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Expression and correlation of endoglin, sEndoglin, and MMP-14 on preeclampsia placenta

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ABSTRACT

Background: Hypertensive disorders, particularly preeclampsia, are major contributors to maternal mortality and neonatal morbidity. Angiogenic imbalance plays a critical role in placental ischemia, a hallmark of preeclampsia.

Purpose: This study aimed to investigate the roles of endoglin, soluble endoglin (sEndoglin), and matrix metalloproteinase-14 (MMP-14) in the angiogenic imbalance observed in preeclampsia placentas compared to normal-term placentas.

Method: A cross-sectional study was conducted using 68 placental samples: 34 from normal-term pregnancies and 34 from preeclampsia cases. Concentrations of endoglin, sEndoglin, and MMP-14 were measured using the sandwich ELISA method, and protein levels were determined using the Christian Warburg method. Data were analyzed using SPSS version 20.

Results: The concentration of endoglin in preeclampsia placentas was slightly higher (1.37 [0.2–2.2] ng/µg protein) compared to normal placentas (1.12 [0.6–14.1] ng/µg protein), although the difference was not statistically significant. In contrast, sEndoglin (0.05 [0.0–0.01] ng/µg protein vs. 0.02 [0.0–0.3] ng/µg protein) and MMP-14 (0.14 [0.1–0.6] ng/µg protein vs. 0.11 [0.1–1.3] ng/µg protein) concentrations were significantly higher in preeclampsia placentas compared to normal placentas. All parameters showed a gradual decrease with advancing gestational age. sEndoglin and MMP-14 demonstrated a strong positive correlation ($r = 0.658$, $p < 0.001$), while endoglin and MMP-14 exhibited a moderate positive correlation ($r = 0.554$, $p < 0.001$).

Conclusion: Endoglin, sEndoglin, and MMP-14 were differentially expressed in preeclampsia placentas, with sEndoglin and MMP-14 significantly elevated. These findings highlight their potential role in angiogenic imbalance and may provide insight into the pathophysiology of preeclampsia.

Keywords: preeclampsia, endoglin, sEndoglin, MMP-14

Introduction

Hypertensive disorders in pregnancy, including preeclampsia, eclampsia, and gestational hypertension, affect approximately 10% of pregnant women worldwide [1]. Among these, hypertension during pregnancy is the leading cause of maternal mortality rate (MMR), followed by hemorrhage, infection, and other coexisting conditions. According to the Voluntary National Review (2017), the MMR decreased from 346 deaths per 100,000 live births in 2010 to 305 in 2015, marking progress toward the Millennium Development Goals (MDGs). However, significant effort and commitment are still required to achieve the global target of 70 maternal deaths per 100,000 live births by 2030, as outlined in the Sustainable Development Goals (SDGs) [2].

Preeclampsia is the most prominent hypertensive disorder in pregnancy due to its profound impact on maternal and neonatal health [1]. It is a syndrome characterized by the new onset of hypertension and proteinuria [1,3] and is recognized as a unique placenta-mediated disease. Despite extensive research, the pathogenesis of preeclampsia remains incompletely understood, and treatment primarily

involves the delivery of the fetus and placenta. Current evidence links preeclampsia to defective placentation, genetic predisposition, immune maladaptation, intolerance to inflammatory changes, angiogenic imbalance, and inadequate nutritional states [4].

Placental ischemia in preeclampsia causes oxidative stress and angiogenic imbalance. Key angiogenic factors implicated include soluble fms-like tyrosine kinase-1 (sFlt-1), endoglin, placental growth factor (PIGF), and vascular endothelial growth factors (VEGFs) [4]. Of particular interest is the role of transforming growth factor (TGF)-β and its interaction with soluble endoglin (sEndoglin), which is crucial in the angiogenic imbalance observed in preeclampsia. Endoglin, a homodimeric transmembrane glycoprotein with a molecular weight of 180 kDa, consists of 633 amino acids and features an extracellular domain, a hydrophobic transmembrane region, and a smaller intracellular tail. Two primary isoforms, long (L) and short (S) endoglin, differ in their intracellular domains, tissue distribution, and phosphorylation [5]. Endoglin functions as a cell surface receptor for TGF-β, modulating its signaling [5,6].

Matrix metalloproteinase-14 (MMP-14) has been proposed as a therapeutic target for reducing circulating sEndoglin and alleviating symptoms of preeclampsia. MMPs are a family of 23 calcium- and zinc-dependent proteases that degrade extracellular matrix components. All MMPs share two structural domains: the C-terminal hemopexin-like domain and the N-terminal propeptide region, as well as catalytic and hinge domains and a linker peptide [7].

This study aims to elucidate the roles of endoglin, sEndoglin, and MMP-14 in the angiogenic imbalance characteristic of the preeclamptic placenta compared to the normal placenta. The findings may provide insights into the pathophysiology of preeclampsia, offering opportunities for improved diagnosis and therapeutic interventions.

Methods

This study employed a cross-sectional design, utilizing 34 placentas from normal-term pregnancies and 34 placentas from preeclampsia cases. Ethical approval for the study was obtained from the Health Research Ethics Committee of FMUI-Ciptomangunkusumo Hospital (No. 104/UN2.F1/ETIK/2016). Placental samples were collected from Ciptomangunkusumo Hospital and Budi Kemuliaan Hospital. Informed consent was obtained from participants after a detailed explanation of the study by the researchers. Placental tissues were stored in a deep freezer (-80°C) until further analysis.

Inclusion and exclusion criteria

The inclusion criteria for the preeclampsia group were placentas obtained from subjects diagnosed with preeclampsia by an obstetrician. For the normal-term group, placentas were collected from pregnancies with gestational ages of 37–40 weeks. Exclusion criteria included preeclampsia cases with concurrent infections or gestational hypertension.

Tissue homogenate preparation

Placental tissues (100 mg) were homogenized using a Potter-Elvehjem homogenizer after the addition of 1 mL phosphate-buffered saline (PBS). The homogenates were centrifuged at 5,000 rpm for 10 minutes, and the resulting supernatant was collected for subsequent measurements [8].

Sandwich ELISA method

The concentrations of endoglin, soluble endoglin (sEndoglin), and MMP-14 in the placental tissue homogenates were measured using the sandwich ELISA method (Elabscience) following the manufacturer's protocol. Serial dilutions of the reference standard (0–10 ng/mL) were prepared using the standard diluent. A 100 µL aliquot of either tissue homogenate supernatant or diluted standards was added to pre-coated 96-well microplates and incubated for 90 minutes at 37°C.

Following incubation, the plates were washed three times with 350 µL/well of wash buffer. Subsequently, 100 µL of biotinylated detector antibody was added to each well and incubated for 60 minutes at 37°C. After a second washing cycle (three washes with 350 µL/well), 100 µL of HRP-conjugate working solution was added and

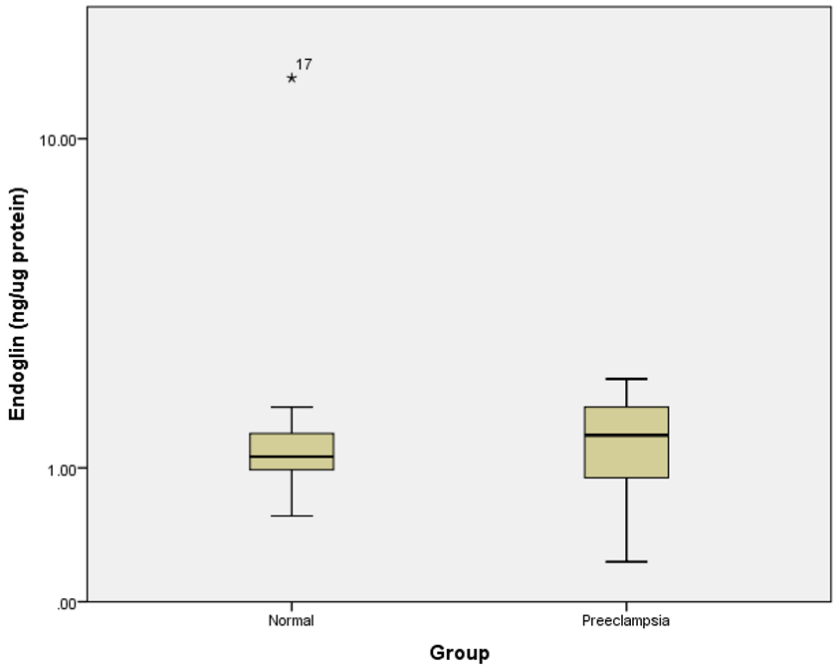

Figure 1. Comparison of endoglin concentration. (n=34 per group, double). No significant difference between normal and pre-eclampsia group (Mann-Whitney, p>0.05)

Table 1. Comparison of age and blood pressure according to gestational age in preeclampsia group

Gestational age	N	Age (year)	SD	Systole (mmHg)	SD	Diastole (mmHg)	Min-max
<32	10	28.1	7.8	172	26	100	90-150
32-36	14	32.9	5.2	155	30	100	80-110
>36	10	30.1	6.8	158	30	100	70-140

incubated for 30 minutes at 37°C. A third washing cycle (five washes with 350 µL/well) was performed, followed by the addition of 90 µL TMB substrate solution. After 15 minutes of incubation, the color of the highest standard turned dark blue. To stop the reaction, 50 µL of stop solution was added to each well. Optical density (OD) values were measured at 450 nm using a microplate reader (Varioskan, Thermo Scientific) [9,11].

Protein concentration determination

The protein concentration of the tissue homogenate supernatant was determined spectrophotometrically at λ280 nm, using bovine serum albumin (BSA) as the standard [12]. The levels of endoglin, sEndoglin, and MMP-14 in the placenta were normalized to the total protein concentration of the placental homogenates and expressed as ng/µg protein.

Statistical analysis

Statistical analyses were performed using SPSS version 20. Normality was assessed with the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Differences between the two groups were analyzed using an independent t-test for parametric data or the Mann-Whitney U test for non-parametric data.

Results

Table 1 displays the characteristics of the study subjects, including age and blood pressure, stratified by gestational age and preeclampsia status. The data indicate that systolic blood pressure at <32 weeks of gestation is higher compared to other groups; however, this difference is not statistically significant. Diastolic blood pressure is similar across all groups.

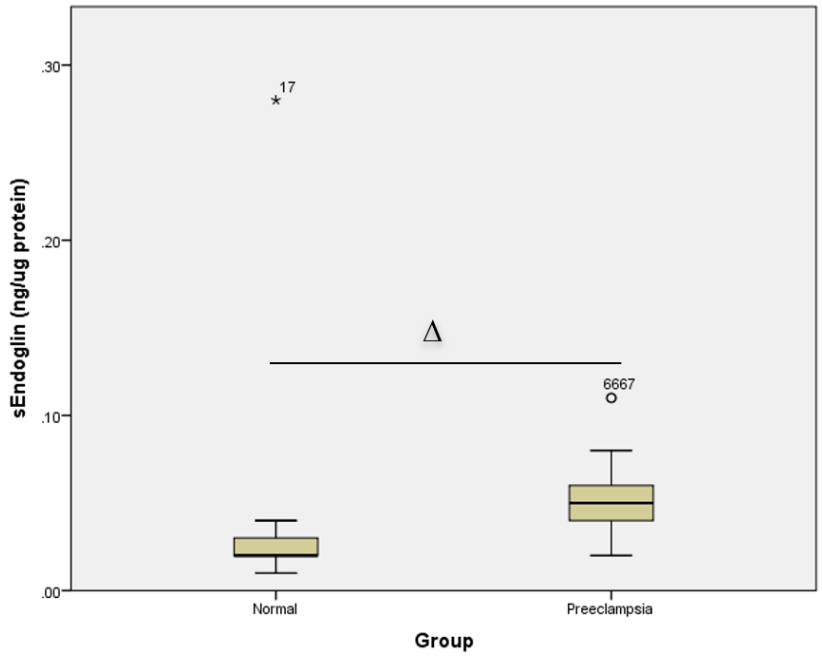

Figure 2. Comparison of sEndoglin concentration. (n=34 per group, double). Δ: a significant difference between normal and pre-eclampsia group (Mann-Whitney, p<0.05)

The concentrations of angiogenic and antiangiogenic factors in placental tissues were assessed. For the angiogenic factor endoglin, the median concentration in the preeclampsia placenta group was slightly higher (1.37 ng/µg protein) than in the normal-term placenta group (1.12 ng/µg protein) (Figure 1). However, this difference was not statistically significant (Mann-Whitney, $p > 0.05$).

sEndoglin, a major antiangiogenic factor commonly investigated in preeclampsia research, demonstrated a significantly higher concentration in the preeclampsia placenta group (0.05 ng/µg protein) compared to the normal-term placenta group (0.02 ng/µg protein) (Figure 2, Mann-Whitney, $p < 0.05$).

Similarly, MMP-14 concentrations were significantly elevated in the preeclampsia placenta group (0.14 ng/µg protein) compared to the normal-term placenta group (0.11 ng/µg protein) (Figure 3, Mann-Whitney, $p < 0.05$).

Correlation analyses revealed a low positive correlation between endoglin and sEndoglin concentrations (Spearman's rho, $rs = 0.390$, $p < 0.05$). A high positive correlation was observed between sEndoglin and MMP-14 concentrations (Spearman's rho, $rs = 0.658$, $p < 0.05$). Additionally, a moderate positive correlation was identified between endoglin and MMP-14 concentrations (Spearman's rho, $rs = 0.554$, $p < 0.05$).

The concentrations of endoglin, sEndoglin, and MMP-14 were highest in the <32 weeks gestational age group compared to other groups (Figure 4). Table 2 highlights a gradual decrease in the levels of all parameters as gestational age increased.

Discussion

Preeclampsia is a systemic disorder characterized by widespread endothelial dysfunction, which increases the risk of organ damage, including cardiovascular and renal complications, in affected women. The pathophysiology of preeclampsia differs based on its onset. Early-onset preeclampsia (<34 weeks) is primarily associated with placental dysfunction, whereas late-onset preeclampsia (>34 weeks) is more closely related to maternal systemic dysfunction. Early-onset preeclampsia is often linked to abnormalities in maternal uterine spiral arteries, resulting in oxidative stress and imbalances in angiogenic factors such as elevated soluble fms-like tyrosine kinase-1 (s-Flt1) and reduced placental growth factor (PlGF) levels [13].

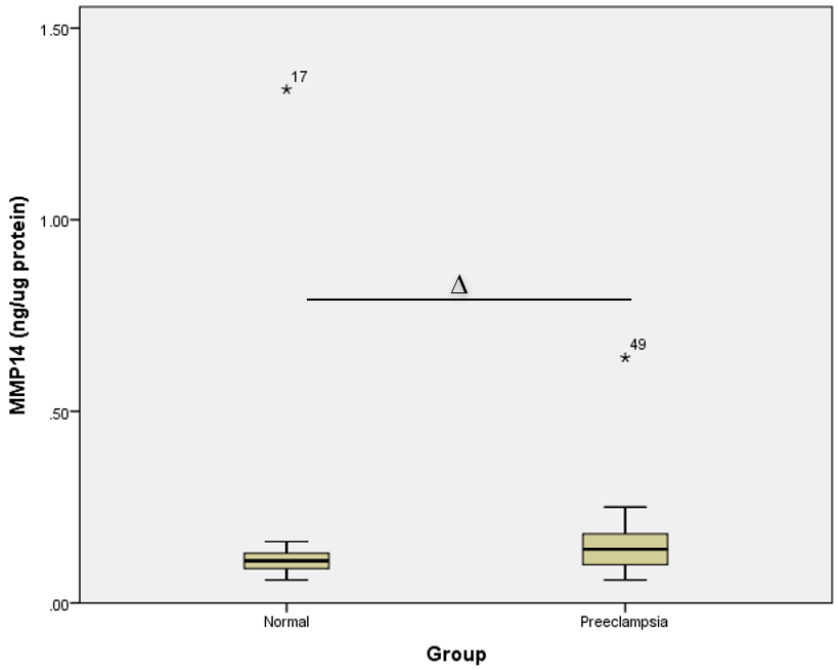

Figure 3. Comparison of MMP-14 concentration. (n=34 per group, double). Δ: significant difference between normal and pre-eclampsia group (Mann-Whitney, p<0.05)

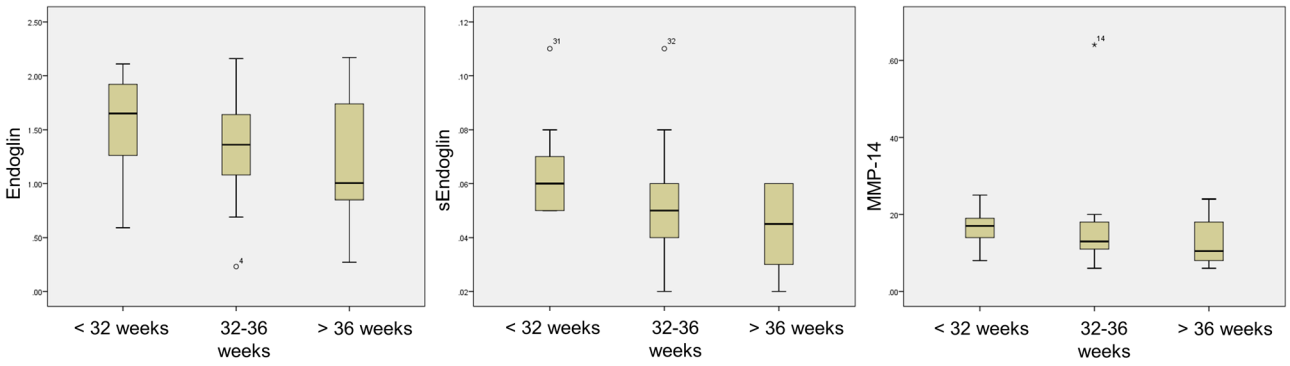

Figure 4. Comparison of endoglin, sEndoglin, and MMP-14 according to gestational age in preeclampsia group

Table 2. Comparison of endoglin, sEndoglin, and MMP14 according to gestational age in preeclampsia group

Gestational age	N	Endoglin (ng/ µg protein)	SD	sEndoglin (ng/µg protein)	Min-max	MMP14 (ng/µg protein)	Min-max
<32	10	1.53	0.53	0.06	0.05-0.11	0.17	0.08-0.25
32-36	14	1.32	0.51	0.05	0.02-0.11	0.13	0.06-0.64
>36	10	1.16	0.58	0.045	0.02-0.06	0.11	0.06-0.24

Angiogenic imbalance is widely considered a key mechanism underlying preeclampsia. In normal pregnancies, angiogenic balance is maintained by factors such as VEGF, TGF-β, and PlGF. However, in preeclampsia, antiangiogenic factors such as soluble endoglin (sEndoglin) and s-Flt1 act as scavengers of VEGF, PlGF, and TGF-β, disrupting their physiological roles and contributing to endothelial dysfunction [13]. Elevated levels of s-Flt1, sEndoglin, and endothelin-1 are also associated with an increased risk of cardiovascular disease [14].

Endoglin, a type I membrane glycoprotein, serves as a co-receptor for TGF-β1 and TGF-β3

and is critical for angiogenesis, endothelial cell differentiation, and vascular tone regulation [13,15]. It is predominantly expressed on endothelial cells and placental syncytiotrophoblasts. In preeclampsia, endoglin mRNA is upregulated, and excess sEndoglin is released into circulation due to proteolytic cleavage of the extracellular domain [16,17]. sEndoglin impairs TGF-β-mediated anti-inflammatory and vasodilatory effects, leading to endothelial dysfunction characterized by vasoconstriction, overexpression of adhesion molecules, and reduced T-cell activity [13].

In this study, the concentration of endoglin in preeclampsia placentas was not significantly different from that in normal-term placentas. This contrasts with findings by Fang et al., who reported significantly increased serum and placental endoglin levels in preeclampsia, particularly in severe cases [18]. However, sEndoglin concentrations in preeclampsia placentas were significantly higher than in normal-term placentas, consistent with previous studies showing elevated sEndoglin levels in serum and plasma from preeclampsia patients [19,20]. sEndoglin has been proposed as a diagnostic biomarker for preeclampsia, with high sensitivity, specificity, and accuracy [20]. The expression of angiogenic and antiangiogenic factors, including VEGF-A, PlGF, endoglin, and their receptors, is essential for establishing uteroplacental circulation [21].

Matrix metalloproteinases (MMPs) are increasingly recognized for their role in vascular remodeling, angiogenesis, and vasodilation during normal pregnancy. Elevated levels of MMP-1, MMP-2, and MMP-9 in preeclampsia suggest their potential as biomarkers for the disease [22]. In this study, MMP-14 concentrations were significantly higher in preeclampsia placentas than in normal-term placentas. MMP-14 is known to mediate endoglin shedding by cleaving it near the transmembrane domain, contributing to angiogenic imbalance. While MMP-14 plays a key role in endothelial cell angiogenesis in other conditions, such as colorectal tumors [23], its role in preeclampsia warrants further investigation. Recent studies have reported increased placental expression of endoglin and MMP-14 in preeclampsia, although the increase in MMP-14 was not statistically significant [24]. Interestingly, MMP-15, despite being phylogenetically related to MMP-14 and upregulated in preeclampsia, does not cleave endoglin [25].

On the other hand, MMP-14 is produced by leukocytes, particularly monocytes and macrophages, and is involved in trans-endothelial migration and T-cell attraction, highlighting its role in modulating inflammatory responses [26]. However, this study did not examine the contribution of leukocytes to the observed increase in MMP-14 levels, presenting an avenue for future research to clarify its role in the angiogenic imbalance associated with preeclampsia.

This study identified a strong positive correlation between sEndoglin and MMP-14 concentrations, suggesting a link between MMP-14-mediated endoglin cleavage and elevated sEndoglin levels in preeclampsia. Both sEndoglin and s-Flt1 may act as scavengers of VEGF, PlGF, and TGF-β, contributing to endothelial dysfunction in preeclampsia [13].

Conclusion

This study confirmed the differential expression and correlation of endoglin, sEndoglin, and MMP-14 in preeclampsia placentas compared to normal-term placentas. The significant increase in these factors in preeclampsia placentas and their gradual decrease with advancing gestational age underscore their role in angiogenic imbalance. Further studies comparing additional angiogenic factors are needed to fully elucidate the mechanisms underlying angiogenic dysregulation in preeclampsia.

Conflict of interest

No potential conflicts of interest were reported by the authors.

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Author contributions

Conceptualization: FCI, ARP; methodology: NM, RP, YP; formal analysis: RP, YP; investigation: NM, RP, YP; writing – original draft preparation: FCI, RP; writing – review & editing: FCI, NM, RP, YP, ARP; visualization: RP; funding acquisition: ARP.

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