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Serum HIF-1α Levels, miR-210 Expressions, and Reactive Oxygen Species Levels in Early Abortion and Normal Pregnancy

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Abstract

BACKGROUND: The blastocyst implants into the endometrium which is in a relatively hypoxic state. Hypoxic state triggers hypoxia-inducible factor-1α (HIF-1α) production, upregulates the transcription factor micro-RNA 210 (miR-210), and stimulates reactive oxygen species (ROS) production by trophoblast cells. HIF-1α also increases the expression of miR-210. High expression of miR-210 suppresses mitochondrial respiration, increasing ROS production. High level of ROS may result in DNA damage or cell dysfunction, thereby impaired trophoblast invasion, leading to early abortion.

AIM: This study aims to determine the differences of serum HIF-1α levels, miR-210 expressions, and ROS levels between early abortion and normal pregnancy.

METHODS: This cross-sectional comparative study was conducted in Dr. M. Djamil Hospital Padang, Andalas University Hospital, and five Public Health Centers in Padang. Fifty patients with gestational age <12 weeks (25 early abortions and 25 normal pregnancies) were included in this study. All samples were tested for HIF-1α and ROS level using enzyme-linked immunosorbent assay method and miR-210 expression using real-time polymerase chain reaction technique. Spearman correlation and Mann–Whitney U-test were used in this study.

RESULTS: Both study groups were equivalent in terms of age, gestational age, and gravidity (p = 0.51, 0.453, and 1.00). The median of HIF-1α level, miR-210 expression, and ROS level was higher in early abortions than normal pregnancies, that is, 3.73 versus 3.42 ng/mL (p = 0.016), 43.55 versus 17.85 copies/ng (p = 0.027), and 1.36 versus 1.20 ng/mL (p = 0.003). The coefficient correlations were 0.16 between HIF-1α level and miR-210 expression (p = 0.267), 0.46 between HIF-1α level and ROS level (p = 0.001), and 0.18 between miR-210 expression and ROS level (p = 0.207).

CONCLUSIONS: HIF-1α level, miR-210 expression, and ROS level were associated with early abortion. HIF-1α level has a correlation with ROS level.

Introduction

The main cause of early pregnancy loss was abortion, with incidence of 10–15%. Eighty percent of abortion occurred in <12 weeks pregnancy (early abortion) [1]. The early abortion is closely related to failure of placentation and impaired trophoblastic invasion in early gestation [2].

Placentation was preceded by blastocyst implantation to endometrium that was in relative hypoxia state [3]. This hypoxic state is important for trophoblastic growth and development [2], [4], early trophoblastic invasion [3], and angiogenesis [5].

Ongoing hypoxic state of endometrium is the first trimester of pregnancy [6]. This condition is the main factor for physiologic trophoblastic invasion [4]. This meant that hypoxic condition determines for successful placentation. Hypoxia-induced production of HIF-1α has role in cell metabolic adaptation to environment lacking oxygen [7]. Hypoxic state also upregulated intracellular trophoblastic micro-RNA 210 (miR-210) [8]. MiR-210 is a main hypoxia-induced MiRNA. miR-210 involved in various biological processes, such as angiogenesis, cell differentiation, cell cycles regulation, growth and proliferation, inflammation, DNA damage repair, and mitochondrial metabolism [9], [10]. Expression of mR-210 also triggered by increased hypoxia-inducible factor-1α (HIF-1α) level [11], [12]. On the contrary, miR-210 has role in maintaining the stability of HIF-1α level [11].

Trophoblast physiological response to hypoxic state in early pregnancy had potential effect to cause pathological conditions. Elevated HIF-1α levels as response to hypoxic state [13] trigger elevated miR-210 expression [12], [14]. Then, miR-210 suppressed oxidative phosphorylation (OXPHO) reaction through the repression of ISCU1/2 genes [10]. This reaction reduces energy production in the form of ATP and increases reactive oxygen species (ROS) production. Increased ROS production also occurs directly due to hypoxic state. Highly elevated ROS cause stress oxidative, result in damage of proteins, lipids, and DNA. This condition resulted in mitochondrial dysfunction, trophoblastic cell dysfunction, or apoptosis [15]. Trophoblastic cell

dysfunction or apoptosis accompanied with inadequate energy might cause impaired first wave of trophoblastic invasion, subsequently causing incomplete placentation and ultimately led to early abortion.

Relation between elevated HIF-1α levels, miR-210 expression, and ROS levels with second-wave trophoblastic invasion had been described by studies in either preeclampsia or intrauterine growth restriction. HIF-1α is higher in severe preeclampsia [16], [17] or in intrauterine growth restriction [18]. miR-210 is associated with severe preeclampsia [19], [20], [21], [22], [23] or with intrauterine growth restriction [23]. Malondialdehyde or ROS is elevated in severe preeclampsia [24], [25], antioxidant is lower in severe preeclampsia [25], [26], [27]. Conclusion from these several studies is increased HIF-1α level, miR-210 expression, and ROS level correlated with failure of second-wave trophoblastic invasion, which manifest clinically as preeclampsia or intrauterine growth restriction. This conclusion induced new hypothesis that HIF-1α level, miR-210 expression, and ROS level could also associate with failure of first-wave trophoblastic invasion, clinically manifest as early abortion. This study aims to prove the differences of HIF-1α level, miR-210 expression, and ROS level between early abortion and normal pregnancy.

Methods

Study design

This was an observational study with a cross-sectional comparative study design in the first trimester pregnancy patients (gestational age <12 weeks), comparing HIF-1α levels, miR-210 expressions, and ROS levels between early abortion and normal pregnancy. Total 50 cases of 1st trimester pregnancy (25 cases of early abortion and 25 cases of normal pregnancy) at Dr. M. Djamil Hospital, Andalas University Hospital, and five Public Health Centers in Padang City were involved in this study. Subjects were classified to be "early abortion" if they had one of criteria proposed by Turrentine [28] as threatened abortion, inevitable abortion, incomplete abortion, or complete abortion. The study material was blood taken from vena cubiti. The ethical clearance was approved by the Research Ethics Committee Medical Faculty, Andalas University, with ethical clearance number 363/KEP/FK/2018.

Examination

Examination of HIF-1α levels: Serum HIF-1α levels were measured by enzyme-linked immunosorbent assay (ELISA), using the "Bioassay Technology Laboratory" kit Human HIF-1α Modulator 1, catalog: E0422Hu and the measurement results are expressed in units of ng/mL. Examination of HIF-1α levels is carried out by the procedure: Number of wells used was determined and unused wells are stored at 2–8°C. About 50 μl of HIF-1α standard was inserted to standard well. Total 40 μl of samples is added to sample wells and additional of 10 μl anti-HIF-1α antibody to sample wells. Afterward, 50 μl of streptavidin-HRP was added to both sample wells and standard wells. Samples were mixed and enclosed with sealer, then incubated for 60 min at 37°C. Plate was then washed with wash buffer for 5 times and 50 μl of substrate solution A was added to each wells followed by substrate solution B to each wells. Plate then reincubated for 10 min at 37°C in dark room. Then, 50 μl of stop solution was added to each wells, and the color of solution would change from blue to yellow. Absorbance value (OD value) of each well was determined using microscope reader 450 nm in 10 min after the addition of stop solution

Examination of miR-210 expressions [9]: Serum miR-210 expressions were measured by RT- polymerase chain reaction (PCR) technique, using miRNeasy Mini kit (Qiagen 217004) for RNA extraction, miScript II RT kit (Qiagen 218161) for cDNA synthesis, and miScript SYBR kit (Qiagen 218073) for qPCR; and the measurement results are expressed in copies/ng. Primer used was morpholino antisense oligo (MO)-210 (5'-AGATCAGCCGCTGTCACACGCACAG-3') and MO-neg (non-targeting antisense oligo) [9]. Examination of miR-210 was carried out by following other publication's procedure [9]: miRNAs were extracted from serum samples through phenol/chloroform extraction followed by column-based purification. Briefly, 750 mL of TRIzol reagent was added to each 50 mL sample of serum then 1 mg of carrier RNA was added. Chloroform (160 mL) added to each sample and mixed vigorously for 15 s. The samples transferred to a phase lock gel tube (50, 2302830) and spin at 12,000 × g for 15 min at 4°C. The result aqueous phase was carefully removed and purified using Qiagen's miRNeasy kit, following the manufacturer's protocol for total RNA isolation. The RNA was eluted in 30 mL of RNase-free water. cDNA was generated from 10 mL of the isolated miRNA using the miScript Reverse Transcription II kit, and qPCR was performed on the 7900HT Real-Time PCR System using the miScript SYBR Green PCR kit, according to the manufacture's protocols. The DDCT method was used for relative expression quantification using the RQ manager software version 2.4. The endogenous reference, RNU6B, was used for miRNA quantification.

Examination of ROS levels: Serum ROS levels were measured by ELISA, using the "Bioassay Technology Laboratory" kit Human ROS Modulator 1, catalog: E2134Hu and the measurement results expressed in units of ng/mL. Examination of ROS levels is carried out by the procedure: Number of wells used was determined and unused wells are stored at 2–8°C. About 50 μl of human ROS standard was inserted to standard well. Total 40 μl of samples was added to sample wells and additional of 10 μl anti-ER antibody

to sample wells. Afterward, 50 μl of streptavidin-HRP added to both sample wells and standard wells. Samples were mixed and enclosed with sealer, then incubated for 60 min at 37°C. Plate washed with washing buffer for 5 times and 50 μl of substrate solution A was added to each wells followed by substrate solution B to each wells. Plate then reincubated for 10 min at 37°C in dark room. Then, 50 μl of stop solution was added to each well, and the color of solution would change from blue to yellow. Absorbance value (OD value) of each well was determined using microscope reader 450 nm in 10 min after the addition of stop solution.

Data analysis

Test of HIF-1α levels, miR-210 expressions, and ROS levels of differences used non-parametric Mann–Whitney U-test due to not normally distributed data with SPSS software.

Results

None of study subjects had a history of abortion.

Subject's characteristics

The characteristics of the subjects are shown in Table 1.

Table 1: Subjects characteristics

Characteristic*	Early abortion (n=25)	Normal pregnancy (n=25)	p
Maternal age (years)			
Mean	31.72	28.60	0.051
SD	6.49	4.23	
Gestational age (weeks)			
Mean	8.2	7.8	0.453
SD	1.53	2.16	
Gravidity			
Primigravida	8 (32%)	8 (32%)	1.00
Multigravidas	17 (68%)	17 (68%)	

*No case of recurrent abortion in the both groups.

Table 1 shows that there were no significant differences in the mean of age, mean of gestational age, and gravidity distribution between the two study groups (p = 0.51, 0.453, and 1.00).

Normality distribution of HIF-1α levels, miR-210 expressions, and ROS levels

The normality of HIF-1α levels, miR-210 expressions, and ROS levels data distribution was tested by Shapiro–Wilk test, with results as shown in Table 2.

Table 2 shows that HIF-1α levels, miR-210 expressions, and ROS levels in the two study groups were not normally distributed, so Mann–Whitney U-test was used as the difference test.

Table 2: The normality distribution test of HIF-1α levels, miR-210 expressions, and ROS levels the distribution of ROS levels data

Variables	x̄+ SD	Min-Max	p*	Normality
HIF-1α (ng/mL)				
EA	4.60±2.43	2.83–10.90	0.000	No
NP	3.37±0.97	0.77–6.52	0.000	No
miR-210 (copies/ng)				
EA	38.9±17.90	1.09–61.63	0.001	No
NP	23.20±20.80	0.86–52.68	0.000	No
ROS (ng/mL)				
EA	4.46±7.66	1.02–26.30	0.000	No
NP	1.23±0.46	0.43–2.75	0.001	No

*Shapiro–Wilk, EA=Early abortion, NP=Normal pregnancy.

HIF-1α Levels, miR-210 expressions, and ROS Levels in Early Abortion and Normal Pregnancies

The result of HIF-1α levels, miR-210 expressions, and ROS levels in early abortion and normal pregnancies is shown in Table 3.

Table 3: HIF-1α levels, miR-210 expressions, and ROS levels in two study groups

Variables	Pregnancy status	Median	Min-Max	p*
HIF-1α (ng/mL)	EA	3.73	2.83–10.90	0.016
	NP	3.42	0.77–6.52	
miR-210 (copies/ng)	EA	43.55	1.09–61.63	0.027
	NP	17.85	0.86–52.68	
ROS (ng/mL)	EA	1.36	1.02–26.30	0.003
	NP	1,20	0.43–2.75	

*Mann–Whitney, EA=Early abortion, NP=Normal pregnancy.

Table 3 shows that the median of HIF-1α level, miR-210 expression, and ROS level in early abortions and normal pregnancies was 3.73 and 3.42 ng/mL (p = 0.016), 43.55 and 17.85 copies/ng (p = 0.027), and 1.36 and 1.20 ng/mL (p = 0.003).

Correlations between variables

The correlations between variables are shown in Table 4.

Table 4: Correlations between variables

Variables	R	p*
HIF-1α and miR-210	0.16	0.267
miR-210 and ROS	0.18	0.207
HIF-1α and ROS	0.46	0.001

p* = Spearman correlation.

Table 4 shows that there was a positive moderate correlation between HIF-1α levels and ROS levels (p = 0.001).

Discussion

Subjects from two groups of the study had similar characteristics of age, gestational age, and gravidity, therefore, the influence of age, gestational age, and gravidity to the differences of HIF-1α level, miR-210 expression, and ROS between study groups

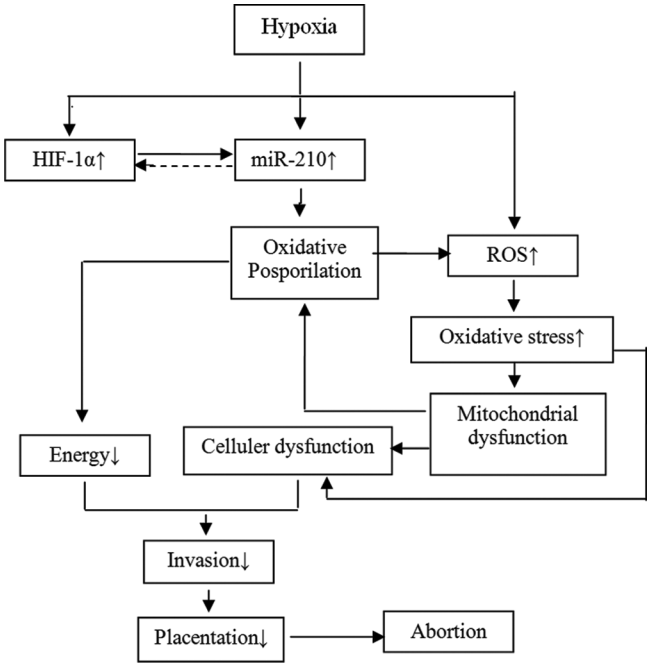

Figure 1: Mechanism of hypoxic-induced early abortion

could be ignored.

High level of HIF-1α in early abortion showed that HIF-1α levels associated with early abortion. This elevated HIF-1α levels in early abortion ensure that there was severe hypoxic state of decidua, since HIF-1α would be produced only if oxygen pressure decreased <1% [13]. Severe hypoxia suppressed mitochondrial function; therefore, energy produced was inadequate. HIF-1α levels induce elevated miR-210 expression, potentially suppressed OXPHO through repression of ISCU 1/2 genes.

High expression of miR-210 in early abortion showed that miR-210 expression associated with early abortion. This elevated miR-210 expression was considered by hypoxic condition, since miR-210 is a hypoxia-induced miRNA[9], [10]; which meant that miR-210 expression was triggered by hypoxic condition. Although the expression of miR-210 may be triggered by high HIF-1α level [12], [14], this study showed no significant correlation between HIF-1α level and miR-210 expression.

High level of ROS in early abortion showed that ROS level associated with early abortion. Elevated ROS level could be resulted directly from hypoxic state, since hypoxic state also suppressed OXPHO, promoting ROS production [29]. Increased ROS production could also resulted from increased miR-210 expression, due to OXPHO suppression by miR-210, but this study showed no significant correlation between miR-210 expression and ROS level. High ROS level lead to oxidative stress, and result in cell dysfunction, impaired trophoblastic invasion, abnormal placentation, and subsequently early abortion.

In facts, high level of HIF-1α, miR-210 expression, and ROS occurred simultaneously in early abortion and induced by hypoxic state, therefore, "hypoxic-induced early abortion" could be described as follows: Hypoxic state induced HIF-1α production, miR-210 expression, and ROS production. High ROS level caused stress oxidative, resulting in mitochondrial dysfunction and cellular dysfunction. Mitochondrial dysfunction suppressed OXPHO, further decreasing energy production and increasing ROS production. Mitochondrial dysfunction caused cellular dysfunction too. Trophoblastic cellular dysfunction accompanied by inadequate energy caused impaired trophoblastic invasion and incomplete placentation, ultimately lead to early abortion. The pathway of "increased HIF-1α level promotes elevated miR-210 expression → suppresses OXPHO → increases ROS production" was still questionable. The mechanism of this hypoxic-induced early abortion is shown in Figure 1.

Conclusions

This study concludes that HIF-1α level, miR-210 expression, and ROS level are associated with early abortion. Decidua (endometrium) was more severe hypoxic state (not "relative hypoxia") in early pregnancy period, impairing the potential of trophoblastic cell to invade. HIF-1α and ROS are key factors in hypoxic-induced abortion.

References

1. Cunningham GF, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CY. Williams Obstetrics. 25th ed. New York: McGraw Hill Companies; 2015.
2. Velicky P, Knöfler M, Pollheimer J. Function and control of human invasive trophoblast subtypes: Intrinsic vs. maternal control. Cell Adh Migr. 2016;10(1-2):154-62. https://doi.org/10.1080/19336918.2015.1089376
   PMid:26418186
3. Horii M, Li Y, Wakeland AK, Pizzo DP, Nelson KK, Sabatini K, *et al*. Human pluripotent stem cells as a model of trophoblast differentiation in both normal development and disease. Proc Natl Acad Sci U S A. 2016;113(27):E3882-91. https://doi.org/10.1073/pnas.1604747113
   PMid:27325764
4. Chakraborty D, Cui W, Rosario GX, Scott RL, Dhakal P, Renaud SJ, *et al*. HIF-KDM3A-MMP12 regulatory circuit ensures trophoblast plasticity and placental adaptations to hypoxia. Proc Natl Acad Sci U S A. 2016;113(46):E7212-21. https://doi.org/10.1073/pnas.1612626113
   PMid:27807143
5. Zimna A, Kurpisz M. Hypoxia-inducible factor-1 in physiological and pathophysiological angiogenesis: Applications and

therapies. Biomed Res Int. 2015;2015:549412. https://doi.org/10.1155/2015/549412
PMid:26146622

6. Wu F, Tian FJ, Lin Y. Oxidative stress in placenta: Health and diseases. Biomed Res Int. 2015;2015:293271. https://doi.org/10.1155/2015/293271
PMid:26693479
7. Lee JW, Ko J, Ju C, Eltzschig HK. Hypoxia signaling in human diseases and therapeutic targets. Exp Mol Med. 2019;51(6):1-13. https://doi.org/10.1038/s12276-019-0235-1
PMid:31221962
8. Biró O, Fóthi Á, Alasztics B, Nagy B, Orbán TI, Rigó J Jr. Circulating exosomal and argonaute-bound microRNAs in preeclampsia. Gene. 2019;692:138-44. https://doi.org/10.1016/j.gene.2019.01.012
PMid:30659946
9. Anton L, Olarerin-George AO, Schwartz N, Srinivas S, Bastek J, Hogenesch JB, *et al*. miR-210 inhibits trophoblast invasion and is a serum biomarker for preeclampsia. Am J Pathol. 2013;183(5):1437-45. https://doi.org/10.1016/j.ajpath.2013.07.021
PMid:24035613
10. Thomas LW, Ashcroft M. Exploring the molecular interface between hypoxia-inducible factor signalling and mitochondria. Cell Mol Life Sci. 2019;76(9):1759-77. https://doi.org/10.1007/s00018-019-03039-y
PMid:30767037
11. Qin Q, Furong W, Baosheng L. Multiple functions of hypoxia-regulated miR-210 in cancer. J Exp Clin Cancer Res. 2014;33(1):50. https://doi.org/10.1186/1756-9966-33-50
PMid:24909053
12. Guan Y, Song X, Sun W, Wang Y, Liu B. Effect of hypoxia-induced microRNA-210 expression on cardiovascular disease and the underlying Mechanism. Oxid Med Cell Longev. 2019;2019:4727283. https://doi.org/10.1155/2019/4727283
PMid:31249644
13. Dobierzewska A, Palominos M, Sanchez M, Dyhr M, Helgert K, Venegas-Araneda P, *et al*. Impairment of angiogenic sphingosine kinase-1/sphingosine-1-phosphate receptors pathway in preeclampsia. PLoS One. 2016;11(6):e0157221. https://doi.org/10.1371/journal.pone.0157221
PMid:27284992
14. Chan SY, Zhang YY, Hemann C, Mahoney CE, Zweier JL, Loscalzo J. MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2. Cell Metab. 2009;10(4):273-84. https://doi.org/10.1016/j.cmet.2009.08.015
PMid:19808020
15. Mendes S, Timóteo-Ferreira F, Almeida H, Silva E. New insights into the process of placentation and the role of oxidative uterine microenvironment. Oxid Med Cell Longev. 2019;2019:9174521. https://doi.org/10.1155/2019/9174521
PMid:31341539
16. Rath G, Aggarwal R, Jawanjal P, Tripathi R, Batra A. HIF-1 alpha and placental growth factor in pregnancies complicated with preeclampsia: A qualitative and quantitative analysis. J Clin Lab Anal. 2016;30(1):75-83. https://doi.org/10.1002/jcla.21819
PMid:25545166
17. Matsubara K. Hypoxia in the pathogenesis of peeclampsia. Hypertens Res Pregnancy 2017;5:46-51. https://doi.org/10.14390/jsshp.HRP2017-014
18. 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(1):2742. https://doi.org/10.1038/s41598-019-39426-5
PMid:30808910
19. Luo R, Wang Y, Xu P, Cao G, Zhao Y, Shao X, *et al*. Hypoxia-inducible miR-210 contributes to preeclampsia via targeting thrombospondin type I domain containing 7A. Sci Rep. 2016;6:19588. https://doi.org/10.1038/srep19588
PMid:26796133
20. 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(1):80. https://doi.org/10.1186/s12958-018-0391-5
PMid:30126412
21. Koushki M, Amiri Dash Atan N, Omidi-Ardali H, Rezaei Tavirani M. Assessment of correlation between miR-210 expression and pre-eclampsia risk: A meta-analysis. Rep Biochem Mol Biol. 2018;7(1):94-101.
PMid:30324123
22. Han L, Zhao Y, Jin Z, Li Y, Zou L. Correlation of miRNA and VEGF expression with the outcome of early-onset severe preeclampsia in patients receiving expectant treatment. Int J Clin Exp Pathol. 2018;11(4):2137-41.
PMid:31938323
23. Wang H, Zhao Y, Luo R, Bian X, Wang Y, Shao X, *et al*. A positive feedback self-regulatory loop between miR-210 and HIF-1α mediated by CPEB2 is involved in trophoblast syncytialization: Implication of trophoblast malfunction in preeclampsia†. Biol Reprod. 2020;102(3):560-70. https://doi.org/10.1093/biolre/ioz196
PMid:31616934
24. Mannaerts D, Faes E, Cos P, Briedé JJ, Gyselaers W, Cornette J, *et al*. Oxidative stress in healthy pregnancy and preeclampsia is linked to chronic inflammation, iron status and vascular function. PLoS One. 2018;13(9):e0202919. https://doi.org/10.1371/journal.pone.0202919
PMid:30204759
25. Shaikh SA, Vijayaraghavan R, Kumar DS, Manidip P. A comparative study of novel biomarkers on preeclampsia in relation to body mass index. Int J Res Pharm Sci. 2020;11(1):913-20. https://doi.org/10.26452/ijrps.v11i1.1914
26. Chamy VM, Lepe J, Catalán A, Retamal D, Escobar JA, Madrid EM. Oxidative stress is closely related to clinical severity of pre-eclampsia. Biol Res. 2006;39(2):229-36. https://doi.org/10.4067/s0716-97602006000200005
PMid:16874398
27. Al-Kuraishy HM, Al-Gareeb AI, Al-Maiahy TJ. Concept and connotation of oxidative stress in preeclampsia. J Lab Physicians. 2018;10(3):276-82. https://doi.org/10.4103/JLP.JLP_26_18
PMid:30078962
28. Turrentine JE. Clinical Protocols in Obstetrics and Gynecology. London: Informa Healthcare; 2008. p. 1-4.
29. Sasaki T, Awaji T, Shimada K, Sasaki H. Increase of reactive oxygen species generation in cerebral cortex slices after the transiently enhanced metabolic activity. Neurosci Res. 2017;123:55-64. https://doi.org/10.1016/j.neures.2017.04.020
PMid:28499835