Acta Pharm. **75** (2025) 687–697
https://doi.org/10.2478/acph-2025-0040

Original research paper

Investigating preeclampsia risk factors and angiogenic profiles in low-screening area

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Accepted December 9, 2025
Published online December 10, 2025

ABSTRACT

Preeclampsia (PE) is a complex pregnancy disorder that may cause adverse outcomes for mother and baby. Combining risk factors with clinical, laboratory, and ultrasonographic data can help identify women at risk. This study investigated the relationship between maternal risk factors, soluble fms-like tyrosine kinase 1 (sFlt-1), placental growth factor (PlGF), their ratio, and pregnancy outcomes, involving 68 women with PE risk factors and 21 controls. There were no significant differences in the frequency of adverse outcomes (PE, foetal death, and infants with abnormal birth weight), sFlt-1, PlGF, the sFlt-1/PlGF ratio, or birth weight centiles between the PE-risk and control groups. The most frequently recorded high-risk factor was gestational diabetes mellitus, whereas moderate risk was a pre-pregnancy body mass index of over 30 kg m^{-2}. The most prominent difference was observed in the subgroups with gestational hypertension and first-time pregnant women as risk factors, with significantly higher sFlt-1/PlGF ratios compared to the control group. Combining multiple risk factors increased the sFlt-1/PlGF ratio compared to both the control group and the group with only one risk factor. The study documented PE risk factors and outcomes at a Croatian hospital where angiogenic markers are not routinely used in screening. Findings highlighted the importance of integrating PE screening into standard practice.

Keywords: preeclampsia, risk factors, screening

INTRODUCTION

Preeclampsia (PE) is a major complication of pregnancy characterised by *de novo* hypertension occurring after 20 weeks of gestation (1). PE affects between 2 and 4 % of pregnancies, resulting in a significant number of adverse maternal outcomes, including maternal hypertension, proteinuria, cerebral oedema and liver dysfunction, and a range of adverse foetal outcomes, including foetal growth restriction, preterm delivery and stillbirth (2). Various studies and clinical practice guidelines have identified multiple maternal

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risk factors associated with high and moderate risks of developing preeclampsia, which can be used to identify pregnant women likely to develop PE and take proactive measures. However, guidelines may vary in how they define risk factors. A personal or family history of preeclampsia, hypertension, diabetes mellitus, multifetal pregnancy, chronic kidney disease, autoimmune diseases with potential vascular complications, nulliparity, and obesity are among the most common risk factors associated with a higher risk of developing PE (3–5).

There is currently no treatment for PE, and delivery remains the only intervention that initiates the resolution of this disorder (7). Although there is no cure, screening, predicting, diagnosing, and monitoring the development of PE enable the implementation of preventive clinical management strategies. Current best practice remains the use of preventative therapy, the management of hypertension and other organ manifestations, and the identification of women at risk (7). PE is a complex disorder characterised by poor placental function and maternal endothelial dysfunction, with altered concentrations of angiogenic factors. Biochemical markers, soluble fms-like tyrosine kinase-1 (sFlt-1), and placental growth factor (PlGF), provide the strongest clinical evidence for diagnosing and monitoring hypertensive disorders in pregnancy. Measuring the concentrations of the anti-angiogenic factor sFlt-1 and the pro-angiogenic PlGF can help identify women at an increased risk of developing PE. Prediction of adverse outcomes may be further improved by combining angiogenic markers with other clinical, laboratory, and ultrasonographic data (8).

This study examined the relationship between risk factors for PE, the biochemical markers sFlt-1 and PlGF, and pregnancy outcomes at a tertiary-care hospital centre without general preeclampsia screening.

EXPERIMENTAL

Study design

The study was carried out on patients visiting the Department of Gynaecology and Obstetrics at Sestre Milosrdnice University Hospital Centre, Zagreb, Croatia. The hospital's Ethics Review Board approved the study. Women with singleton pregnancies were recruited during regular check-ups after the 20th week of pregnancy and who were expected to deliver in our institution. Women were divided into two groups: the PE-risk group and the control group. The inclusion criteria for the PE-risk group were at least one of the high-risk factors: chronic (CH) or gestational hypertension (GH), autoimmune disease (lupus or antiphospholipid syndrome), gestational diabetes mellitus (GDM), renal disease, or a history of PE. Any additional moderate risk factors were recorded, including nulliparity, age 40 years or older, prior placental abruption, prior stillbirth, prior foetal growth restriction, pre-pregnancy obesity (body mass index greater than 30 kg m^{-2}), family history of preeclampsia, pregnancy interval of more than 10 years, assisted reproductive technology (ART), and thrombophilia. The inclusion criteria for the control group were the absence of any high- or moderate-risk factors. The choice of inclusion criteria was defined as the most suitable for our local clinical practice, while keeping in mind the most frequent risk factors outlined in the international guidelines.

Recorded and measured data

For both groups, we recorded basic features (maternal age, blood pressure, pre-pregnancy body mass index (BMI), pregnancy weight gain and smoking) and additional information about pregnancy (gestational week, number of previous pregnancies and births). We recorded gestational age at birth, mode of delivery, birth weight and length, calculated centile (according to Nicolaides *et al.*), sex of the newborn and adverse outcomes (PE, foetal death, birth weight $\leq 5^{th}$ and $\geq 95^{th}$ percentile) (9). A blood sample was taken to measure the concentration of sFlt-1, PlGF and sFlt-1/PlGF ratio. Samples were collected in a fasting state by venipuncture into test tubes containing clot activator (4 mL Vacuette, Greiner Bio-One GmbH, Austria). After clotting, the blood samples were centrifuged at 2200 × g for 10 minutes. Measurements were carried out on a Roche cobas e801 analyser (Roche Diagnostics GmbH, Germany) using an electrochemiluminescent immunoassay with dedicated Roche Elecsys sFlt-1 and PlGF reagent kits. As the concentrations of sFlt-1 and PlGF change during pregnancy, and the study involved sampling from various stages, to compare concentrations of sFlt-1, PlGF, and the sFlt-1/PlGF ratio, each result was transformed to a multiple of the median (MoM) by dividing each patient's result by the median of the population result according to Verlohren *et al.* (10). The median values published by Verlohren *et al.* are based on samples from multiple European perinatal centres and were analysed using a Roche electrochemiluminescent immunoassay (10).

Definitions

The diagnosis of GDM was based on the International Association of Diabetes and Pregnancy Study Group criteria, as any glucose intolerance with onset or first recognition during pregnancy (11). GH and PE were defined according to the American College of Obstetricians and Gynaecologists criteria (3). GH was defined as systolic pressure ≥ 140 mmHg and/or diastolic pressure ≥ 90 mmHg on two separate occasions at least 4 hours apart after 20 weeks of gestation in a woman with previously normal blood pressure. PE was defined as GH with proteinuria or GH without proteinuria, accompanied by organ dysfunction. If hypertension was recognised before pregnancy or before the 20th week of gestation, it was classified as chronic hypertension.

Statistical analysis

All statistical analyses were performed using MedCalc software (MedCalc Software Ltd., Belgium). Nonparametric tests were employed due to the small sample size. For comparisons of quantitative variables between two groups, the Mann-Whitney U test was used. When multiple comparisons on the same dataset were made, Bonferroni correction was applied, and the adjusted significance level was set at $p < 0.05/n$, where n indicates the number of comparisons. Results are presented as medians with interquartile ranges in brackets. For comparisons of categorical variables between two groups, Fisher's exact test was utilised. Results are expressed as frequencies with percentages in brackets. For comparisons among three groups, the Kruskal-Wallis test was used. If the overall p-value was < 0.05, post hoc pairwise comparisons were carried out using the Conover test. A p-value < 0.05 was considered statistically significant for all tests.

RESULTS AND DISCUSSION

The study included 21 pregnant women in the control group and 68 in the PE-risk group. Maternal and neonatal characteristics for both groups are presented in Table I. The most common high-risk factors were GDM (n = 46), followed by GH (n = 25), a personal history of PE (n = 8), and autoimmune disease (antiphospholipid syndrome) (n = 1). None had renal disease or chronic hypertension. The majority of subjects (57/68) had only one high-risk factor, while 11 had two or more. The most common moderate risk factor was pre-pregnancy BMI ≥ 30 kg m^{-2} (n = 20), nulliparity (n = 10), age ≥ 40 years (n = 7), pregnancy interval ≥ 10 years (n = 5), thrombophilia (n = 4), ART (n = 3), prior stillbirth (n = 2) and prior foetal growth restriction (n = 1). None had a family history of PE or a previous history of placental abruption. Around half of patients (33/68) had no moderate risk factors, 22 had 1, and 13 had 2 or more. Recorded high-risk factors included GH (4/6), previous PE (2/6), and GDM (2/6), along with moderate-risk factors nulliparity (3/6) and high BMI (3/6).

PE was diagnosed in 6/68 women, with one pregnancy ending with foetal death. There was no PE or foetal death in the control group. Small gestational-age infants (birth

Table I. Maternal and neonatal characteristics for the control and PE-risk group

	Control group (n = 21)	PE-risk group (n = 68)	p-value
Maternal characteristics			
Age (years)	33 (29–36)	33 (30–37)	0.877
Prepregnancy BMI (kg m^{-2})	22.1 (20.7–24.1)	26.9 (22.9.0–30.3)	< 0.001
Pregnancy weight gain (kg)	13 (10–15)	11 (8–15)	0.129
sBP (mmHg)	110 (110–116)	120 (110–130)	0.014
dBP (mmHg)	70 (60–70)	73 (70–80)	0.032
Smoking, number	1 (5 %)	8 (12 %)	0.680
Induced labour	9 (43 %)	41 (60 %)	0.210
Epidural anaesthesia	7 (33 %)	21 (31 %)	1.000
Caesarean delivery	1 (5 %)	13 (19 %)	0.173
Neonatal characteristics			
Gestation week at delivery	40 (39–41)	39 (38–39)	0.004
Preterm delivery < 37	1 (5 %)	2 (3 %)	0.559
Sex, male	12 (57 %)	27 (40 %)	0.210
Birth weight (g)	3800 (3460–4075)	3420 (3170–3763)	0.010
Birth length (cm)	51 (50–52)	50 (48–51)	0.003

The values presented as median (interquartile range) were analysed using the Mann-Whitney U test, and the values shown as number (percentage) were analysed with Fisher's exact test. The level of statistical significance was set at p < 0.05.PE, preeclampsia; BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure.

weight ≤ 5th percentile) were born in two deliveries in the PE-risk group, whereas none were born in the control group. Macrosomia (defined as birth weight ≥ 95th percentile) was recorded in 5 cases in the PE-risk group and 3 in the control group. However, Fisher's exact test showed no statistically significant differences in the frequency of adverse outcomes, including PE, foetal death, and birth weight ≤ 5th and ≥ 95th percentile between the control and total PE-risk group.

The median gestation week for blood sampling was 40 for the control group and 39 for the PE-risk group, with interquartile ranges of 39–41 and 34–39, respectively. There were no statistically significant differences between the control and total PE-risk groups in sFlt-1 MoM, PlGF MoM, sFlt-1/PlGF ratio MoM and birth weight centiles. Since the PE-risk group is heterogeneous, we investigated whether there are differences in sFlt-1 MoM, PlGF MoM, sFlt-1/PlGF ratio MoM, and birth weight centiles for each risk factor individually. Due to the multiple comparisons (n = 10), the Bonferroni correction was applied, and p-values < 0.005 were considered statistically significant (0.05/10). The results are shown in Table II.

Additionally, all patients were divided into three groups based on the number of high--risk factors: group 0 (control group, n = 21), group 1 (one high-risk factor, n = 57), and group 2 (two or more high-risk factors, n = 11). The Kruskal-Wallis test showed no differences between these groups in sFlt-1 MoM (p = 0.196) and birth weight centiles (p = 0.214). However, there were statistically significant differences between groups for PlGF MoM (p = 0.007) and sFlt-1/PlGF ratio MoM (p = 0.024), with post-hoc analysis showing differences between both group 0 and 1 compared to group 2. Box-plot diagrams are presented in Fig. 1.

Main findings

This study gives an overview of the most common risk factors for PE in a tertiary-care hospital centre and their relationship to sFlt-1 and PlGF concentrations and pregnancy outcomes. Overall, the PE-risk group showed no differences in sFlt-1 MoM, PlGF MoM, sFlt-1/PlGF ratio MoM and birth weight centile of the newborns. However, differences were notable in several PE subgroups based on risk factors, with the most significant differences in groups with GH and nulliparity as risk factors.

Influence of high-risk factors on sFlt-1 PlGF and ratio

Both GDM and PE are characterised by endothelial dysfunction, and women with GDM are at higher risk of developing PE. Nuzzo *et al.* found slightly lower concentrations of both sFlt-1 and PlGF in GDM compared to healthy pregnant women, but with no statistically significant difference in concentration or their ratio (12). Researchers found that the ratio of sFlt-1/PIGF in women with PE was significantly higher compared to the group with PE in the background of GDM (GDM-PE). They hypothesised that sFlt-1 overproduction in GDM-PE patients increases the risk of preeclampsia, but due to a less pronounced decrease in PIGF, it resulted in less severe endothelial dysfunction and a lower sFlt-1/PIGF ratio compared to the PE group. Like Nuzzo *et al.*, we found no difference in ratio when comparing the GDM and control groups (0.71 *vs.* 0.77). As our study recorded only 2 out of 6 women who developed PE in a background of GDM, the data were too limited to analyse potential differences between PE and GDM-PE groups.

Table II. Comparison of the control group with total and individual PE-risk groups

	n	sFlt–1 MoM	PlGF MoM	sFlt–1/PlGF ratio MoM	Birth weight centile
Control	21	0.87 (0.70–1.11)	1.07 (0.84–2.03)	0.77 (0.26–1.27)	78 (63–90)
Control *vs.* total PE-risk group					
Total PE–risk	68	0.78 (0.54–1.26)	0.79 (0.51–1.80)	0.93 (0.32–2.05)	64 (33–85)[a]
		$p = 0.575$	$p = 0.091$	$p = 0.320$	$p = 0.080$
Control *vs.* PE-risk group with high risk factors					
GDM	46	0.70 (0.50–1.18)	1.09 (0.55–2.27)	0.71 (0.26–1.62)	65 (34–90)[a]
		$p = 0.152$	$p = 0.552$	$p = 0.957$	$p = 0.218$
GH	25	1.13 (0.84–1.51)	0.54 (0.36–0.78)	1.75 (1.03–3.63)	58 (31–77)[a]
		$p = 0.086$	$p < 0.001$	$p = 0.001$	$p = 0.029$
Previous PE	8	0.81 (0.69–1.06)	0.76 (0.51–0.90)	1.18 (0.77–2.04)	63 (52–80)
		$p = 0.661$	$p = 0.019$	$p = 0.143$	$p = 0.272$
Control *vs.* PE-risk group with moderate risk factors					
BMI ≥ 30 kg m^{-2}	20	1.00 (0.61–1.48)	0.47 (0.35–0.77)	2.05 (0.75–3.68)	72 (36–88)
		$p = 0.676$	$p < 0.001$	$p = 0.011$	$p = 0.489$
Nulliparity	10	1.53 (1.13–1.79)	0.43 (0.30–0.52)	3.01 (2.06–4.70)	45 (20–70)[a]
		$p = 0.002$	$p < 0.001$	$p < 0.001$	$p = 0.024$
Age ≥ 40 y	7	0.90 (0.42–1.20)	0.76 (0.62–1.30)	0.81 (0.52–1.60)	83 (47–87)
		$p = 0.770$	$p = 0.185$	$p = 0.614$	$p = 0.937$
Interval ≥ 10 y	5	0.80 (0.64–1.29)	0.78 (0.31–1.86)	0.86 (0.42–3.19)	70 (48–90)
		$p = 0.820$	$p = 0.346$	$p = 0.537$	$p = 0.754$
Thrombophilia	4	0.58 (0.44–1.24)	1.12 (0.98–1.30)	0.51 (0.33–1.29)	36 (17–57)
		$p = 0.182$	$p = 1.00$	$p = 0.824$	$p = 0.024$
ART	3	1.17 (0.88–1.26)	0.46 (0.41–0.51)	2.30 (2.10–2.30)	73 (26–84)
		$p = 0.206$	$p = 0.013$	$p = 0.033$	$p = 0.432$

Each *p*-value represents a comparison between the control group and the PE-risk groups: total PE-risk group, high-risk PE groups (GDM, GH, and previous PE), and moderate-risk PE groups (BMI ≥ 30, nulliparity, age ≥ 40 years, between-interval pregnancy ≥ 10 years, thrombophilia, and ART).

The values are presented as median (interquartile range Q1–Q3) and tested using the Mann-Whitney U-test. Due to the small sample size, ART data are presented as medians (with minimum and maximum values). Due to the multiple comparisons ($n = 10$), the Bonferroni correction was applied, and p-values < 0.005 were considered statistically significant (0.05/10).

[a] Number of data for the birth weight centile is adjusted for one pregnancy with foetal death as an outcome. PE, preeclampsia; GDM, gestational diabetes mellitus; GH, gestational hypertension; BMI, body mass index; ART, assisted reproductive technology; MoM, multiple of the median; sFlt-1, soluble fms-like tyrosine kinase 1; PlGF, placental growth factor

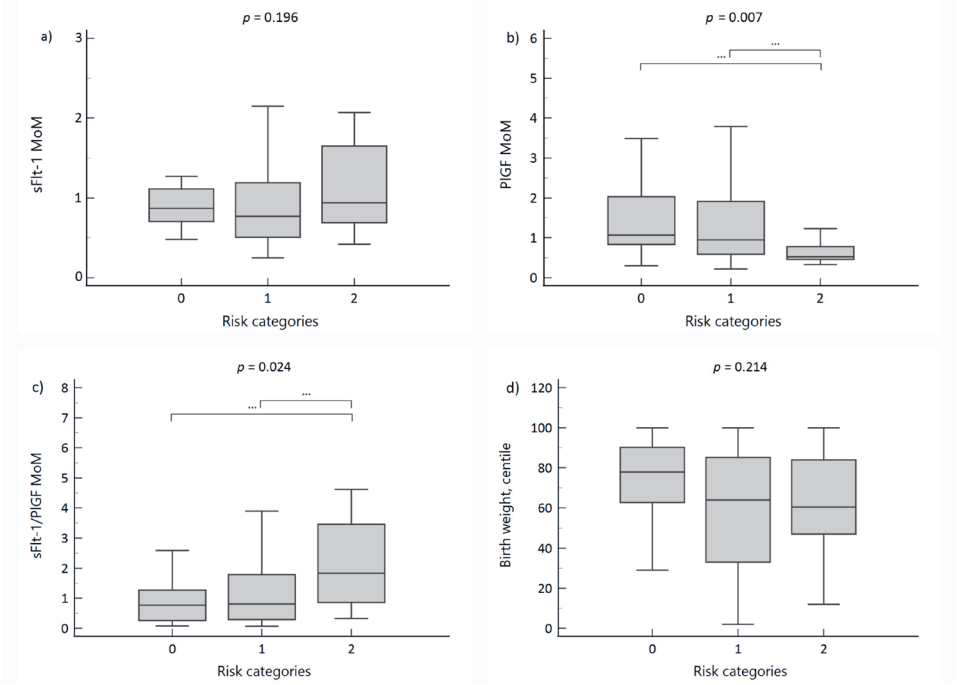

Fig. 1. Differences between risk groups. Box and whiskers plot comparing: a) sFlt-1MoM; b) PlGF MoM; c) sFlt-1/PlGF MoM and d) birth weight centile among different risk groups: group 0 (control group), group 1 (one high-risk factor), and group 2 (≥ two high-risk factors). Results were compared using the Kruskal-Wallis test followed by Conover post-hoc analyses, with statistically significant differences marked with an asterisk (***). MoM, multiple of the median; sFlt-1, soluble fms-like tyrosine kinase 1; PlGF, placental growth factor.

GH is a key factor and criteria in preeclampsia. GH was defined as a high-risk factor when recruiting women for the study, although there is no quality evidence to confirm that GH is a risk factor for PE (13). On the other hand, chronic hypertension is strongly associated with a high risk of developing PE factor, but no women with CHT were included in the study (14). GH and concordantly higher sFlt-1/PlGF ratio were a result of PE pathophysiology, rather than a cause of it. In our study, women with GH had a higher sFlt-1/PIGF ratio, resulting from increased sFlt-1 and decreased PIGF concentrations.

PE during the first pregnancy increases the risk of developing PE in the second pregnancy by 7 times (15). After nulliparity and high pre-pregnancy BMI, prior PE is the third most common risk factor with an attributable fraction of 22.8 % (16). The studied group with a history of PE showed a higher sFlt-1/PlGF ratio due to lower PlGF MoM than the control group, but these differences were not statistically significant after Bonferroni correction.

Influence of moderate risk factors on sFlt-1, PlGF and ratio

Obesity is defined as a BMI ≥ 30 kg m^{-2} and it has been on the rise over the past several years in pregnant women. Maternal obesity increases the risk of preeclampsia by three to

four times when compared to normal-weight mothers (17). Weight gain during pregnancy has also been linked to PE, and the combined effect of maternal obesity and gestational weight gain further heightens the risk (18). High sFlt-1/PlGF ratio in the second trimester occurred 3 times more frequently in pregnant obese women than in pregnant women with normal weight (19). As high prepregnancy BMI was one of the inclusion criteria, it is not surprising that the total PE-risk group had a higher BMI compared to the control group (26.9 *vs.* 22.1 kg m^{-2}), but there was no difference in gestational weight gain (11 *vs.* 13 kg). The sFlt-1/PIGF ratio was 3 times higher in the obese women subgroup due to a decrease in PIGF rather than an increase in sFlt-1 concentration. These results are in line with those of Beck *et al.*, who demonstrated that ratios above 38 were attributed to a lower-than-expected PlGF concentration. However, they are opposite to those of Karge *et al.*, who found only a lower sFlt-1 concentration in obese women (19, 20).

First-time pregnant women are the largest population with attributable risk factors for preeclampsia. Researchers are proposing a link to immunological interactions related to minimal exposure to paternal antigen prior to conception. Preeclampsia was diagnosed more often in nulliparous women than in women with subsequent pregnancies (21). It is suggested that the maternal immune response and adaptations to pregnancy may differ in nulliparous women compared to multiparous women (22). Besides nulliparity, each pregnancy with a different partner is considered a risk factor, which supports the theory of an immunological mechanism of PE development. Nulliparous women in our study had significantly higher sFlt-1/PlGF ratios (3.45 *vs.* 0.77), similar to previously published studies (23). The nulliparity group had the highest sFlt-1 (1.53) and lowest PIGF MoM (0.43) of all risk factors included in this study. Also, 3 of 6 women who developed PE in this study were nulliparous. Although nulliparity was considered a moderate risk factor, the findings demonstrate that it significantly influences the sFlt-1/PIGF ratio and may contribute to adverse pregnancy outcomes.

ART increases the incidence of obstetric problems compared to spontaneously conceived pregnancies. Since the *in vitro* fertilisation (IVF) procedure frequently leads to pregnancies with corpus luteum defect, women who conceive by IVF may have pathophysiological changes in the placenta. Compared to spontaneous pregnancies, IVF-conceived pregnancies had higher levels of sFlt-1 and lower levels of PlGF in the second and third trimesters (24). We only had three pregnancies conceived with ART, with a higher ratio compared to the control group, but with no statistically significant differences. Pregnancies at advanced maternal age (> 35) and very advanced maternal age (> 40) have become more prevalent over the last few decades. The mechanism by which maternal age contributes to an increased risk of preeclampsia is not fully understood, but it is independent of maternal comorbidities. Although advanced maternal age and IVF are independent risk factors for PE, they are often found together as advancements in assisted reproductive technologies have contributed to an increase in the incidence of advanced maternal age. Our study found no difference in sFlt-1 and the sFlt-1/PlGF ratio, with a slight decrease in PlGF MoM in pregnant women over 40.

Only two guidelines listed thrombophilia as a risk factor (25), and it is considered a moderate risk factor for PE, although the quality of evidence for this is low (13). In our study, there were no significant differences in MoM values between the control group and women with thrombophilia. However, this may be due to the study's low prevalence of thrombophilia.

Influence of high and moderate risk factors on birth weight centile

Birth weight is an important indicator of the nutrition and growth progress of the foetus during pregnancy. Poor or overnutrition can lead to small (SGA) or large infants for gestational age. Although PE is associated with SGA, we found no difference in frequency between the total PE-risk and the control group. However, we recorded more cases of macrosomia, but this outcome is probably due to the high number of pregnancies with gestational diabetes and obesity as risk factors. The whole PE-risk group had lower birth weight than the control group: 3420 *vs.* 3800 g and 64th *vs.* 78th birth weight centiles. Although not statistically significant, all risk factor groups, except women over 40, showed lower birth weight centiles than the control group, with more pronounced differences in subgroups with GH (58th percentile), nulliparity (45th percentile), and thrombophilia (36th percentile). Interestingly, pregnant women in the small risk group with thrombophilia (n = 4) delivered infants with the most considerable difference compared to the control group (36th *vs.* 78th).

Multiple risk factors

Multiple maternal risk factors can have a synergistic effect on sFlt-1 and PlGF concentrations, increasing sFlt-1/PlGF ratio and the risk for developing PE (26). Our data showed that women with multiple risk factors had a higher sFlt-1/PlGF ratio than both the control group and the group with only one risk factor, confirming that multiple risk factors strongly influence the sFlt-1/PlGF ratio. The higher ratio was primarily due to a significantly lower PlGF MoM, while there was no difference in sFlt-1 MoM between the groups.

CONCLUSIONS

As PE is a major pregnancy disorder with possible adverse outcomes for both the mother and baby, recognising women at risk is crucial for timely detection and treatment. This study recorded PE risk factors in conjunction with angiogenic markers and pregnancy outcomes in a Croatian hospital centre. Although the studied group was not large, the results confirmed previous studies and emphasised the need to introduce PE screening into routine practice.

Ethical approval. – Ethical approval for this study was obtained from the Ethics Committee of Sestre Milosrdnice University Hospital Centre. Participants were free to decline participation at any point during the study. Data were collected and stored under specific codes, with guarantees of anonymity and confidentiality. All procedures were conducted in accordance with relevant guidelines and regulations.

Consent to participate. – Informed consent for participation was obtained from all subjects before data collection.

Availability of data and materials. – The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest. – The authors declare that the research was conducted without any commercial or financial relationships that could be perceived as a potential conflict of interest.

Funding. – This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors contributions. – Conceptualisation, A.B; methodology, A.B. and D.B; analysis, A.B. and M.G.R; writing, original draft, A.B., M.Č., and I.D.; writing, review and editing, D.B. and M.G.R; supervision, D.B. and M.G.R. All authors have read and agreed to the published version of the manuscript.

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