

eISSN : 2579-8324

pISSN : 2579-8323

**Address for Correspondence:**Editorial Room Andalas Obstetrics and Gynecology Journal, 3<sup>rd</sup> floor of KSM of Obstetrics and Gynecology, RSUP DR. M. Djamil Padang, Jl. Perintis Kemerdekaan Padang, Sumatera Barat 25127**Website:**<http://jurnalobgin.fk.unand.ac.id/index.php/JOE>**RESEARCH**

## The Difference in Average of Maternal Serum Hypoxia-Inducible Factors-1 $\alpha$ Levels between Early Onset and Late-Onset Severe Preeclampsia

David Perdana<sup>1</sup>, Defrin<sup>2</sup>, Firdawati<sup>3</sup>

**Affiliation author :** 1. *Obstetrics and Gynecology, Faculty of Medicine, Andalas University, Ibnu Sina Simpang Empat Hospital West Pasaman;* 2. *Sub Division of Maternal Fetal Medicine, Obstetrics and Gynecology Department, Faculty of Medicine, Andalas University, Dr. M. Djamil Central General Hospital Padang;* 3. *Public Health Department, Faculty of Medicine, Andalas University Padang*

**Correspondence to:** David Perdana, email: reddaveil@gmail.com, Hp: 081363479983

**Abstract**

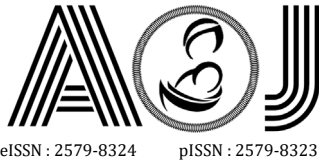
The purpose of this study is to know the difference average of maternal serum levels of HIF-1 $\alpha$  between early-onset and late-onset severe preeclampsia. This study used a cross sectional comparative study design that conducted in Februari 2020 - Agustus 2020 in the SMF / Obstetrics and Gynecology department of RSUP dr. M. Djamil Padang, RSUD Achmad Mochtar, RSUD Pariaman, RSUD M Zein Painan. We used consecutive sampling method which consists of 60 pregnant women who fulfill the inclusion and exclusion criteria. They were divided into two groups early-onset severe preeclampsia and late-onset severe preeclampsia. HIF-1 $\alpha$  tests were done using ELISA method. The average of maternal serum levels of HIF-1 $\alpha$  in late-onset severe preeclampsia is found to be the highest when compared to the early-onset severe preeclampsia,  $1,37 \pm 1,08$  ng/ml vs  $0,69 \pm 0,11$  ng/ml. This difference is significant with the Mann-whitney non parametrical statistical test ( $p < 0.05$ ). There is a significant difference average of maternal serum levels of HIF-1 $\alpha$  between early-onset and late-onset severe preeclampsia

**Keywords:** early onset severe preeclampsia, late onset preeclampsia late onset, maternal serum levels of HIF-1 $\alpha$

**INTRODUCTION**

The World Health Organization (WHO) systematically reviews maternal mortality worldwide, and in developed countries, 16 percent of maternal deaths were attributed to hypertensive disorders. In the United States from 2011 to 2013, 7.4 percent of 2009 pregnancy-related maternal deaths were caused by preeclampsia or eclampsia.<sup>1,2</sup> Preeclampsia has been characterized by some investigators into 2 different disease entities: early-onset preeclampsia and late-onset preeclampsia. Early-onset preeclampsia is usually defined as preeclampsia that develops before 34 weeks of gestation, whereas late-onset preeclampsia develops at or after 34 weeks of gestation.<sup>3,4</sup>

Early-onset preeclampsia occurs in 5-20% of all cases of preeclampsia, is considered a fetal disorder that is typically associated with placental dysfunction, reduction in placental volume, intrauterine growth restriction, abnormal uterine and umbilical artery Doppler



eISSN : 2579-8324

pISSN : 2579-8323

**Address for Correspondence:**Editorial Room Andalas Obstetrics and Gynecology Journal, 3<sup>rd</sup> floor of KSM of Obstetrics and Gynecology, RSUP DR. M. Djamil Padang, Jl. Perintis Kemerdekaan Padang, Sumatera Barat 25127**Website:**<http://jurnalobgin.fk.unand.ac.id/index.php/JOE>

evaluation, low birth weight, multiorgan dysfunction, perinatal death, and adverse maternal and neonatal outcomes. Late-onset preeclampsia is around 75-80% related to a maternal disorder; a result of an underlying maternal constitutional disorder (such as metabolic syndrome, impaired glucose tolerance, obesity, dyslipidemia, chronic hypertension), it is more often associated with a normal placenta, larger placental volume, normal fetal growth, normal uterine and umbilical artery Doppler evaluation, normal birth weight, and more favorable maternal and neonatal outcomes.<sup>4,5,6</sup>

Although the etiology of preeclampsia is not yet clear, abnormalities in the placenta are undoubtedly the pathogenesis of preeclampsia. The presence of abnormal placentation, including invasion of incomplete spirals of the trophoblast spirals, plays an important role as the pathogenesis and pathophysiology of preeclampsia. It may be due to insufficient adaptation of decidual and intramyometrial portion of spiral arterioles or due to shallow trophoblastic invasion, resulting in reduced uteroplacental blood flow leading to placental hypoxia.<sup>7,8</sup>

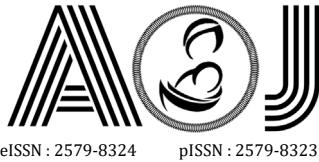
Placental hypoxia results in the release of several mediators into maternal circulation, which causes endothelial dysfunction prevailing in preeclampsia. The key mediator of the hypoxic condition is the hypoxia inducible factor 1 (HIF-1). HIF-1 is involved in transcription of many oxygen-dependent genes that encode for proteins associated with angiogenesis and cell metabolism. During low oxygen conditions, HIF-1 $\alpha$  is highly expressed and helps in development of placenta in early gestation. Overexpression of HIF-1  $\alpha$  has been observed in many inflammatory disorders, including cancer and preeclampsia.<sup>8,9</sup>

Hypoxia induces angiogenesis by regulating angiogenic proteins such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and FLT-1. HIF-1 $\alpha$  levels that promote enhanced transcription of genes encoding soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng), and endothelin-1 (ET-1), all known to contribute to preeclampsia. The change in concentration of these proteins causes angiogenic imbalance, leading to endothelial damage and the onset of preeclampsia.<sup>8,9,10</sup>

The expression of HIF-1 $\alpha$  is regulated not only by hypoxia but also by inflammation stimulation (for example, thrombin, peptide cytokine, like tumour necrosis factor (TNF), and reactive oxygen species (ROS), especially mediated by nuclear factor  $\kappa$ B (NF- $\kappa$ B), as the promoter of HIF-1 $\alpha$  contains binding site of NF- $\kappa$ B.<sup>7,11,12</sup>

There is still little research on the differences of maternal serum levels of HIF-1 $\alpha$  in severe preeclampsia, and the results are still controversial. The goals of this study were to know the maternal serum levels of HIF-1 $\alpha$  in severe preeclampsia and to compare the difference between early-onset and late-onset severe preeclampsia.

**METHODS**



eISSN : 2579-8324

pISSN : 2579-8323

**Address for Correspondence:**Editorial Room Andalas Obstetrics and Gynecology Journal, 3<sup>rd</sup> floor of KSM of Obstetrics and Gynecology, RSUP DR. M. Djamil Padang, Jl. Perintis Kemerdekaan Padang, Sumatera Barat 25127**Website:**<http://jurnalobgin.fk.unand.ac.id/index.php/JOE>

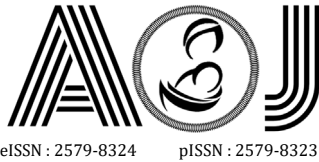
The study was conducted at Dr M. Djamil Hospital Padang and network hospitals. The study used a cross-sectional comparative study design by comparing the two study groups, namely early-onset severe preeclampsia and late-onset severe preeclampsia to evaluate the maternal serum levels of HIF-1 $\alpha$  in severe preeclampsia and normal and to compare the difference between early-onset and late-onset severe preeclampsia. Severe preeclampsia is defined as hypertension with minimum criteria for systolic blood pressure  $\geq$  160 mmHg and/or diastolic blood pressure  $\geq$  110 mmHg after 20 weeks' gestation, and one or more proteinuria  $\geq$  300 mg / 24 hours or urine / creatinine protein ratio  $\geq$  30 mg / dl (1 + urine dipstick), renal insufficiency, hematological abnormalities, liver disorders, cerebral disorders, pulmonary edema. The number of samples was 60 samples that met the inclusion criteria (single pregnancy women, gestational age > 20 weeks, there were no severe medical abnormalities, and no chorioamnionitis), and exclusion criteria damage to blood samples during the research process.

Patients who meet the inclusion and exclusion criteria will be interviewed to obtain characteristic data including name, age, identity number, address, contact number, history of pregnancy, the first day of the last day or ultrasound examination to assess gestational age. The size of the samples is calculated according to the simple formula to estimate the proportion of a population. The sampling formula used in unpaired numerical comparative research. The sampling technique is done by the consecutive method, i.e. the samples are taken sequentially. By the inclusion criteria and exclusion until the required number of samples is fulfilled. After the patient signed a letter of informed consent, venous blood specimens were collected in the median cubital vein by folding the elbow by 5 ml. Next blood is sent to the laboratory for examination HIF-1 $\alpha$  levels. Examination of HIF-1 $\alpha$  levels using reagents from *Human Hypoxia-Inducible Factor 1* ELISA kit BT LAB, Shanghai, Cina, with *Standard Curve Range* : 0.05 ng/ml – 15 ng/ml, dan sensitivity : 0.01 ng/ml. In this study, patients were not followed up until birth.

**RESULTS**

The study was conducted on 60 patients consisting of 30 early-onset severe preeclampsia, 30 late-onset severe preeclampsia. Sample characteristic of 60 patients, based on age, gestational age, parity, systole and diastole blood pressure, and birth weight was shown in Table 1.

**Table 1.** Clinical characteristics of the study population



eISSN : 2579-8324

pISSN : 2579-8323

**Address for Correspondence:**Editorial Room Andalas Obstetrics and Gynecology Journal, 3<sup>rd</sup> floor of KSM of Obstetrics and Gynecology, RSUP DR. M. Djamil Padang, Jl. Perintis Kemerdekaan Padang, Sumatera Barat 25127**Website:**<http://jurnalobgin.fk.unand.ac.id/index.php/JOE>

Characteristics	Severe Preeclampsia onset		p value
	Early Onset	Late Onset	
Age	30,17 ± 4,98	33,27 ± 5,28	0,02
Age of pregnant women (year)	30,70 ± 2,07	36,37 ± 1,19	0,0001
Sistolic (mmHg)	180 ± 24,57	172 ± 18,02	0,15
Diastolic mmHg)	107 ± 14,49	100 ± 9,78	0,04
Parity (%)			
Nullipara	6 (20 %)	5 (16,7%)	1,00
Multipara	24 (80 %)	25 (83,3%)	

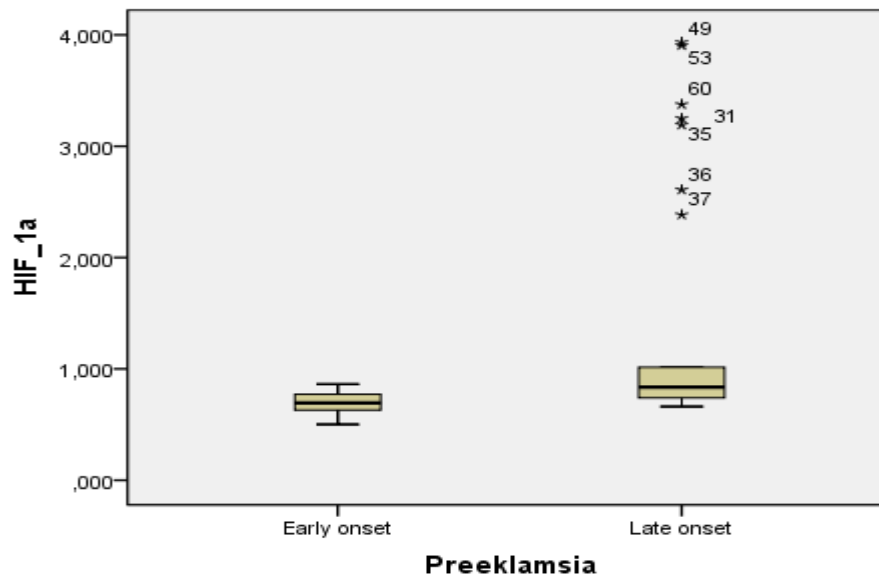
Based on the table, it is known that age average of late onset severe preeclampsia group are older than early onset severe preeclampsia group (33,27 ± 5,28 vs 30,17 ± 4,98) . In the early onset severe preeclampsia group, the systolic blood pressure is higher than the late onset severe preeclampsia group (180 ± 24,57 vs 172 ± 18,02). The diastole blood pressure found in the early onset severe preeclampsia group was higher than late onset severe preeclampsia group (107 ± 14,49 vs 100 ± 9,78). Both grup also show multipara are more than nullipara.

**Table 2.** HIF-1α Levels and the onset severe preeclamsia

Characteristics	Early Onset PEB	Late Onset PEB	p-value
	Mean ± SD	Mean ± SD	
HIF-1α (ng/ml)	0,69 ± 0,11	1,37 ± 1,08	0,0001

As described in Table 2, there was the difference in maternal serum HIF-1α between groups early-onset severe preeclampsia and late-onset severe preeclampsia, in which the mean maternal serum levels of HIF-1α was 0,69 ± 0,11 ng/ml and 1,37 ± 1,08 ng/ml. The results of the study showed that the mean maternal HIF-1α in late onset severe preeclampsia was higher than early onset severe preeclampsia. The mean of maternal HIF-1α level was statistically different between early-onset severe preeclampsia and late-onset severe preeclampsia (p-value < 0.05).

There is a significant difference between the levels of HIF-1α and the onset of severe preeclampsia, as shown in Figure 1.



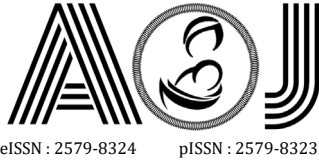
**Figure 1.** Boxplot Levels of HIF-1 $\alpha$  with the Onset of severe Preeklamsia

## DISCUSSION

The results of this study showed that the mean HIF-1 $\alpha$  levels of late-onset severe preeclampsia groups were significantly higher than early-onset severe preeclampsia group. Statistically, there is a significant difference with a value of  $p = 0.0001$  ( $p < 0.05$ ) between levels of late-onset severe preeclampsia groups and early-onset severe preeclampsia group.

Abnormalities of the placenta, which serves as the interface between the maternal and fetal environment are postulated to be central to the etiology of preeclampsia.<sup>7</sup> The transcription factor HIF-1 $\alpha$  has emerged as a key molecule in placental development, regulating angiogenesis and trophoblastic differentiation. HIF-1 $\alpha$  is a key transcription factor that plays a central role in the cellular response to low oxygen tension under physiological and pathological conditions.<sup>8,13</sup> Also recent studies have shown that HIF-1 $\alpha$  levels can be regulated by means that are independent of hypoxia. Inflammatory cytokines and growth factors have been shown to induce HIF-1 $\alpha$  gene expression and HIF-1 $\alpha$  is emerging as one of the “signalling drivers” of inflammation.<sup>7,12</sup>

The role of HIF-1 $\alpha$  in preeclampsia pathogenesis has been investigated in numerous immunohistochemical and serum analyses both in vitro and in vivo animal studies. Akhilesh et al showed that the HIF-1 $\alpha$  levels of the mothers with preeclampsia were higher compared to normal controls.<sup>13</sup> Bobek et al detected in mice that HIF-1 $\alpha$  induced by an imbalance in inflammatory cytokines is implicated in the pathogenesis of preeclampsia.<sup>14</sup> Recently, Iriyama et al. demonstrated in the placentas of two independent animal models of preeclampsia that hypoxia-independent stimulation of HIF-1 $\alpha$  gene expression in the placenta is a common pathogenic mechanism promoting disease progression.<sup>10</sup> Our results demonstrated that



eISSN : 2579-8324

pISSN : 2579-8323

**Address for Correspondence:**Editorial Room Andalas Obstetrics and Gynecology Journal, 3<sup>rd</sup> floor of KSM of Obstetrics and Gynecology, RSUP DR. M. Djamil Padang, Jl. Perintis Kemerdekaan Padang, Sumatera Barat 25127**Website:**<http://jurnalobgin.fk.unand.ac.id/index.php/JOE>

maternal serum HIF-1 $\alpha$  levels of late-onset severe preeclampsia group were significantly higher than early-onset severe preeclampsia group. We speculate that inflammation and the oxygen-independent mechanisms are the predominant features of HIF-1 $\alpha$  increase in late-onset severe preeclampsia group.

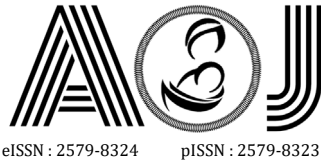
In addition, recent evidence suggests that oxygen is not the only factor that regulates the activity of HIF-1 $\alpha$ . For example, CO and NO block the hypoxic induction of HIF-1 $\alpha$ , whereas several divalent cations (Co<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>) and iron chelators induce HIF-1 $\alpha$ .<sup>15</sup> Furthermore studies have implicated a role for stress-related cytokines in the regulation of HIF-1 $\alpha$ , and it has been shown that mitogen activated protein kinase (MAPK), the insulin-like growth factor-1 receptor (IGF-1R), and other growth factors positively regulate HIF-1 $\alpha$  expression. Additionally, it has been shown in vascular smooth muscle cells that up-regulation of VEGF expression by HIF-1 $\alpha$  can be induced by hormones in an oxygen independent manner. Therefore, we hypothesize that factors other than hypoxia may contribute to the aberrant expression of HIF-1 $\alpha$  in preeclamptic placentae.<sup>15,16,17</sup>

An alternative hypothesis is that the reduced utero-placental perfusion may create a situation of oxidative stress inducing the release of reactive oxygen species (ROS) by the placenta into the maternal circulation. Studies have shown that trophoblasts from pre-eclamptic pregnancies show decreased expression of the ROS scavenger superoxide dismutase (SOD), increased xanthine dehydrogenase/xanthine oxidase (XDH/XO) expression, and increased xanthine oxidase (XO) activity. Moreover, pre-eclamptic placentae show increased production of lipid peroxides. Overall, these changes indicate an increased ability of pre-eclamptic placentae to generate ROS, which are known to alter endothelial function and, therefore, could be responsible for the characteristic widespread maternal endothelial dysfunction that is associated with pre-eclampsia.<sup>15,17,18</sup>

Although the cause of aberrant expression in pre-eclamptic placentae remains to be elucidated, it appears that HIF-1 $\alpha$  activation may not simply be due to hypoxic conditions alone. Instead, an interaction of hypoxic conditions, factors associated with oxidative stress, altered oxygen sensing and release of inflammatory cytokines may influence HIF-1 $\alpha$  expression.<sup>15,19,20</sup>

## CONCLUSION

The difference between HIF-1 $\alpha$  with the onset of severe preeclampsia was found to be the highest level in late onset severe preeclampsia, which was  $1,37 \pm 1,08$  ng/ml. There was a significant difference ( $p < 0.0001$ ) between late-onset severe preeclampsia and early-onset severe preeclampsia.



eISSN : 2579-8324

pISSN : 2579-8323

**Address for Correspondence:**Editorial Room Andalas Obstetrics and Gynecology Journal, 3<sup>rd</sup> floor of KSM of Obstetrics and Gynecology, RSUP DR. M. Djamil Padang, Jl. Perintis Kemerdekaan Padang, Sumatera Barat 25127**Website:**<http://jurnalobgin.fk.unand.ac.id/index.php/JOE>**REFERENCE**

1. Cunningham L, Bloom, Dashe. Hypertensive Disorders. Williams Obstetric 24 ed. New York: Mc Graw Hill; 2018. p.1086-8
2. ACOG. Hypertension in pregnancy. Washington : the American College of Obstetricians and Gynecologists; 2013.p. 22
3. Saito S. Preeclampsia, Basic, Genomic, and Clinical. Springer. 2018
4. Adhi Pribadi. Preeklampsia "Stoppable". Sagung Seto. 2019
5. Sibai BM. Hypertension. In: Gabbe SG, Niebyl JR, Simpson JL, Landon MB, Galan HL, Jauniaux ERM, et al, editors. Obstetrics normal and problem pregnancies. 7th ed. Philadelphia: Elsevier; 2017. p. 661-705.
6. Raymond D, Peterson E. A Critical Review of Early-Onset and Late-Onset Preeclampsia. Obstetrical and Gynecological Survey by Lippincott Williams & Wilkins. 2011. Volume 66, Number 8.
7. Sriyanti R, Mose JC, Masrul, N. S. The difference in maternal serum Hypoxia-Inducible Factors-1a levels between early onset and late onset preeclampsia. Journal of Medical Science. 2019;7(13):2133-7.
8. Rath G, Aggarwa , Jawanjal P, Tripathi R, Batra A. HIF-1 Alpha and Placental Growth Factor in Pregnancies Complicated With Preeclampsia: A Qualitative and Quantitative Analysis. J of Clin Lab Anal. 2016; 30(75-83).
9. Wantania J, Bakri S, Pandelaki K, Chalid M. (2013). Altered level of Soluble fms-like Tyrosine Kinase 1 (sFlt1) and Hypoxia Inducible Factor-1 $\alpha$  in Normotensive Pregnancy and Preeclampsia. Indones Biomed J 2013; 5(2):121-8.
10. Iriyama T, Wang , Parchim N, Song , Blackwell SC, Sibai B, et al. Hypoxia-Independent Upregulation of Placental Hypoxia Inducible Factor-1 $\alpha$  Gene Expression Contributes to the Pathogenesis of Preeclampsia. Hyper AHA J. 2015 April.
11. Tal R. The Role of Hypoxia and Hypoxia-Inducible Factor-1Alpha in Preeclampsia Pathogenesis. Bio of Rep. 2012; 87(6).
12. Davutoglu EA, Firat AA, Ozel A, Yilmaz N, Uzun I, Yuksel IT, et al. Evaluation of maternal serum hypoxia inducible factor-1a, progranulin and syndecan-1 levels in pregnancies with early and late onset preeclampsia. The Journal of Maternal Fetal & Neonatal. 2017;2017:1-7.
13. Akhilesh M, Mahalingam V, Nalliah S, Ali RM, Ganesalingam , Haleagrahara N. Participation of hypoxia-inducible factor-1a in the pathogenesis of preeclampsia-related placental ischemia and its potential as a marker for preeclampsia. Biomarker and Genom Med. 2014 June; 6(121-125).
14. Bobek G, Surmon L, Mirabito KM, et al. Placental regulation of inflammation and hypoxia after TNF-a infusion in mice. Am J Reprod Immunol. 2015;74: 407-418.



eISSN : 2579-8324

pISSN : 2579-8323

**Address for Correspondence:**

Editorial Room Andalas Obstetrics and Gynecology Journal, 3<sup>rd</sup> floor of KSM of Obstetrics and Gynecology,  
RSUP DR. M. Djamil Padang, Jl. Perintis Kemerdekaan Padang, Sumatera Barat 25127

**Website:**

<http://jurnalobgin.fk.unand.ac.id/index.php/JOE>

15. Caniggia I, Mostachfi H, Winter J, Gassmann M, Lye SJ, Kuliszewski M, Post M. Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta(3). *J Clin. Invest* 2000; 105(5):577–587.
16. Galbiati S, Inversetti A, Causarano V, Stenirri S, Soriani N. HIF1A and MIF as potential predictive mRNA biomarkers of pre-eclampsia: a longitudinal prospective study in high risk population. *Clin Chem Lab Med.* 2015; 53(9).
17. Vermaa S, Pillay P, Naickerb T, Moodley J, Mackraj I. Placental hypoxia inducible factor -1a & CHOP immuno-histochemical expression relative to maternal circulatory syncytiotrophoblast micro-vesicles in preeclamptic and normotensive pregnancies. *Euro J of Obs & Gyn Rep Bio.* 2018; 220(18-24).
18. Liu W, Wang SJ, Lin QD. Study on the Expressions of PHD and HIF in Placentas from Normal Pregnant Women and Patients with Preeclampsia. *Int. J. Biol. Sci.* 2014; 10
19. Rolfo A, Many A, Racano A, Tal R, Tagliaferro A, et al. Abnormalities in Oxygen Sensing Define Early and Late Onset Preeclampsia as Distinct Pathologies. *PLoS ONE* 2010; 5(10): e13288.
20. Galbiati S, Inversetti A, Causarano V, Stenirri S, Soriani N, Ambrosi A. HIF1A and MIF as potential predictive mRNA biomarkers of pre-eclampsia: a longitudinal prospective study in high risk population *Clin Chem Lab Med.* 2015;53(9):1339-47.