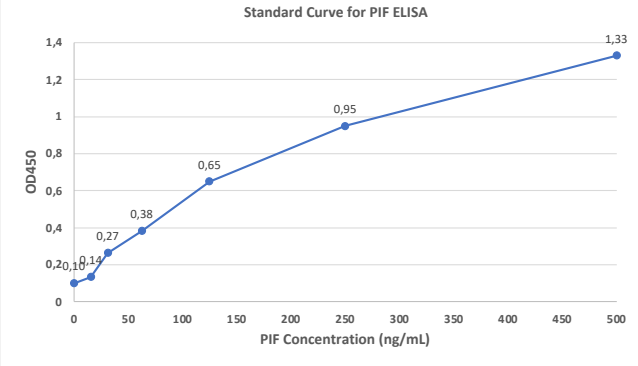

Figure 1. *Standard Curve for Competitive Preimplantation Factor (PIF) ELISA*

Statistical Analysis

Statistical analyses were performed using SPSS™ version 17.0 package software. The correlation between variables was analyzed using the Spearsman's rho correlation test. Descriptive analyses and categorical variables were given using the mean and standard deviation range. Variables not normally distributed were compared using the Mann-Whitney U test. For categorical variables, comparisons between groups were made using Chi-Square-Fisher tests. P-values less than 0.05 ($p<0.05$) were interpreted as statistically significant.

3. RESULTS

Considering the inclusion criteria in our study, 39 cases were included in the study group and 45 cases were included in the control group out of a total of 110 samples collected for evaluation. Age, body mass index (BMI), gravidity, parity, gestational age (GA), estimated fetal weight (EFW) and maternal serum PIF values were evaluated in all groups. Gestational age and EFW are given in correlated days for better explanation and statistical analyses. The results of these parameters are shown in Table I.

Table I. *Evaluation of Descriptive Variables between the groups*

Variable	Study (N1=39)	Control (N2=45)	p
Age	31.03 ± 6.46	29.29 ± 6.18	0.202
Gravida	2.44 ± 1.93	1.96 ± 1.22	0.41
Parity	1.05 ± 1.34	0.53 ± 0.79	0.088
BMI	*30.2 ± 3.88*	*27.2 ± 4.14*	*0.001[a]*
Gestational Age	214.64 ± 20.38	209.44 ± 24.1	0.348
EFW	205.46 ± 21.71	207.18 ± 22.94	0.713

a: Statistically significant, EFW: Estimated Fetal Weight in correlated days, BMI: Body Mass Index
All values are given as Mean ± Standard Deviation p-value less than 0.05 is considered to be statistically significant

Age, gravidity, parity, GA and EFW were similar between the preeclamptic patients and healthy controls (p=0.202, p=0.41, p= 0.088, p=0.348, p=0.713, respectively, all p>0.05).

The mean ± std BMI was 30.2 ± 3.88 kg/m2 in the study group (pregnant women diagnosed with PE) and 27.2 ± 4.14 kg/m2 in the control (healthy pregnant women) group and a statistically significant difference was found between the two groups in terms of BMI (p= 0.001 p< 0.05).

When the groups were evaluated in terms of maternal serum PIF values, mean maternal serum PIF value was statistically significantly higher in the study group (100.36 ± 41.92 vs. 83.14 ± 51.27 p=0.016) (Table II).

Table II. *Comparison of PIF Value Between Groups*

	Study (N1=39)	Control (N2=45)	
PIF	100.36 ± 41.92	83.14 ± 51.27	0.016[a]

a: Statistically significant, PIF: Preimplantation Factor

When the common complications of PE in the study group were evaluated; fetal growth restriction (FGR) was found in 15 cases (38.46%), abruptio placenta in 2 (5.1%), progression to HELLP syndrome in 3 (7.6%) cases. Doppler ultrasonography

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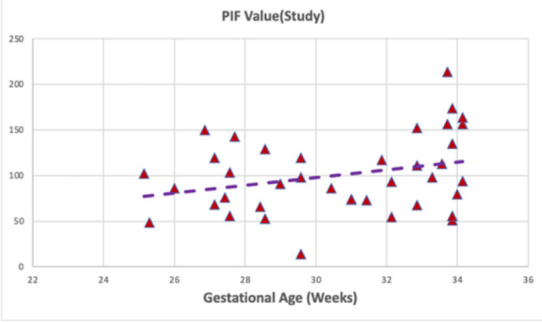
evaluation revealed bilateral uterine artery notch in 15 (7.6%) of 39 patients of cases (Table III).

Table III. *Distribution of Common Complications in Preeclampsia in the Study Group*

Variable	N	Percentage
FGR	15/39	38.5%
Ablatio Placenta	2/39	5.1%
HELLP	3/39	7.7%
Bilateral Uterine Artery Notch	15/39	38.5%

FGR: Fetal Growth Restriction, HELLP: Hemolysis Elevated Liver Enzymes Low Platelets Syndrome

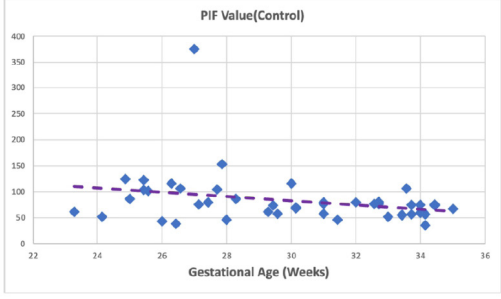
The distribution of maternal serum PIF values through GA in weeks for both groups is given in Figure 2 and 3. Cases diagnosed with PE are marked in the figure as triangles (Figure 2), and healthy controls are shown as diamonds (Figure 3). The interrupted line shows the trend through GA in weeks.



Interrupted line shows the trend in gestational age in weeks.

PIF: Preimplantation Factor

Figure II. *PIF Values of Preeclamptic Patients and Distribution through Gestation*



Interrupted line shows the trend in gestational age in weeks

PIF: Preimplantation Factor

Figure III. *PIF Values of Healthy Controls and Distribution through Gestation*

Correlation between maternal serum PIF and GA, EFW and maternal weight are shown in Table VI. In the analysis performed, there was no significant correlation between maternal serum PIF values and GA (p=0.097 p > 0.05) in the study group, whereas a significant and inverse (negative) correlation was observed between maternal serum PIF and GA in the control group. When all cases were evaluated, no statistically significant correlation was found between GA and maternal serum PIF (p=0.642 p > 0.05).

Table IV. *Correlation between PIF-Gestational Age, Fetal Weight and Maternal Weight*

		Study (N1=39)	Control (N2=45)	All groups (N=84)
Gestational Age	rho	0.27	-0.337	-0.051
	p	0.097	0.023[a]	0.642
EFW	rho	0.347	-0.35	0.047
	p	0.03[b]	0.018[a]	0.67
Maternal Weight	rho	-0.038	0.045	0.153
	p	0.817	0.767	0.166

a: Significant negative correlation, b: Significant positive correlation, EFW: Estimated Fetal Weight, p: p-value less than 0.05 is considered to be statistically significant rho: Spearman correlation between two variables

The correlation of maternal serum PIF value with EFW is also in Table IV. In the analysis performed, a significant and positive correlation was observed between maternal serum PIF values and EFW in the study group (p=0.03 p < 0.05), while a significant and inverse (negative) correlation was observed between maternal serum PIF and EFW in the control group as it was with GA. When all groups were evaluated, no statistically significant correlation was found (p=0.67 p > 0.05).

There was a predictable difference between the BMI values of the 2 groups. In the analysis made for a more valid explanation; there was no statistically significant correlation between maternal serum PIF values and BMI in the study group, control group and all cases (respectively p=0.817, p=0.767, p= 0.166, p>0.05 for all) (Table V).

When the maternal serum PIF values of the patients diagnosed with PE with and without bilateral notch in the uterine artery and/or FGR were compared, no statistical significant difference was found (p=1.00,p=0.2.respectively) (Table V).

Table V. *Correlation of Notch in Bilateral Uterine Artery Doppler and Fetal Growth Restriction with PIF Measurements*

	BUAN	N	Mean ±SD	P
PIF	+	15	102 ±43.67	1
	-	24	100 ±39.22	
	FGR	N	Mean ±SD	P
PIF	+	15	89.82 ±45.97	0.2
	-	24	106.94±38.71	

BUAN: Notch in Bilateral Uterine Artery Doppler, FGR: Fetal Growth Restriction

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4. DISCUSSION

The study was carried out in 2 groups consisting of healthy pregnant women and pregnant women diagnosed with early onset PE at similar gestational weeks. Considering demographic characteristics the study group was in the range of "obese" description and the mean value of the control group was in the "overweight" group [17]. Obesity alone is a risk factor for PE and obese women are at a higher risk for developing PE [18]. A local study among 986 pregnancies calculated the mean ± std for maternal weight in the third trimester 28.2 ± 4 and stated a rise of 4.6 in the BMI [19]. Pregnant women with PE tend to gain more weight cause of due to the oedema resulting from systemic vascular inflammation. The Norwegian fit for delivery trial shows a significant weight gain and a rise in the total body water in the third trimester in pregnancies diagnosed with PE when compared to the healthy ones [20]. These explain well why we have overweight patients even in the control group and correlates with our results in the controls.

Maternal serum levels of some pregnancy associated proteins and their associations with obesity have been studied before. Maternal serum Alpha-fetoprotein levels are lower in pregnant women with higher maternal weight than in those with normal maternal weight [21]. Obese pregnant women have lower maternal serum levels of human chorionic gonadotropin, pregnancy associated plasma protein-A, unconjugated estriol, most probably due to higher plasma volume and its dilutional effects [22]. For a better analysis we performed a correlation analysis between maternal weight and maternal serum PIF levels in all 84 cases. No significant correlation was found between maternal serum PIF value and maternal weight in both the study, control group and also in evaluation of all cases (respectively p=0.817, p=0.767, p= 0.166, p>0.05 for all) (Table IV). We found a significantly higher level of maternal serum PIF in the study group.

We found that maternal serum PIF levels become lower in healthy controls with advancing gestational age (r= – 0.337, p=0.023, p<0.05) (Table IV). This negative correlation is parallel to the findings of the study performed by Barnea et.al. [7]. In this study, correlation statistical analyses showed that the downward trend changed to an upward trend in the pregnant group diagnosed with PE. (r= 0.270, p=0.097, p>0.05) (Figure 2). This may support the altered maternal serum PIF level in PE group. The same shift in the downwards trend was calculated significantly between the correlation of EFW and PIF levels. (r= – 0.350, p=0.018, p<0.05 vs. r= 0.347, p=0.03, p<0.05) (Table 4).

Since, PIF plays a regulating role in the immunomodulation, its levels might be rising in PE due to the marked inflammation. Many pro-inflammation factors such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and Interleukin-6 are found to be elevated in PE [23]. This may be an explanation for the significantly high maternal serum PIF levels in the PE group. Simone et al., conducted a study on female Swiss mice, they modelled a lipopolysaccharide (LPS) induced placental inflammation and measured endogenous PIF levels and 22 cytokines/chemokines. They found that LPS induced inflammation causes a rise in placental PIF expression and results in increased levels of TNF-α (prime pro-inflammatory cytokine), growth related oncogene (GRO: neutrophil-attractive chemokine) and Interleukin – 18 (an inflammasome-related cytokine). This correlates with our findings in the PE group. They also add synthetic-PIF (s-PIF) and it was shown that s-PIF reversed the inflammatory response [24].

The one and only study conducted on human placenta samples related to PIF was carried out by Moindjie et al.[25]. By using PIF immunostaining, they compared placenta samples obtained from 8 normal pregnancies with samples of 8 pregnancies with diagnosis of FGR and 4 pregnancies with diagnosis of PE. They found lower PIF protein expression in normal third-trimester than in first-trimester placental villis. However, relative quantification of PIF by immunostaining indicated that PIF protein staining was lower in FGR and PE samples than in third-trimester control samples. This conflicts with our finding of higher maternal serum PIF levels in the PE group. The theoretical reason for that might be an altered/damaged trophoblastic layer due to inflammation which may raise the maternal serum levels, but lower the placental PIF protein content. This may even support our result, since we evaluated two very different samples. If we refer to the AFP example mentioned earlier, while its levels in fetal plasma and amniotic fluid starts to decline after the first trimester, the maternal serum levels of AFP keep increasing till the end of 35th week of gestation [26].

In our study, no statistically significant difference was found between FGR, BNUA and PIF levels. Considering the sample size of our study and the number of cases complicated with FGR, HELLP and abruptio placentae, it may have been insufficient to obtain a meaningful result.

The effect of corticosteroid injections on PIF levels remains unclear. There are no data concerning this issue. We collected the blood samples just after the antenatal steroid administration, but the maximum effects of betamethasone occur after 24-48 h of administration [27]. Secondly, considering other placental proteins like human placental lactogen and other placental steroids, antenatal steroid administration does not seem to effect placental lactogen levels even after 48 h of administration [28], and even lowers the levels of other placental steroids [29]. Considering its vital benefits [27], the antenatal steroid was not delayed in our patient group. An animal model might be useful for the further investigation of the possible effects of betamethasone on serum PIF levels.

Placental abruption occurred in 2 pregnant women included in the study group. The serum PIF values of these two pregnant women were found to be remarkably low. Their samples were collected just before emergency caesarean section, one patient had 14.1 ng/ml and the other one 48.7 ng/mL. These results are quite low considering the average serum PIF level of study group 100.4 ± 41.9. Since, there is no data considering the clearance and distribution of PIF in maternal serum, it is hard to interpret but given the fact that PIF is released by only living cells, this alteration can be regarded as an expected result [25].

Limitations

Our study has some limitations;

1. There was no research funding and the study was only supported by the research team, limiting the size and power of this study,
2. For Competitive ELISA (Elabscience Biotechnology Co™ USA) the commercial manufacturer did not provide large scale validation results, lowering the reliability of our results,
3. Finally, as mentioned in the methods, we collected our blood samples after the corticosteroid injections. Although, not considered important, since PIF targets Kv1.3β – cortisone target, this may have altered our results.

Conclusion

We found higher maternal serum PIF levels in the preeclampsia group. Despite its limitations, our study is the first work regarding PIF levels in maternal serum. Larger scaled and multicenter studies may reveal the true connection between PIF and pregnancy complications. This can also shed light on the studies on the use of sPIF in the prevention and treatment of obstetric complications.

Compliance with the Ethical Standards

Ethical Approval: This study was approved by the Zeynep Kamil Training and Research Hospital, Clinical Research Ethics Committee with the decision number 54 dated 10.03.2017. T

Financial Support: The authors have no relevant financial information to disclose.

Conflict of Interest: The authors have no potential conflicts of interest to declare.

Authors' Contributions: MAO and HAT: Concept and design of the study, MAO, HAT and EK: Acquisition and analysis of data, MAO, HAT and EK: Drafting the manuscript, tables and figures. All authors read and approved the final version of the article.

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