Acta Biochimica Indonesiana

https://doi.org/10.32889/actabioina.61

RESEARCH ARTICLE

Open Access

The correlation between malondialdehyde concentration and FOXO3 and CASP3 mRNA expression changed in early-onset preeclampsia placenta

Ni Made Wiasty Sukanty[1], Febriana Catur Iswanti[2,3], Syarifah Dewi[2,3], Muhammad Faruqi[4], Alyssa Shafa Andiana[4], Ani Retno Prijanti[2,3*]

[1]Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia
[2]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia
[3]Centre of Hypoxia and Oxidative Stress Studies, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia
[4]Undergraduate Medical Education Program, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia
[*]Correspoding author: Jl. Salemba Raya No. 6, Central Jakarta 10430, phone (021) 31930373. Email: aniretno@gmail.com

ABSTRACT

Background: Preeclampsia is one of the factors causing the high maternal mortality rate. The risk of morbidity and mortality is higher in Early Onset Preeclampsia (EOPE). Failure of spiral artery remodeling can cause oxidative stress that can inhibit placental development and increase trophoblast apoptosis.

Objective: This study aims to analyze the oxidative stress and apoptosis of EOPE placentas.

Methods: This study is an observational study with a cross-sectional design. A total of 31 EOPE placentas and 31 normal term placentas were used to measure the concentration of malondialdehyde (MDA) and the relative mRNA expression of FOXO3 and CASP3 using the spectrophotometric and RT-qPCR methods.

Results: There was no difference in MDA concentration ($p = 0.580$) and FOXO3 ($p = 0.467$) and CASP3 ($p = 0.243$) mRNA expression in the normal and EOPE groups. There was a strong positive correlation between FOXO3 and CASP3 mRNA expression in the normal ($p= 0.0001$; $r = 0.938$) and EOPE groups ($p = 0.0001$; $r = 0.855$). There was no correlation between MDA concentration to FOXO3 ($p = 0.124$; $r = 0.282$) and CASP3 ($p = 0.569$; $r = 0.106$) mRNA expression in normal placenta. There was positive correlation between MDA concentration to FOXO3 ($p = 0.016$; $r = 0.429$) and CASP3 mRNA expression in EOPE placenta ($p = 0.028$; $r = 0.395$).

Conclusion: These results indicate that cell integrity is still maintained through the autophagy process and the level of apoptosis in EOPE placenta is regulated by ROS through FOXO3.

Keywords: apoptosis, oxidative stress, placenta, pre-eclampsia

Introduction

The hypertensive disorder is one of the pregnant women's problems that causes the mortality of mothers and babies [1]. According to WHO, one of the causes is preeclampsia. In 2017, the maternal mortality rate in developed countries reached 11/100000 live births, while in developing countries, it was higher, at 462/10000000 [2]. In addition, the International Federation of Gynecology and Obstetrics (FIGO) states that every year there are 76000 women and 500000 babies die from preeclampsia [3].

Preeclampsia is characterized by hypertension with or without proteinuria at about 20 weeks of gestation. This disorder can progress to eclampsia, causing hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome [4]. Early-onset preeclampsia (EOPE) is a type of preeclampsia that appears before 34 weeks. EOPE provides a higher risk of morbidity and mortality than late-onset preeclampsia (LOPE) [5].

Hypoxia affects placental development. In early pregnancy, physiological hypoxia occurs

to support the remodeling of the spiral arteries that increase the diameter of the blood vessels, forming oxygen-rich blood flow from the mother to the fetus [5]. Although the pathogenesis of EOPE is unclear, it is believed that failure of spiral artery remodeling causes continued hypoxia and contributes to increased placental trophoblast apoptosis [1,5,6]. Hypoxia is generally accompanied by an increase in ROS synthesis. High ROS cause oxidative stress and cell damage by affecting various macromolecules, such as lipid [7–9]. In the presence of ROS, polyunsaturated fatty acids can undergo peroxidation and initiate the formation of lipid adducts. Malondialdehyde (MDA) is a secondary product of lipid peroxidation. It is commonly used as a surrogate marker for oxidative stress [10].

Several studies have tested the MDA parameters in the placenta. Sahay et al. showed that MDA levels in preeclamptic placentas were higher than normal placentas, implying that ROS increase in the preeclamptic placenta [11]. In contrast, Prijanti et al. showed that the MDA levels of preeclamptic placentas were lower than those of the normal placenta [12]. Nonetheless, both studies were conducted on preeclamptic placentas. In this study, we wanted to focus on EOPE placentas.

Placental development is affected by autophagy. Several factors that cause autophagy have been identified, such as nutritional deficiencies, hypoxia, and oxidative stress. The mechanism of autophagy can lead to apoptosis [13,14]. In preeclampsia, placental trophoblast apoptosis is increased. Caspase-3 (encoded by *CASP3*), an executor of apoptosis, acts as a protease effector regulated by Forkhead Box O3 (FOXO3) [14,15]. Previous studies have shown that hypoxia-induced HTR8/SVneo cells have increased apoptosis associated with nuclear FOXO3 [6]. However, the relationship between FOXO3 and CASP3 is not yet known in EOPE placentas.

This study aims to determine the oxidative stress and apoptosis in EOPE placentas by measuring, comparing, and correlating the MDA concentrations and the relative expression of FOXO3 and CASP3 mRNA in normal and EOPE placentas.

Methodology

This study is an observational study with a cross-sectional design. A total of 62 placental tissues used in this study were 62 stored biological material obtained from each 31 pregnant women with normal pregnancies and pregnant women with EOPE syndrome at Cipto Mangunkusumo Hospital and Budi Kemuliaan Hospital, Jakarta. The normal group samples were obtained from pregnant women with normotensive blood pressure at gestational age ≥ 37 weeks, while the EOPE group sample was obtained from pregnant women with preeclampsia syndrome and normotensive antenatal blood pressure before experiencing preeclampsia. These tissues were taken at < 34 weeks' gestation. The samples were stored at -80°C. This research was conducted at the Laboratory of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, and the Laboratory of Molecular Biology and Proteomic Core Facilities at Indonesian Medical Education and Research Institute (IMERI) Universitas Indonesia (UI).

MDA level measurement

One hundred milligrams of placental tissue was homogenated using 1 mL of 0.05 M PBS pH 7.4. The homogenate was centrifuged at 5000 rpm for 10 minutes, and the supernatant formed was taken. MDA was measured using the Wills method by adding 200 µL of 20% trichloroacetate (TCA). The sample was then centrifuged. Then 400 µL of 0.67% thiobarbituric acid (TBA) was added to the supernatant, and the sample was heated at 96°C for 10 minutes. The sample was allowed to cool at room temperature, and the absorbance was measured at 530 nm.

Relative FOXO3 and CASP3 mRNA expression measurement

RNA preparation and isolation were carried out according to the Quick-RNATM Miniprep Plus Kit R1058 procedure from ZYMO Research. RNA concentration and purity were measured using µdrops at 260 nm and 280 nm. We used 0.1

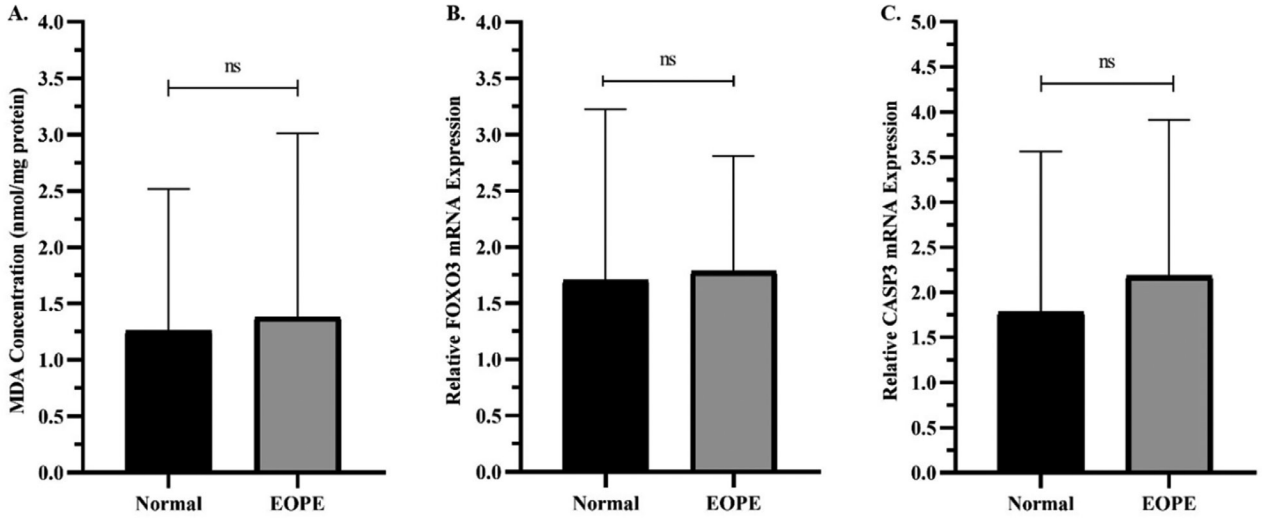

Figure 1. The MDA level and relative expression of FOXO3 and CAPS3. (a) The comparison of MDA concentration in normal and EOPE groups (p = 0.580). (b) The relative FOXO3 mRNA expression in normal and EOPE groups (p = 0.467). (c) The relative CASP3 mRNA expression in normal and EOPE groups (p = 0.243).

g of RNA sample, BIO-72005 SensiFAST SYBR No-ROX One-Step Kit from Meridian Bioscience, and primers (Table 1) to measure the relative expression of FOXO3, CASP3, and 18S rRNA mRNAs. The annealing temperature for the 18S rRNA, FOXO3, and CASP3 genes was 50ºC. The relative mRNA expression was carried out using the Livak method according to the CT values.

Table 1. Forward and reverse primers.

Gene	Primer
18S rRNA	F : CACGGACAGGATTGACAGATT
	R : GCCAGAGTCTCGTTCGTTATC
FOXO3	F : GCAGACCATCCAAGAGAACA
	R : GCTAAGTGAGTCCGAAGTGAG
CASP3	F : CTTGGCGAAATTCAAAGGATGG
	R : CCCGGGTAAGAATGTGCATAA

Data analysis

We used SPSS version 23 software to perform statistical tests and Graphpad Prism 8 to made the graph. First, the normal distribution and homogeneity test were carried out, then proceed to the independent sample t-test to see the differences of each parameter in the EOPE and normal groups. The correlation between MDA and FOXO3 and CASP3 was tested using the Spearman's rho correlation test, while the correlation between FOXO3 and CASP3 mRNA expressions was tested using the Pearson correlation test. The normally distributed data are shown in mean ± SD with $p < 0.05$ indicating a significant difference.

Results

MDA is a marker of oxidative stress. To determine the presence of oxidative stress in EOPE placentas, we tested MDA concentrations in normal and EOPE placental samples. Independent sample t-test results showed that the average concentration of MDA in the normal group was 1.257 ± 1.260 nmol/mg protein, while the EOPE group was 1.381 ± 1,630 nmol/mg protein (p = 0.580) (Figure 1A).

To determine the extent of apoptosis occurring in EOPE placentas, we measured CASP3 mRNA expression. As a link between oxidative stress and apoptosis, we chose to assay FOXO3 mRNA expression because FOXO3 is a regulator of oxidative stress and is involved in the mechanism of apoptosis. The expression of FOXO3 mRNA in the normal group was 1.705 ± 1.520 and in the EOPE group was 1.783 ± 1.027 (p = 0.467) (Figure 1B). As for CASP3, the mRNA expression in the normal group was 1.782 ± 1.780) and in the EOPE group, it was 2.180 ± 1.732 (p = 0.243) (Figure 1C). The

statistical test results for FOXO3 and CASP3 also showed no significant results ($p<0.05$).

The relationship between oxidative stress and apoptosis in the EOPE placenta was tested using Spearman's rho correlation test. We tested the correlation between the parameters. FOXO3 and CASP3 mRNA expression had a strong positive correlation in both the normal group ($p < 0.05$) and the EOPE group ($p < 0.05$) (Figure 2A-B). The MDA concentration and FOXO3 mRNA expression did not correlate in normal placentas ($p > 0.05$) (Figure 2C), nor did the correlation of MDA and CASP3 (Figure 2E). Meanwhile, in the EOPE placenta, FOXO3 and CASP3 mRNA expressions both showed a positive correlation to MDA concentration ($p < 0.05$) (Figure 2D and 2F).

Discussion

The placenta is the primary tissue that connects the mother and the fetus. Its vascular system is used to transport the nutrients, gases, and various waste materials that support fetal growth and development. This system is formed through the spiral artery remodeling that occurs early in pregnancy [16,17]. Failure in the spiral artery remodeling can lead to vascular dysfunction [18]. Narrow blood vessels initiate hypoperfusion, hypoxia, and trigger ROS synthesis, which leads to oxidative stress [19]. Although there is no definite theory regarding the pathogenesis of preeclampsia, it is believed that vascular dysfunction is one of the factors that cause preeclampsia [18].

Increased levels of MDA can be used as a marker of oxidative stress [10]. Although oxidative stress is often associated with preeclampsia, we did not find any difference in MDA concentrations in EOPE and normal placentas ($p>0.05$) in this study. This result differs from Sahay et al., which showed higher MDA levels in preeclamptic placental samples [11]. Likewise, Huang et al. used a rat model of preeclampsia and showed an increase in serum MDA concentrations compared to control rats. It is caused by high ROS synthesis due to active metabolism in mitochondria [18]. The opposite result is shown by Prijanti et al. that showed a decrease of MDA levels in preeclampsia placental samples [12].

The synthesis of ROS can be inhibited through antioxidant mechanisms [10]. One of the proteins that play a role in this process is hypoxia-inducible factor-1α (HIF-1α) [20]. We also tested HIF-1α concentrations in both sample groups (data in progress to publish). The much higher concentration of HIF-1α in EOPE placentas may be the reason for the absence of an increase in MDA in EOPE placentas. In addition to HIF-1α, FOXO3 also increases the expression of antioxidant enzymes [21]. There was no difference in MDA concentration in the two groups in this study, indicating that in the EOPE placenta, antioxidants were still able to overcome the ROS formed. It seems aimed to preserve vascular function.

The spiral artery remodeling is supported by autophagy, a catabolic process that degrades cell components to maintain cell homeostasis. Autophagy in stressful conditions such as hypoxia and oxidative stress can lead to two possibilities, cell survival or cell death [22]. FOXO3 is a member of the FOXO family that plays a role in regulating autophagy and apoptosis. The disturbances in basal autophagy can inhibit the degradation of FOXO3. Consequently, FOXO3 activates autophagy-related genes so that autophagy can continue their process. However, if this process fails, FOXO3 will inhibit the expression of antiapoptotic proteins such as Bcl-2 and activate apoptosis-related genes such as Bcl-2-like protein 11 (BIM) and Bcl2-associated X protein (BAX) [21,23].

Apoptosis is programmed cell death [24]. Apoptosis during pregnancy aims to assist in differentiation, fusion, and trophoblast degeneration [25]. The balance between pro-apoptotic proteins such as caspase 3 and antiapoptotic proteins such as Bcl2 can help to establish a proper spiral artery remodeling process [26]. Excessive apoptosis can cause endothelial cell dysfunction. In some studies, there was a higher rate of apoptosis in preeclamptic placentas than in normal placentas [25,26].

To confirm the apoptosis in EOPE placentas, we tested the FOXO3 and CASP3 mRNA expression.

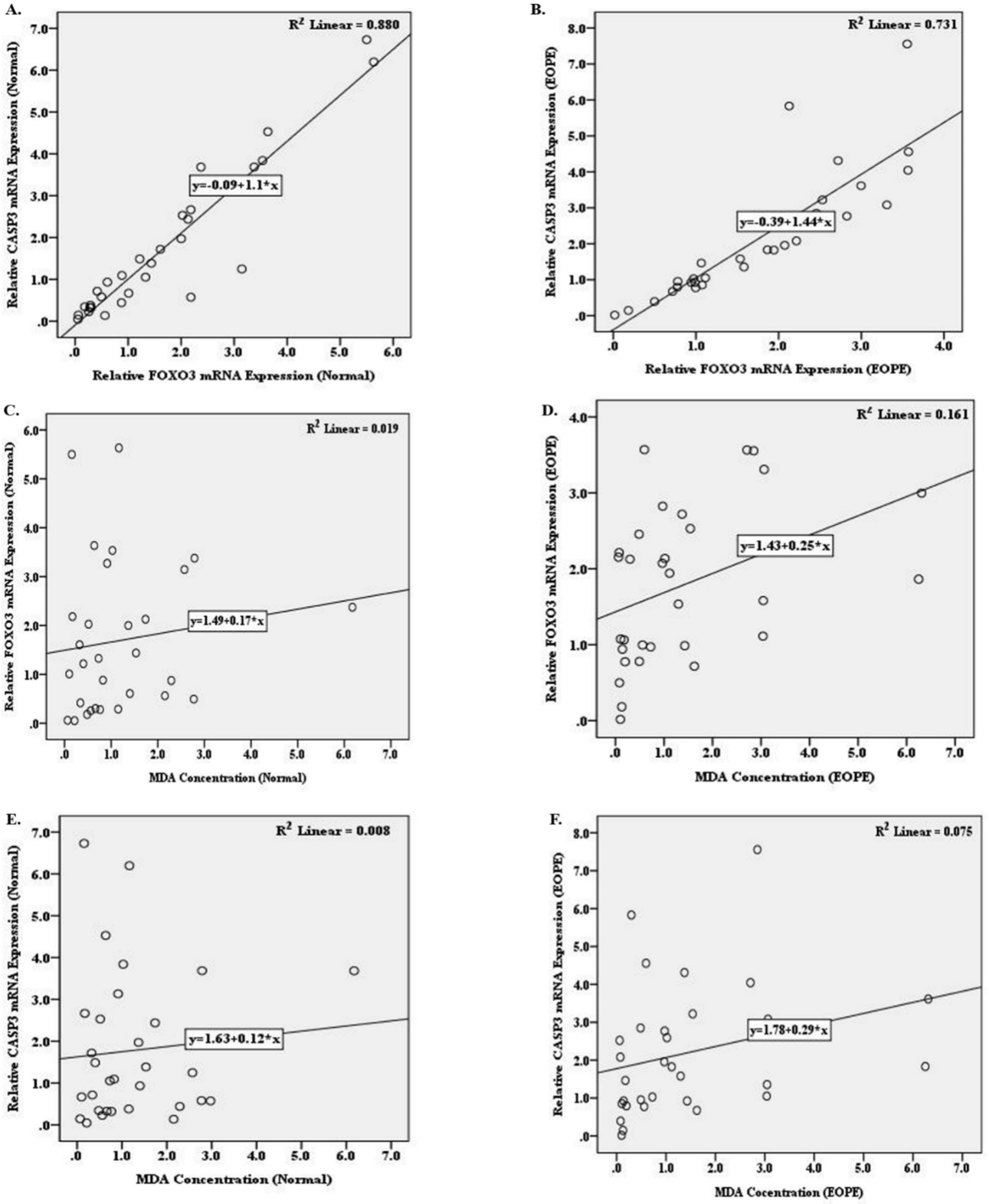

Figure 2. The correlation of MDA, FOXO, CASP3 in normal and EOPE groups. (A) The correlation of FOXO3 and CASP3 in normal group (p = 0.0001; r = 0.938). (B) The correlation of FOXO3 and CASP3 in EOPE group (p = 0.0001; r = 0.855). (C) The correlation of MDA and FOXO3 in normal group (p = 0.124; r = 0.282). (D) The correlation of MDA and FOXO3 in EOPE group (p = 0.016; r = 0.429). (E) The correlation of MDA and CASP3 in normal group (p = 0.569; r = 0.106). (F) The correlation of MDA and CASP3 in EOPE group (p = 0.028; r = 0.395).

The statistical test results showed no differences in the FOXO3 and CASP3 mRNA expressions in both groups. The results of this study are different from Zhang et al. They used the preeclamptic placenta and HTR8/SVneo cells with high HIF-1α and found an increase in FOXO3 expression and trophoblast apoptosis. It is thought to be a factor in the occurrence of preeclampsia [6]. As a downstream in the apoptotic stage, caspase 3 acts as an apoptotic executor. There was no difference in CASP3 expression in the two groups (Figure 1C). It was in line with FOXO3 mRNA expression and can be seen from the correlation test that showed a strong positive correlation in both groups (Figure 2A-B). It is due to the role of FOXO3 as a transcription factor that regulate CASP3 expression [15]. This result is different from Cali et al.'s and Pramatirta et al.'s studies. They showed a significant increase in caspase 3 in preeclamptic placentas and preeclampsia-serum-induced trophoblast cells [26,27]. On the other hand, Kadyrov et al. and Stepan et al. showed a decrease in apoptosis in preeclamptic placentas. Stepan et al. suspected there is a change from apoptosis to necrosis in preeclampsia that leads to decreased apoptosis. This process is associated with a hypoxic state. Chronic hypoxia in preeclampsia is well tolerated and has been suggested to cause decreased trophoblast apoptosis [28]. The absence of differences in the FOXO3 and CASP3 mRNA expression in EOPE and normal placentas indicated no increase in apoptosis in EOPE placentas. We suspect this process involves a hypoxic state. Hypoxia can be regulated by FOXO3 via autophagy homeostasis. The complex formation between FOXO3, HIF1, and p300 can inhibit the transcriptional activity of HIF1 in the genes involved in apoptosis [21].

To investigate the association between oxidative stress and apoptosis in EOPE placentas, we tested the correlation of MDA with FOXO3 and CASP3 mRNA expression. There is no correlation between MDA with FOXO3 and CASP3 in the normal group. It indicates that in the normal placenta, the expression of FOXO3 and CASP3 was not affected by ROS. In normal pregnancy, ROS is involved in angiogenesis [29]. In contrast, the correlation between MDA and FOXO3 in EOPE placentas was moderately positive, while it was weak in the correlation between MDA and CASP3. It indicates that in EOPE placentas, the expression of FOXO3 and CASP3 was affected by ROS. High ROS cause endothelial dysfunction through deleterious effects [29].

Although there were no differences in MDA, FOXO3, and CASP3 in this study, the correlation of MDA with FOXO3 and CASP3 became new findings to better understand the relationship between oxidative stress and apoptosis, especially in EOPE placental. Shaker et al. proved that in preeclamptic placentas, high concentrations of MDA and an increase in apoptosis due to oxidative damage were found. Nevertheless, there was no correlation between MDA and caspase 9 as an initiator of apoptosis in the tested preeclamptic placentas [30].

Apoptosis occurs gradually. Initially, there is an initiation process in which the cell receives the death signal, mitochondrial decision, and caspase activation. It continued to cleave protein and DNA fragmentation. As a caspase initiator, activation of caspase 9 can lead to activation of caspase 3, an effector caspase [30]. Unlike Shaker et al.'s study that analyzed caspase 9, in this study, caspase 3 expressions in placental EOPE was positively correlated to MDA. It suggests that oxidative stress is involved in the regulation of apoptosis. Shaker et al. also showed a positive relationship between gestational age and preeclampsia placental MDA levels [30]. The correlation between MDA with FOXO3 and CASP3 in EOPE placentas without differences in their expression compared to normal placentas may also be influenced by the gestational age and pathogenesis of EOPE.

Conclusion

There was no difference in MDA concentration and mRNA expression of FOXO3 and CASP3 in normal and EOPE placentas, but there was a strong positive correlation between FOXO3 and CASP3 in both groups and a positive correlation between MDA and FOXO3 and CASP3 in EOPE placenta. The results obtained in this study may indicate

that cell integrity is still maintained through the autophagy process, and the level of apoptosis in the EOPE placenta is regulated by ROS through FOXO3.

Acknowledgment

This study was funded by Hibah PUTI-Q2 2020.

Author contributions

Conceptualization, ARP, FCI, SD; Methodology, ARP, FCI, SD; Investigation, NMWS, MF, ASA; Formal analysis, NMWS, MF, ASA; Writing – Original Draft, ARP, NMWS; Writing – Review & Editing, ARP, NMWS; Supervision, FCI, SD; Visualization, NMWS; Validation and data curation and funding acquisition, ARP.

Declaration of interest

The authors do not have any conflict of interest.

Received: 19 December 2021
Accepted: 31 December 2021
Published online: 31 December 2021

References

1. Anton L, DeVine A, Polyak E, Olarerin-George A, Brown AG, Falk MJ, et al. HIF-1α Stabilization Increases miR-210 Eliciting First Trimester Extravillous Trophoblast Mitochondrial Dysfunction. Front Physiol. 2019;10: 699. https://doi.org/10.3389/fphys.2019.00699
2. Maternal mortality [Internet]. [cited 8 Jan 2022]. Available: https://www.who.int/news-room/fact-sheets/detail/maternal-mortality
3. FIGO releases new guidelines to combat Pre-Eclampsia | Figo [Internet]. [cited 8 Jan 2022]. Available: https://www.figo.org/figo-releases-new-guidelines-combat-pre-eclampsia
4. Nevalainen J, Skarp S, Savolainen E-R, Ryynänen M, Järvenpää J. Intrauterine growth restriction and placental gene expression in severe preeclampsia, comparing early-onset and late-onset forms. J Perinat Med. 2017;45: 869-877. https://doi.org/10.1515/jpm-2016-0406
5. Gathiram P, Moodley J. Pre-eclampsia: its pathogenesis and pathophysiolgy. Cardiovasc J Afr. 2016;27: 71-78. https://doi.org/10.5830/CVJA-2016-009
6. Zhang Z, Huang C, Wang P, Gao J, Liu X, Li Y, et al. HIF-1α affects trophoblastic apoptosis involved in the onset of preeclampsia by regulating FOXO3a under hypoxic conditions. Mol Med Rep. 2020;21: 2484-2492. https://doi.org/10.3892/mmr.2020.11050
7. Li H-S, Zhou Y-N, Li L, Li S-F, Long D, Chen X-L, et al. HIF-1α protects against oxidative stress by directly targeting mitochondria. Redox Biol. 2019;25: 101109. https://doi.org/10.1016/j.redox.2019.101109
8. Lacher SE, Levings DC, Freeman S, Slattery M. Identification of a functional antioxidant response element at the HIF1A locus. Redox Biol. 2018;19: 401-411. https://doi.org/10.1016/j.redox.2018.08.014
9. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014: 360438. https://doi.org/10.1155/2014/360438
10. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2017;524: 13-30. https://doi.org/10.1016/j.ab.2016.10.021
11. Sahay AS, Sundrani DP, Wagh GN, Mehendale SS, Joshi SR. Regional differences in the placental levels of oxidative stress markers in pre-eclampsia. Int J Gynaecol Obstet. 2015;129: 213-218. https://doi.org/10.1016/j.ijgo.2015.03.001
12. Prijanti AR, Marissa N, Paramita R, Humaira S, Nabila EN, Wijaya AE, et al. Analysis of oxidative stress markers malondialdehyde, glutathione, nitric oxide, and prorenin level in preeclampsia placental tissues. Asian J Pharm Clin Res. 2018;11: 158. https://doi.org/10.22159/ajpcr.2018.v11i1.18330
13. Oh S-Y, Roh C-R. Autophagy in the placenta. Obstet Gynecol Sci. 2017;60: 241-259. https://doi.org/10.5468/ogs.2017.60.3.241
14. Kasture V, Sahay A, Joshi S. Cell death mechanisms and their roles in pregnancy related disorders. Adv Protein Chem Struct Biol. 2021;126: 195-225. https://doi.org/10.1016/bs.apcsb.2021.01.006
15. Zhu D, Yuan D, Guo R, Zhang L, Guo T, Zhao Y, et al. Overexpression of miR-148a inhibits viability and invasion of ovarian cancer OVCAR3 cells by targeting FOXO3. Oncol Lett. 2019;18: 402-410. https://doi.org/10.3892/ol.2019.10321
16. Gao L, Qi H-B, Kamana KC, Zhang X-M, Zhang H, Baker PN. Excessive autophagy induces the failure of trophoblast invasion and vasculature: possible relevance to the pathogenesis of preeclampsia. J Hypertens. 2015;33: 106-117. https://doi.org/10.1097/HJH.0000000000000366
17. Albers RE, Kaufman MR, Natale BV, Keoni C, Kulkarni-Datar K, Min S, et al. Trophoblast-Specific Expression of Hif-1α Results in Preeclampsia-Like Symptoms and Fetal Growth Restriction. Sci Rep. 2019;9: 2742. https://doi.org/10.1038/s41598-019-39426-5
18. Huang J, Zheng L, Wang F, Su Y, Kong H, Xin H. Mangiferin ameliorates placental oxidative stress and activates PI3K/

Akt/mTOR pathway in mouse model of preeclampsia. Arch Pharm Res. 2020;43: 233-241. https://doi.org/10.1007/s12272-020-01220-7

19. Chen C-P, Chen C-Y, Wu Y-H, Chen C-Y. Oxidative stress reduces trophoblast FOXO1 and integrin β3 expression that inhibits cell motility. Free Radic Biol Med. 2018;124: 189-198. https://doi.org/10.1016/j.freeradbiomed.2018.06.006
20. Xu G. HIF-1-mediated expression of Foxo1 serves an important role in the proliferation and apoptosis of osteoblasts derived from children's iliac cancellous bone. Mol Med Rep. 2018;17: 6621-6631. https://doi.org/10.3892/mmr.2018.8675
21. Fasano C, Disciglio V, Bertora S, Lepore Signorile M, Simone C. FOXO3a from the Nucleus to the Mitochondria: A Round Trip in Cellular Stress Response. Cells. 2019;8. https://doi.org/10.3390/cells8091110
22. Carvajal L, Gutiérrez J, Morselli E, Leiva A. Autophagy process in trophoblast cells invasion and differentiation: similitude and differences with cancer cells. Front Oncol. 2021;11: 637594. https://doi.org/10.3389/fonc.2021.637594
23. Fitzwalter BE, Thorburn A. FOXO3 links autophagy to apoptosis. Autophagy. 2018;14: 1467-1468. https://doi.org/10.1080/15548627.2018.1475819
24. Wu F, Tian F-J, Lin Y. Oxidative stress in placenta: health and diseases. Biomed Res Int. 2015;2015: 293271. https://doi.org/10.1155/2015/293271
25. Hung T-H, Burton GJ. Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia. Taiwan J Obstet Gynecol. 2006;45: 189-200. https://doi.org/10.1016/S1028-4559(09)60224-2
26. Cali U, Cavkaytar S, Sirvan L, Danisman N. Placental apoptosis in preeclampsia, intrauterine growth retardation, and HELLP syndrome: an immunohistochemical study with caspase-3 and bcl-2. Clin Exp Obstet Gynecol. 2013;40: 45-48.
27. Pramatirta AY, Laksono B, Fauziah PN, Anwar AD, Widiarsih E. Effects of Low Dose Aspirin on Caspase 3, TNF-α and Apoptotic Index Levels in Preclampsia Maternal Serum-Induced Placental Trophoblast Cell Line In Vitro. International Journal of PharmTech Research. 2016;
28. Stepan H, Leo C, Purz S, Höckel M, Horn LC. Placental localization and expression of the cell death factors BNip3 and Nix in preeclampsia, intrauterine growth retardation and HELLP syndrome. Eur J Obstet Gynecol Reprod Biol. 2005;122: 172-176. https://doi.org/10.1016/j.ejogrb.2005.01.017
29. Lu J, Wang Z, Cao J, Chen Y, Dong Y. A novel and compact review on the role of oxidative stress in female reproduction. Reprod Biol Endocrinol. 2018;16: 80. https://doi.org/10.1186/s12958-018-0391-5
30. Shaker OG, Sadik NAH. Pathogenesis of preeclampsia: Implications of apoptotic markers and oxidative stress. Hum Exp Toxicol. 2013;32: 1170-1178. https://doi.org/10.1177/0960327112472998