Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2023.82529

Turk J Obstet Gynecol 2023;20:22-8

Increase in transforming growth factor-β didnot affect trombospondin1 in preeclampsia placentas

Transforming growth faktör-β'daki artış, preeklampsili plasentalarda trombospondin1'i etkilemedi

Ani Retno Prijanti[1], Nissa Thoyyiba Oktavia[2], Febriana Catur Iswanti[1], Ninik Mudjihartini[1], Yuditiya Purwosunu[3]

[1]*Department of Biochemistry and Molecular Biology, and Center of Hypoxia and Oxidative Stress Studies, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*

[2]*Master's Programe in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia*

[3]*Department of Obstetrics and Gynecology, Faculty of Medicine-cipto Mangunkusumo Central Hospital, Universitas Indonesia, Jakarta, Indonesia*

Abstract

Objective: The abnormalities of the placental growth process are a theory causing pre-eclampsia. Antiangiogenic factors contributed to it, such as thrombospondin-1 (TSp-1) that could stimulate transforming growth factor-beta (TGF-β), or vice versa. Some research showed that an increase in TGF-β did not always figurized its signaling. Therefore, we conducted a study to examine the TGF-β signaling proteins through its receptors and TSp-1 expression in preeclampsia placentas.

Materials and Methods: This observational study used 33 normal and 33 pre-eclampsia placental storaged samples, for examination of TGF-β and TGF-βR 1 and 2, SMAD2 using ELISA, and SMAD2 and TSp-1 mRNA using the reverse transcription polymerase chain reaction method. Data were analyzed using SPSS version 20.0, normality test by Kolmogorov-Smirnov, and significancy was analyzed using nonparametric Mann-Whitney test, or t-test for parametric, with confidence interval 95%. Spearman correlation was used for non-parametric data, besides the Pearson correlation for parametric data.

Results: Results showed that there were significant differences between preeclampsia and normal placenta in TGF-β, its receptors, SMAD2, and TSp-1 mRNA. Normal-TGF-β=1.19 (0.713-2.051) pg/mg; preeclampsia-TGFB=2.69 (0.906-10.252) pg/mg; p=0.001; normal-TGFBR1=1.025 (0.622-1.402) ng/mg; preeclampsia-TGFBR1=1.223 (0.372-2.553) ng/mg; p=0.004; Normal-TGF-βR2=0.959 (0.644-1.634) pg/mg; preeclampsia-TGFBR2=1.490 (0.775-3.645) pg/mg; p=0.0001; normal-SMAD2=2.087 (1.279-4.300) ng/mg; preeclampsia-SMAD2=3.508 (1.842-22.489) ng/mg; p=0.0001. The SMAD2 mRNA relative expression (Livax) in the normal placenta was=0.71 (0.03-7.25); pre-eclampsia placenta (PE)=0.49 (0.01-40.71); p=0.075, the normal TSp-1 mRNA expression=1.08 (0.09-5.31); PE=0.21 (0.002-24.06); p=0.002. The correlation test showed a strong correlation between TGF-β with TGFBR1 and 2 in the normal placenta, conversely, there was no correlation in the preeclampsia placenta. There was also no correlation between SMAD2 and TSp-1 mRNA in both normal and pre-eclampsia.

Conclusion: TGF-β signaling in the preeclampsia placenta was changed due to the increased of the protein signaling it self without correlation between TGF-β to its receptors and TSp-1 relative expression.

Keywords: Pre-eclampsia placenta, TGF-β, TGF-βRs, SMAD2, and thrombospondin-1

Öz

Amaç: Plasental büyüme sürecindeki anormalliklerin preeklampsiye neden olması bir teoridir. Transforming growth faktör beta'yı (TGF-β) uyarabilen trombospondin-1 (TSp-1) gibi antianjiyojenik faktörler buna katkıda bulunmuştur veya bunun tersi de geçerlidir. Bazı araştırmalar, TGF-β'daki bir artışın her zaman onun sinyalini yansıtmadığını göstermektedir. Bu nedenle, preeklampsili plasentalarda reseptörleri ve TSp-1 ekspresyonu yoluyla TGF-β sinyal proteinlerini incelemek için bu çalışma planlanmıştır.

PRECIS: The levels of TGF-β, TGFBR1 and 2, SMAD2 were increased in the preeclampsia placenta, but conversely the thrombospondin1 mRNA relative expression, it considered that there was no correlation between TGF-β and its receptors.

Address for Correspondence/Yazışma Adresi: Assoc. Prof. Ani Retno Prijanti,
Department of Biochemistry and Molecular Biology, and Center of Hypoxia and Oxidative Stress Studies, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
Phone: +62213910734 **E-mail:** aniretno@gmail.com **ORCID ID:** orcid.org/0000-0003-0663-2714
Received/Geliş Tarihi: 15.07.2022 **Accepted/Kabul Tarihi:** 01.02.2023

Gereç ve Yöntemler: Bu gözlemsel çalışmada, 33 normal ve 33 preeklampsili plasental depolanmış numunede, TGF-β, TGF-βR 1 ve 2 ve SMAD2 düzeyleri ELISA metodu kullanılarak ve SMAD2 ve TSp-1 mRNA transkripsiyonları ters transkripsiyon polimeraz zincir reaksiyonu yöntemi kullanılarak ölçüldü. Veriler SPSS versiyon 20.0 ile analiz edildi. Kolmogorov-Smirnov normallik testi kullanıldı. Anlamlılık, %95 güven aralığı ile, normal dağılmayan verilerde parametrik olmayan Mann-Whitney testi kullanılarak ve normal dağılan verilerde parametrik t-testi kullanılarak analiz edildi. Normal dağılmayan veriler için Spearman korelasyonu, normal dağılan veriler için Pearson korelasyonu kullanıldı.

Bulgular: Preeklampsi ve normal plasenta grupları arasında TGF-β, reseptörleri, SMAD2 ve TSp-1 mRNA ölçümleri açısından farklılıklar saptandı. Normal-TGF-β=1,19 (0,713-2,051) pg/mg; preeklampsi-TGFB=2,69 (0,906-10,252) pg/mg; p=0,001; normal-TGFBR1=1,025 (0,622-1,402) ng/mg; preeklampsi-TGFBR1=1,223 (0,372-2,553) ng/mg; p=0,004; normal-TGF-βR2=0,959 (0,644-1,634) pg/mg; preeklampsi-TGFBR2=1,490 (0,775-3,645) pg/mg; p=0,0001; normal-SMAD2=2,087 (1,279-4,300) ng/mg; preeklampsi-SMAD2=3,508 (1,842-22,489) ng/mg; p=0,0001. Normal plasentadaki SMAD2 mRNA göreceli ifadesi (Livax)=0,71 (0,03-7,25); preeklampsili plasentada=0,49 (0,01-40,71); p=0,075; normal TSp-1 mRNA ifadesi=1,08 (0,09-5,31); preeklampsili plasentada=0,21 (0,002-24,06); p=0,002. Korelasyon testi normal plasentada TGF-β ile TGFBR1 ve 2 arasında güçlü bir korelasyon gösterdi, aksine preeklampsili plasentada korelasyon yoktu. Hem normal plasentalarda hem de preeklampsili plasentalarda SMAD2 ve TSp-1 mRNA ekspresyonu arasında bir korelasyon yoktu.

Sonuç: Preeklampsili plasentadaki TGF-β sinyali, TGF-β, TGF-β'nın reseptörleri ve göreceli TSp-1 ekspresyonu arasında korelasyon olmaksızın, kendi kendine sinyal veren proteinin artması nedeniyle değişmiştir.

Anahtar Kelimeler: Preeklampsili plasenta, TGF-β, TGF-βR'ler, SMAD2 ve trombospondin-1

Introduction

In Indonesia, maternal mortality rate (MMR) is one indicator of success to achieve Sustainable Development Goals in 2030[1]. The MMR in Indonesia was still relatively high, equal to 133 per 100,000 live birth[2]. Among them, 27.1% with hypertension in pregnancy[1]. Maternal hypertension in pregnancy can be caused by the presence of preeclampsia, and characterized by hypertension and proteinuria at over 20 weeks of gestation[3]. Every year there was approximately 10 million pregnant women in the world with preeclampsia and 76,000 cases were death[4]. One theory developed about the cause of preeclampsia was the presence of an ischemic placenta, placental failured to supply oxygen and nutrients that result in a pathophysiological disorder. The failure of the placenta was due to the failure of the placental development process, both in the invasion of trophoblastic cells in the maternal decidua during implantation and spiral artery remodeling. Both of these processes involved many proteins that play a role in angiogenesis, proangiogenesis, and antiangiogenesis[5,6].

One protein that plays a role in angiogenesis in the placenta is TGF-β, a multifunctional signal that activates the intracellular signal transduction pathway by binding to its serine/threonin transmembrane receptor, TGF-β receptor 1 (TGFBR1) and TGF-β receptor 2 (TGFBR2)[7,8]. Some previous studies, TGF-β has been shown to be antiangiogenic and its expression is increased in preeclampsia[9-11]. The association between TGF-β with both of TGFBR1 and TGFBR2 triggers the activation of the SMAD proteins, one of them is the SMAD2, and signaling continues into to the nucleus and plays a role on regulation of gene expression[12,13]. Various tissues demonstrated the role of SMAD2, as an antiantiogenic peptide, it regulates the expression of the thrombospondin-1 (TSp-1) and sFLT-1 genes, an antiangiogenesis factor[14]. Therefore, it is considered that an increase in SMAD2 mRNA expression because of TGF-β signaling via TGF-β receptor 2 failured the placental vascular development in preeclampsia. This study was conducted to observe wether any different expression and correlation of TGF-β, TGFBR1 and 2, SMAD2, and Tsp-1 expression between normal and preeclampsia placentas, also to examine the possibility of TGF-β signal transduction disturbed through SMAD2 relative expression.

Materials and Methods

This observational study was a cross-sectional design, conducted in Molecular Biology Laboratory for Oxidative Stress, Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia. The samples used were biological material stored as many as 33 normal cases and 33 cases of pre-eclampsia. This research has been approved by the Ethic Committe of the Faculty of Medicine, University of Indonesia, number 0878/UN2.F1/ETIK/2018.

The examination of protein concentration: The first placenta was made to be homogenate. A total of 100 mg of placental tissue was balanced (Sartorius) and placed into a 1.5 mL microtube and then added with 500 µL PBS to be homogenized. After the tissue was completely blended, 500 µL PBS was added again and the homogenization process was resumed. The homogenate was then centrifuged for 10 min at 5.000 g, the supernatant formed was taken for the total protein content measured by spectrofluorophotometer (Varioscan) and compared to the standard curve formula of bovine serum albumin.

Examination of TGF-β and TGF-β protein receptor 1 and 2 levels was performed using the ELISA method. A total of 100 µL of standard solutions of all dilutions and samples were put into the microplate wells, sealed and incubated for 90 min at 37 °C. After that, the sample and standard solutions were removed from the wells, and filled with 100 µL Biotinylated Detection Ab Working Solution that was already diluted 100 times, and then incubated for 60 min at 37 °C. After, the solution was discharged, the well was washed using a 350 mL Wash Buffer (diluted 25 times), and repeated 3 times. In each standard and samples, 100 mL HRP Conjutae Working Solution (diluted 100

times) were put, and incubated for 30 min at temperature 37 °C. Then, after the solution was removed, the wells washed again with 350 µL wash buffer and repeated 5 times. A total of 90 µL Substrate Reagent was added to each well and incubated for 15-30 min to develop color, and the last, reaction was discontinued by adding 50 µL Stop Solution. The target protein concentration was examined by measuring the absorbance at a 450 nm wavelength and the result is calculated using the equation formula of the standard curve to obtain the concentration.

Measurement of SMAD2 and TSp-1 mRNA relative expression by reverse transcription polymerase chain reaction method (RT-PCR) method was performed using Sensifast™ SYBR No-ROX One Step Kit RT-PCR (Meridian Bioscience). Before performing the relative expression, total RNA isolation was measured using a total RNA minikit (Geneaid). The primers used are forward primer and reverse primer: SMAD2 Forward primer: 5'-ACC-GAA-ATG-CCA-CGG-TAG-AA-3', Reverse primer: 5'-TGG-GGC-TCT-GCA-CAA-AGA-T-3'; TSp-1 Forward primer: 5'-AGC-ATG-GTC-CTG-GAA-CTC-AG-3', Reverse primer: 5'-CAG-CTC-ATT-GGC-CAA-CTC-TT-3'; 18s rRNA Forward primer: 5'-AAA-CGG-CTA-CCA-CAT-CCA-AG-3', Reverse primer: 5'-CCT-CCA-ATG-GAT-CCT-CGT-TA-3'.

To obtain the relative expressions of mRNAs, optimization of the primary attachment temperature was prepared. The protocol of mRNA relative expression was in the following steps: Into each well the mix solution was added, it consisted of 10 µL Sensifast™ SYBR No-ROX One Step mix, 0.8 µL forward primer, 0.8 µL reverse primer, 0.4 µL Ribosafe RNase Inhibitor, 0.2 µL Reverse Transcriptase, 4 µL mRNA template, and DEPC-H_2O maximum 16 µL. Each sample was run in triplo and using NTC as a negative control. The samples were then incubated on a qRT-PCR machine (PCR max Ecosains) with the appropriate protocol kit temperature.

The result of one-step qRT-PCR is then analyzed further to determine the relative expression of the gene using the following formula:

$$\text{Relative Expresssion} = 2^{-\Delta\Delta Cq}$$

Statistical Analysis

Statistical analysis was performed using the statistical package for social science (SPSS) software for window version 2.0. All data were tested for their normality using Kolmogorov-Smirnov test. Furthermore, homogeneity tes, the normal and homogeneous data will be presented in mean and standard deviation using the unpaired t-test. Besides, the data that were not normally distributed, the Mann-Whitney test was used. Assessment of the correlation between two variables, the Pearson correlation

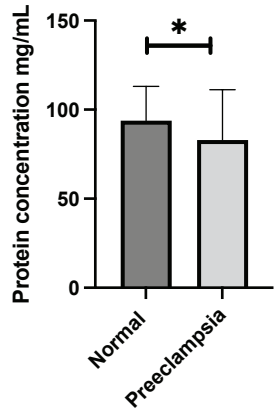

Figure 1. Placental protein concentration

It showed that the protein concentration in the normal placenta was significantly higher that the preeclampsia placenta, p=0.01

Table 1. Data distribution and significances

Placental Markers	Median (min-max)	p#	p$
TGFB-N (ng/mg)	1.19 (0.713-2.051)	0.0001	0.0001*
TGFB-PE (ng/mg)	2.69 (0.906-10.252)	0.0001	
TGFBR1-N (ng/mg)	1.025 (0.622-1.402)	0.0001	0.002*
TGFBR1-PE (ng/mg)	1.223 (0.372-2.553)	0.0001	
TGFBR2-N	0.959 (0.644-1.634)	0.0001	0.0001*
TGFBR2-PE	1.490 (0.775-3.645)	0.0001	
SMAD2-N (ng/mg)	2.087 (1.279-4.300)	0.0001	0.0001*
SMAD2-PE (ng/mg)	3.508 (1.842-22.489)	0.0001	
SMAD2 mRNA-N (ratio)	0.71 (0.03-7.25)	0.0001	0.075
SMAD2mRNA-PE (ratio)	0.49 (0.01-40.71)	0.0001	
TSp-1mRNA-N (ratio)	1.08 (0.09-5.31)	0.0001	0.0001*
TSp-1mRNA-PE (ratio)	0.21 (0.002-24.06)	0.0001	

#: Shapiro-Wilk test; $: Mann-Whitney test; *: Significantly different; N: Normal placenta; PE: Pre-eclampsia placenta, min: Minimum, max: Maximum, TSp-1: Thrombospondin-1

was used for normally distributed data and the Spearman test was used for the data thet were not normally distributed. The significant limit used was p<0.05.

Results

Protein Level of TGF-β

Before calculating the TGF-β concentration, the total protein of the placentas was measured. There was a significant different between normal and preeclampsia placenta protein contents (Figure 1). Measurement of TGF-β using the ELISA sandwich method showed that using the Mann-Whitney test it was proffed a significantly different between the relative expression of normal and preeclampsia markers. TGF-β levels in the preeclampsia placenta was 2.69 (0.906-10.252) ng/mg, it was increased significantly compared with normal placenta, 1.19 (0.713-2.051) ng/mg, p=0.0001, (Table 1, and Figure 2).

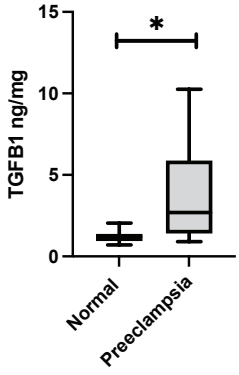

Figure 2. TGF-β protein levels in the placenta of normal pregnancy and pre-eclampsia. There was a significant difference between normal protein TGF-β levels and pre-eclampsia (Mann-Whitney, p=0.0001)

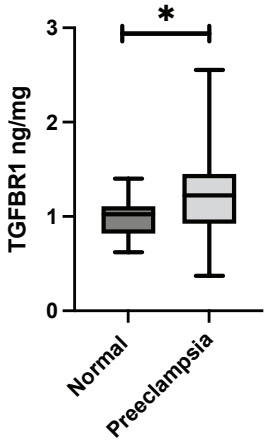

Figure 3. TGFBR1 protein levels in the placenta of normal pregnancy and pre-eclampsia. There was a significant difference between normal protein TGF-β levels and preeclampsia, (Mann-Whitney test, p=0.002)

Protein Level of TGF-β Receptor 1

Measurement of TGF-BR1 showed that its concentration was increased significantly in preeclampsia 1.2239 (0.372-2.553 ng/mg) compared to normal placenta 1.025 (0.622-1.402 ng/mg), p=0.002, (Figure 3 and Table 1).

Protein Level of TGF-β Receptor 2

TGF-β receptors 2 (TGFBR2) protein level were examined using the sandwich ELISA method (Figure 4). It showed that levels of TGF-β receptor 2 in the preeclamptic placenta 1.490 (0.775-3.645) ng/mg, it was higher significantly than normal 0.959 (0.644-1.6340) ng/mg. The Mann-Whitney test results showed a significant difference between the TGFBR2 relative expression of normal and preeclampsia with p=0.0001 (p<0.05), (Table 1).

Relative Expression of SMAD2 mRNA

The SMAD2 mRNA relative expression of preeclampsia was not significantly different compared to normal placentas, (Mann-Whitney test, p=0.075), Figure 5.

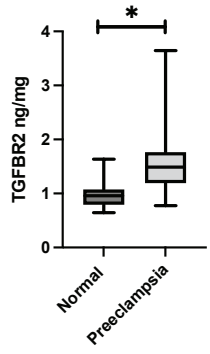

Figure 4. Protein levels of TGF-β receptor 2 in placental tissue normal pregnancy as control and pre-eclampsia. There was a significant difference between TGF-β protein levels. Normal receptor 2 and pre-eclampsia (Mann-Whitney test, p=0.0001)

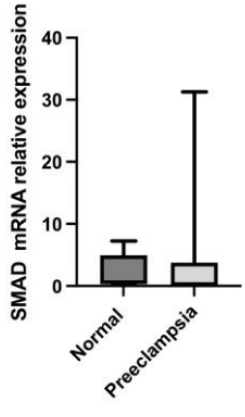

Figure 5. SMAD2 mRNA Relative Expression on normal placenta and pre-eclampsia. There was no significant difference between the relative expression of normal placental SMAD2 mRNA and preeclampsia (p=0.075)

Protein Level of SMAD2

SMAD 2 protein concentration showed that its concentration in the preeclampsia placenta 3.508 (1.842-22.489) ng/mg was significantly higher than normal 2.087 (1.279-4.300) ng/mg, (Mann-Whitney, p=0.0001), (Table 1, and Figure 6).

Thrombospondin mRNA Relative Expression

The relative expression of TSp-1 mRNA was decreased significantly in the preeclampsia placenta compared to normal, (Mann-Whitney, p=0.0001), (Table 1, and Figure 7).

Comparison of Protein Markers Correlation Between Preeclampsia and Normal Placentas

The correlation between TGF-β and TGF-BR1 in the normal placenta was strong, (Spearman correlation, R=0.789, p=0.0001), but in the preeclampsia placenta, there was no correlation between TGF-β and TGF-BR1 in the normal placenta, (Spearman correlation, R=0.053, p=0.76), (Table 2). A similar results shown in the correlation between TGF-β and TGF-BR2. In the preeclampsia placenta, there was no correlation between TGF-β and TGF-BR2 (Spearman correlation R=0.028, p=0.878), but in the normal placenta it showed a strong correlation (Spearman correlation R=0.623, p=0.0001), (Table 2). Correlation between TGF-BR1 and TGF-BR2 showed strong correlation both in normal (Spearman correlation, R=0.799) and preeclampsia placenta, (Table 2).

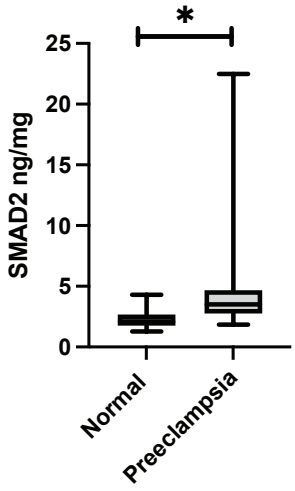

Figure 6. SMAD2 placental protein. There was a significant difference between normal and preeclampsia placenta, (Mann-Whitney, p=0.0001)

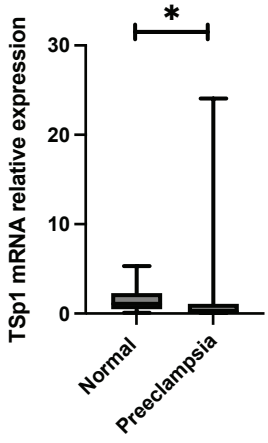

Figure 7. Thrombospondin-1 mRNA relative expression. There was a significant different between TSp-1 mRNA relative expression between preeclampsia and the normal placenta

Mann-Whitney test, p=0.0001.

Discussion

Preeclampsia is a disorder in pregnancy characterized by the presence of hypertension (blood pressure ≥140/90 mmHg) and proteinuria (urinary protein level ≥300 mg/24 h) at gestational age above 20 weeks[15,16]. Preeclampsia has a negative impact on the mother may lead to eclampsia and lead to HELLP syndrome, a set of syndromes characterized by microangiopathy, elevated liver enzymes (liver dysfunction) and low platelets[16,17]. Fetus of preeclampsia, may suffered to retardation during fetal growth, premature birth, low birth weight, and neonatal death[18,19].

There were several developing theories about the pathophysiology of preeclampsia, but in developmental research on pre-eclampsia was focused on the placenta. Placental ischemia in preeclampsia is placental failure in supplying oxygen and nutrients that results in pathophysiological disorders and affects fetal development and maternal health. The failure of the placenta is due to an interruption of the placental development process. In the process of placental development, during the invasion, trophoblasts induce spiral artery remodeling to form a low -resistance vascular system and it is essential for fetal development. This developmental stage is characterized by a physiological change in oxygen pressure at the base of the intervillous region[20].

During the first few weeks of pregnancy, the trophoblast is in a relatively low-oxygen environment. Maternal blood flow to the placenta tends to be low for trophoblast invasion, although this low oxygen pressure state is important for normal embryo and placental development. As soon as the spiral artery remodeling is completed, maternal blood flow to the fetus increases and

Table 2. Correlation between placental TGFB signaling proteins

Groups	Normal		Pre-eclampsia	
	R	p	R	p
TGFB and TGFBR1	0.789	**0.0001***	0.053	0.76
TGFB and TGFBR2	0.623	**0.0001***	0.028	0.878
TGFBR1 and TGFBR2	0.799	**0.0001***	0.783	**0.0001***
TGFBR1 and SMAD2	0.704	**0.0001***	0.675	**0.0001***
TGFBR2 and SMAD2	0.650	**0.0001***	0.539	**0.001***
SMAD2 and TSp-1 mRNA	0.013	0.943	0.151	0.401

*: Spearman correlation, TSp-1: Thrombospondin-1

hypoxia may be discontinued[20]. However, this condition does not occur in the placenta with impaired in trophoblast invasion, such as preeclampsia. Lack of optimum trophoblast invasion, cause the spiral artery remodeling process does not occur properly, leading to a decrease in maternal blood flow to the fetus, and hypoxia in pregnancy continues, leading to preeclampsia[3].

The trophoblastic invasion process involved tissues, multiple cytokines, growth factors, and angiogenesis factors that are mutually coordinated. These molecules interact with each other to provide biological effects, by triggering the transcription of certain proteins. Therefore, failure in coordination and interaction between cytokines and growth factors may impact the disruption of trophoblast invasion that triggers placental abruption. A cytokine that play a role in the development of the placenta is TGF-β. In this study, we found that there was an increase in TGF-β levels of placental pre-eclampsia. This agreed with previous studies suggesting that TGF-β protein levels are higher in placenta preeclampsia because TGF-β has an anti-angiogenesis effect through smad2 but proangiogenesis through smad3[21,22]. Additionally, Extra Villous Trophoblastic cells treated with TGF-β1, TGF-β2, and TGF-β3 showed decreased invasion by decreasing protease activity[22,23].

Increased levels of TGF-β in the placenta pre-eclampsia are also triggered by the presence of placental hypoxia. In a hypoxic state, the cells produce HIF-1 proteins that regulate the transcription of certain genes in response to their environment. This increase in HIF-1 also triggers an increased expression of TGF-β mRNA in trophoblasts[24]. Increased levels of TGF-β by HIF-1 may be intended to trigger angiogenesis to reduce hypoxi; it is considered that TGF-β via another receptor signaling molecule may also be proangiogenic. However, when the state of hypoxia did not goes down, the TGF-β levels increased excessively and eliminated its proangiogenic effec; also, the increased in SMAD2 led to suppress the trophoblast ability to make network[25].

Similar results were found in the examination of levels of TGF-β receptor 2 protein, which were higher in the preeclampsia than in the normal placenta. The interaction of the TGF-β ligand with its receptor triggers both of these receptor types for association, which are then followed by the phosphorylation of type 1 receptors by the type 2 receptors in the domain of the serine-threonin kinase. This activated type 1 receptor followed by passing the signal into an intracellular mediator called SMADs[6]. In line with elevated levels of TGF-β and TGF-β receptor 2 proteins, the relative expression of SMAD2 mRNA in preeclamptic placentas also significantly increased compared to normal placentas. This increase in SMAD2 mRNA expression was not very high compared with elevated levels of TGF-β and TGF-β receptor 2 proteins, it was considered that may be other factors affecting the regulation of SMAD2 mRNA expression.

In this study, the correlation analysis showed a weak positive correlation relationship between TGF-β protein and the relative expression of SMAD2 mRNA in the normal placenta. Conversely, a correlation analysis of both proteins in the preeclamptic placenta groups did not show any correlation. In the other side, a correlation test also performed on TGF-β receptor 2 protein levels with SMAD2 mRNA expression, it also showed no correlation between both parameters, either on normal or pre-eclampsia placentas.

In various studies on the TGF-β signaling pathway in preeclampsia, it mentioned that this protein will bind to both receptors, which can then give a pleiotropic effect: Proangiogenesis or antiangiogenesis[26]. It was also mentioned that TGF-β signaling via TGF-β receptor 1 (ALK5) and TGF-β Receptor 2 phosphorylates the antiangiogenesis SMAD2 protein[27,28]. This suggests that the TGF-β signaling pathway was more influential on the activation of latent SMAD2 protein, compared with its mRNA expression. We consider that any other molecule affects SMAD2 mRNA expression, based on the result that there was no correlation between TGF-β protein levels and their receptor with SMAD2 mRNA expression, presented in this study. Although, both groups of these parameters were increased in the preeclampsia placenta compared with normal.

Study Limitation

There was a limitation in this study due to using storaged placentas that not yet be parafined therefore the immunohystochemistry preparation couldnot be performed to observe TSp-1, and fibrotic area due to the high level of the TGF-β.

Conclusion

TGF-β protein level, TGF-β receptors and SMAD2 were increased in the preeclampsia placenta. Additionally, there was a mild positive correlation between TGF-β protein and relative expression of SMAD2 mRNA in the normal placenta, but was not found in the preeclamptic placenta. There may be other factors contributing to the regulation of SMAD2 mRNA expression.

Ethics

Ethics Committee Approval: This research has been approved by the Ethic Committe of the Faculty of Medicine, University of Indonesia, number 0878/UN2.F1/ETIK/2018.

Informed Consent: Not necessary.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.R.P., N.T.O., F.C.I., N.M., Y.P., Concept: A.R.P., N.T.O., F.C.I., N.M., Y.P., Design: A.R.P., N.T.O., F.C.I., N.M., Y.P., Data Collection or Processing: A.R.P., N.T.O., F.C.I., N.M., Y.P., Analysis or Interpretation: A.R.P., N.T.O., F.C.I., N.M., Y.P., Literature Search: A.R.P., N.T.O., F.C.I., N.M., Y.P., Writing: A.R.P., N.T.O., F.C.I., N.M., Y.P.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Lundine J HR, Sudrajat T. Indonesia's progress on the 2015 Millennium Development Goals. Indonesia 360. 2013; VOLUME 3/NUMBER 3.
2. Cameron L, Contreras Suarez D, Cornwell K. Understanding the determinants of maternal mortality: An observational study using the Indonesian Population Census. PLoS One 2019;14:e0217386.
3. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: Pathophysiology, Challenges, and Perspectives. Circ Res 2019;124:1094-112.
4. Kuklina EV, Ayala C, Callaghan WM. Hypertensive Disorders and Severe Obstetric Morbidity in the United States. Obstet Gynecol 2009;113:1299-306.
5. Roberts JM. Pathophysiology of ischemic placental disease. Semin Perinatol 2014;38:139-45.
6. Ji L, Brkic J, Liu M, Fu G, Peng C, Wang YL. Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. Mol Aspects Med 2013;34:981-1023.
7. Huang FCY. Regulation of TGF-b receptor activity.pdf. Cell & Bioscience 2012;2:9 http://wwwcellandbiosciencecom/content/2/1/9. 2012.
8. Shi Y, Massagué J. Mechanisms of TGF-β Signaling from Cell Membrane to the Nucleus. Cell 2003;113:685-700.
9. Ni N, Li Q. TGFbeta superfamily signaling and uterine decidualization. Reprod Biol Endocrinol 2017;15:84.
10. Knoller M, Pollheimer J. Human placental trophoblast invasion and differentiation: a particular focus on Wnt signaling. Front Genet 2013;4:190.
11. Yockey LJ, Iwasaki A. Interferons and Proinflammatory Cytokines in Pregnancy and Fetal Development. Immunity 2018;49:397-412.
12. Schmierer B, Hill CS. TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. Nat Rev Mol Cell Biol 2007;8:970-82.
13. Ten Dijke P, Arthur HM. Extracellular control of TGFbeta signaling in vascular development and disease. Nat Rev Mol Cell Biol 2007;8:857-69.
14. Nakagawa T, Li JH, Garcia G, Mu W, Piek E, Bottinger EP, et al. TGF-beta induces proangiogenic and antiangiogenic factors via parallel but distinct Smad pathways. Kidney Int 2004;66:605-13.
15. Nikkei P, Rajaei M, Roozbeh N, Mohseni F, Poordarvishi F, Azad M et al. Diagnostic accuracy of sFlt1/PlGF ratio as a marker for preeclampsia. BMC Pregnancy Childbirth 2020;20:80.
16. Steegers EAP, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. The Lancet 2010;376:631-44.
17. Mustafa R, Ahmed S, Gupta A, Venuto RC. A comprehensive review of hypertension during pregnancy. J Pregnancy 2012;2012:105918.
18. Sharma D, Shastri S, Sharma P. Intrauterine Growth Restriction: Antenatal and Postnatal Aspects. Clin Med Insights Pediatr 2016;10:67-83.
19. Surico D, Borodino V, Cantaluppi V, Mary D, Gentilli S, Oldani A, et al. Pre-eclampsia and intrauterine growth restriction: Role of human umbilical cord mesenchymal stem cells-trophoblast cross-talk. PLoS One 2019;14:e0218437.
20. Nishi H, Nakada T, Hokamura M, Osakabe Y, Itokazu O, Huang LE, et al. Hypoxia-inducible factor-1 transactivates transforming growth factor-beta3 in trophoblasts. Endocrinology 2004;145:4113-8.
21. Cheng JC, Chang HM, Leung PC. Transforming growth factor-beta1 inhibits trophoblast cell invasion by inducing Snail-mediated down-regulation of vascular endothelial-cadherin protein. J Biol Chem 2013;288:33181-92.
22. Davies JE, Pollheimer J, Yong HE, Kokkinos MI, Kalionis B, Knoller M et al. Epithelial-mesenchymal transition during extravillous trophoblast differentiation. Cell Adh Migr 2016;10:310-21.
23. Zhang Y, Yang X. The Roles of TGF-beta Signaling in Cerebrovascular Diseases. Front Cell Dev Biol 2020;8:567682.
24. Furuta C, Miyamoto T, Takagi T, Noguchi Y, Kaneko J, Itoh S, et al. Transforming growth factor-beta signaling enhancement by long-term exposure to hypoxia in a tumor microenvironment composed of Lewis lung carcinoma cells. Cancer Sci 2015;106:1524-33.
25. Brkic J, Dunk C, Shan Y, O'Brien JA, Lye P, Qayyum S et al. Differential Role of Smad2 and Smad3 in the Acquisition of an Endovascular Trophoblast-Like Phenotype and Preeclampsia. Front Endocrinol (Lausanne) 2020;11:436.
26. Budi EH, Mamai O, Hoffman S, Akhurst RJ, Derynck R. Enhanced TGF-beta Signaling Contributes to the Insulin-Induced Angiogenic Responses of Endothelial Cells. iScience 2019;11:474-91.
27. Lash GE, Otun HA, Innes BA, Bulmer JN, Searle RF, Robson SC. Inhibition of trophoblast cell invasion by TGFB1, 2, and 3 is associated with a decrease in active proteases. Biol Reprod 2005;73:374-81.
28. Walshe TE, Saint-Geniez M, Maharaj AS, Sekiyama E, Maldonado AE, D'Amore PA. TGF-beta is required for vascular-barrier function, endothelial survival, and homeostasis of the adult microvasculature. PLoS One 2009;4:e5149.