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HIF2α Placental Expression in Intrauterine Growth Restriction Preeclampsia

Nurfianti Indriana, Nugrahanti Prasetyorini, Imam Wahyudi
Departement of Obstetrics and Gynecology Laboratory of Medical Faculty of Brawijaya University/Saiful Anwar Hospital Malang

Abstrak:
Tujuan: *Mengetahui adanya perbedaan ekspresi HIF2α pada plasenta kehamilan preeklampsia IUGR dan kehamilan preeklampsia non-IUGR. Untuk membuktikan adanya hubungan antara ekspresi HIF2α dengan berat badan bayi lahir dan derajat IUGR.*
Metode: *Penelitian cross sectional. Penelitian menggunakan spesimen sel trofoblas pada plasenta dari preeklampsia IUGR dan preeklampsia non-IUGR. Sampel yang diambil sebanyak 42 (nPE-IUGR=21, nPE-NonIUGR= 21). Sampel plasenta dikumpulkan di laboratorium Biomedik, kemudian diperiksa ekspresi HIF2α. Hasil diuji dengan Shapiro-Wilk test dan Levene test dilanjutkan dengan Mann-Whitney test dan Spearman test.*
Hasil: *Ekspresi HIF2α pada preeklampsia IUGR lebih tinggi dibanding preeklampsia non IUGR didapatkan perbedaan secara bermakna dengan [p(0,000)< 0.05]. Ekspresi HIF2α memiliki hubungan yang bermakna dengan berat badan lahir bayi dengan [p(0,000)< 0.05]. Ekspresi HIF2α memiliki hubungan yang bermakna dengan derajat IUGR dengan [p(0,000)< 0.05].*
Kesimpulan: *Terdapat perbedaan yang bermakna antara ekspresi HIF2α pada plasenta kehamilan preeklampsia IUGR dibandingkan dengan kehamilan preeklampsia non-IUGR. Terdapat hubungan yang signifikan antara ekspresi HIF2α dengan berat badan lahir bayi. Terdapat hubungan yang signifikan antara ekspresi HIF2α dengan derajat IUGR.*
Kata kunci: *HIF2α, preeclampsia, IUGR*

Abstract:
Objective*: Analizing the difference expression of HIF2α between placenta preeclampsia IUGR and preeclampsia non-IUGR. Analizing correlation between expression of HIF2α with body weight, degree of IUGR.*
Methods*: Cross sectional study. The speciments were placental trophoblast cell from preeclampsia IUGR and preeclmapsia non-IUGR. Samples taken 42 (nPE-IUGR=21, nPE-NonIUGR= 21). Samples were collected in the laboratory, then examined expression of HIF2α. The result were analyzed by Shapiro-Wilk test dan Levene test continued with Mann-Whitney test dan Spearman test.*
Results*: Expression of HIF2α significantly higher in preeclampsia IUGR than preeklampsia non IUGR with [p(0,000)< 0.05]. Expression of HIF2α significantly have correlation with body weight with [p(0,000)< 0.05]. Expression of HIF2α significantly have correlation with degree of IUGR with [p(0,000)< 0.05].*
Conclusion*: Expression of HIF2α significantly higher in placental preeclampsia IUGR. Expression of HIF2α significantly have correlation with body weight. Expression of HIF2α significantly have correlation with degree of IUGR*
Keywords: *HIF2α, preeclampsia, IUGR*

I. Introduction

Intrauterine growth restriction (IUGR) is a disorder of growth and development that occurs in the fetus. The incidence of IUGR preganancies with approximately 7% in the world, 90% in developing countries (Damir, 2011). The incidence of IUGR pregnancies with approximately 7-14% in Indonesia (Depkes, 2012). The incidence of pregnancies with IUGR were 26 cases in 2012 in Saiful Anwar Hospital (RSSA, 2012). IUGR infants have an increased risk of mortality and morbidity are higher than normal infants (WHO, 2008). IUGR infant growth is not in accordance with standart growth charts. In IUGR baby growth chart is below the 10^{th} percentile (Andrea, 2012)

The cause of IUGR is associated with the type of IUGR. There are two types of IUGR, symmetric and asummetric. In symmetric IUGR interference occurs early in pregnancy. This disorder causes a decrease in the number and size of cells (Cunningham, 2010). This disorder is caused by an infection during pregnancy, chromosomal abnormalities and congenital anomalies. Tupe of asymmetric IUGR occurs around 70-80%. Type of asymmetric IUGR caused by placental insufficiency. Starting with ischemia on throphoblast causes a

decrease in uteroplacental perfusion. The clinical manifestations of ischemia trophoblast appears in the second trimester of pregnancy, but the pathophysiologic process begins during the first trimester (Figen. 2010).

In IUGR, there were decreased of trophoblast villi branch number, volume and surface of villi due to apoptosis. The presence of apoptosis in villi appear as aggregates syncytial (Scifres, 2009). Increased apoptosis causes decreased perfusion in the syncytiotrophoblast, nutrient transport disruption and release of placental hormones (Alexander, 2011).

According Gourvas 2010, the process of cell apoptosis that occurs in a cellular response is transcribed by Hypoxia Inducible Factor (HIF). Hypoxia Inducible Factor (HIF) is a transcription factor that is commonly found in mammalian cells due to low oxygen. HIF has heterodimer HIFα (1,2 and 3) and HIFβ or ARNT (Aryl Hydrocarbon Nuclear Translocation Protein). HIFα subunit are found in the cytoplasm. In the process of transcription, HIFα transported into the nucleus and forming subunits with ARNT (Gourvas, 2010).

HIF1α and HIF2α have different responses to hypoxia exposure. HIF1α and HIF2α response depends on the length of hypoxia and hypoxia level. In mild hypoxic conditions (5% O2) HIF2α rise higher than HIF1α. While the conditions of severe hypoxia (1% O2) levels increased HIF1α higher that HIF2α. In the long mild hypoxic conditions, HIF2α will work actively in the process of gene expression (Pringle, 2010).

According to Pringle, 2010, HIFα expressed more in the villi of the placenta pregnancy preeclampsia compared to normal pregnancy. Another study by Helske stated that HIFα levels increased in pregnancies with preeclampsia and IUGR. Number HIFα expression in trophoblast in pregnancy with preeclampsia same with HIFα expression in trophoblast during the first trimester when there is no exposure to oxygen. In preeclampsia, down regulation of protein HIF1α and HIF2α disrupted due to the proteosome dysfunction leads to increased formation and decreased degradation of HIFα (Pringle, 2010).

To find out how pathomeccanism occurrence of IUGR, it is necessary also to know how the risk factors for IUGR. according to Andrea in 2012 mentioned that there are three factors that cause IUGR, maternal, fetal and placenta factors. Maternal factors that cause IUGR such as small mother and low weight gain. Fetal factors that cause IUGR, congenital infection due to TORCH, chromosomal abnormalities and discordant growth due to multiple pregnancies. Placental factors that cause IUGR, uteroplacental insufficiency, malformations of the uterus, placenta separation, infarction, postterm. With so many risk factors that cause the IUGR, this research aimed to determine the occurrence of IUGR pathomeccanism with use a uniform sample. Factors that uniform is a placenta factor as the cause of IUGR. One cause of IUGR placental factors are pregnancy with preeclampsia. From this background, the researcher wants to know how the expression of the HIF2α transcription factor which have specificity in mild and chronic hypoxia condition that occurs in IUGR and non-IUGR preeclampsia placenta of pregnancy. Researchers also want to know the relationship between the expression HIF2α with birthweight outcomes and degree of IUGR.

This study uses the placental trophoblast cel speciments from preecalmptic pregnancies IUGR and non-IUGR. placental samples were taken and then proceed and followed by measuring HIF2α expression by immunohistochemistry. The result then performed normality test with Shapiro-Wilk test, homogenity test with Levene test and analysis test with Mann-Whitney, found a significant difference. Relationship analysis test HIF2α levels and birthweight with Spearman test found a significant relationship. Relationship analysis test HIF2α levels with the degree of IUGR found a significant relationship.

II. Materials And Methods

Research Design

This study was an observational analytic study, with cross sectional study. This study selected by purposive sampling in Saiful Anwar Hospital and Iskak Hospital. The research was conducted in the Saiful Anwar Hospital and the Central Laboratory of Biomedic in Malang Brawijaya University, Faculty of Medicine. This research are held in 9-month, from November 2013 until Juli 2014. The study population is a mother who deliver her baby in Saiful Anwar Hospital and Iskak Hospital.

Inclusion criteria for study subjects were mother with preeclampsia-IUGR and preeclampsia non-IUGR delivery with sectio cesarean. As exclusion criteria: mother with unclear gestational age, pregnancy with congenital anomaly fetus, mother with anemia, heart failure, mother who lived in high altitude, mother with pulmonary disease, hematological disease, and history of hormonal contraception before pregnancy.

The number of samples taken as many as 42 samples, is determined by the formula
n=number of samples

Zα=desired confidence level with (Z (5%) = 1.64).
Zα=desired confidence level with (Z (10%) = 1.28).
r=correlation number = 0,6

$$n1 = n2 = \left\{ \frac{(Z\alpha + Z\beta)}{} \right\}^2 + 3$$

0,5lm [1+r)/(1-r)]
n1 = n2 = 20,6 = 21

In this study population, the subject will be devided to the criteria of inclusion and exclusion criteria. Samples who participate in the study were signing the informed consent agreement. Placenta samples were taken sized 2x2 cm and placed in formalin tube with label. Samples were taken to the laboratory Biomedical and performed preparat preparation until it be measured with a specific antibody immunohystochemistry of HIF2α. If all sample already collected, immunohystochemistry procedure will be held after it.

III. Result

Prerequisite parametric test results

In this study the data analysis is using SPSS statistical software release 21. Normality test performed by the Shapiro-Wilk test, the homogenity test used the Levene. The same decision criteria, that is, when the Sig or the p-value is greater than $\alpha = 0.05$, the data were normally distributed, and when the Sig or the p-value is less than $\alpha = 0.05$, it means that data not normally distributed.

In this study 21 samples were pre-eclampsia non-IUGR and 21 samples were preeclampsia IUGR. In the normality test of HIF2α, samples obtained for preeclampsia non-IUGR not normally distributed, preeclampsia IUGR is normally distributed. While the results of the normality test of baby born weight combined sample of preeclampseia non-IUGR and preeclampsia IUGR are not normally distributed.

Based on the results of normality test data, for normally distributed data analysis will be performed by independent sample t test to make comparison mean of 2 free sample group. However if the test result are not normally distributed, the data analysis will be performed by Mann-Whitney test for comparison of mean 2 free sample groups.

The results of the comparison HIF2α expression

Based on the results of Mann-Whitney test on the data HIF2α group significant difference in the two groups of preeclampsia IUGR and preeclampsia non-IUGR. In Table 1, it appears significant increased the mean of HIF2α from preeclampsa IUGR and preeclampsia non-IUGR with (p-value = 0.000< α).

	Kelompok	N	Mean Rank	Sum of Ranks
HIF2-alpha	non-IUGR	21	11.81	248.00
	IUGR	21	31.19	655.00
	Total	42		

Test Statistics[a]

	HIF2-alpha
Mann-Whitney U	17.000
Wilcoxon W	248.000
Z	-5.122
Asymp. Sig. (2-tailed)	.000

a. Grouping Variable: Kelompok

Table 1. Comparison of HIF2α expression

Result of Analyzing test HIF2α expression and baby born weight

Based on the Spearman correlation test on the results obtained by analysis of r-count value of -0,705 with a significance value of 0,000. Significance value is worth less than 5% alpha so it can be concluded that there is a significant correlation between the expression HIF2α with birth weight. The correlation value is negative so it can be interpreted that if HIF2α expression is high then the lower the birth weight. Conversely, if the HIF2α expression is low then the higher the birth weight. In detail can be seen in the figure below.

Correlations

			HIF2A	IUGR
Spearman's rho	Berat bayi	Correlation Coefficient	1,000	-,705**
		Sig. (2-tailed)	.	,000
		N	42	42
	IUGR	Correlation Coefficient	-,705**	1,000
		Sig. (2-tailed)	,000	.
		N	42	42

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2. Result Spearmen test of HIF2α correlation with birth weight.

Result of Analyzing test HIF2α expression and degree expression of IUGR

Based on the Spearman correlation test on the results obtained by analysis of r-count value of 0,804 with a significance value of 0,000. Significance value is less than 5% alpha so it can be concluded that there is a significant positive so that it can be interpreted that if the HIF2α expression is low, the more mild degree of IUGR. Conversely, if the expression of HIF2α is high, the severe degree of IUGR. In detail can be seen in the figure below.

Correlations

			HIF2A	IUGR
Spearman's rho	HIF2α	Correlation Coefficient	1,000	,804**
		Sig. (2-tailed)	.	,000
		N	42	42
	IUGR	Correlation Coefficient	,804**	1,000
		Sig. (2-tailed)	,000	.
		N	42	42

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3. Result Spearmen test of HIF2α correlation with degree of IUGR.

IV. Discussion

IUGR is fetal growth disturbances that occur in pregnancies where the baby's weight at birt was below the 10th percentile corresponding gestational age. Babies born with IUGR morbidity and mortality will increase both the short term and long term effects. To find out how pathomechanism occurrence of IUGR it is necessary also to know the risk factors for IUGR. according to andrea in 2012 mentioned that there are three factors that cause IUGR, maternal, fetal and placenta factors. Maternal factors that cause IUGR such as small mother and low weight gain. Fetal factors that cause IUGR, congenital infection due to TORCH, chromosomal abnormalities and discordant growth due to multiple pregnancies. Placenta factors that cause IUGR, uteroplacental insufficiency, malformations of the uterus, placenta separation, infarction, postterm.

With so many risk factors that cause the IUGR, this research airmed to determine the patomecchanism occurrence of IUGR use a uniform sample. Factors that uniform is the placenta factor that cause of IUGR. One cause placental factor is pregnancy with preeclampsia. The incidence of pregnancy with preeclampsia as the cause of asymmetric IUGR is about 70-80% higher than symmetric IUGR about 20-30%.

The population in this study were pregnant women who gave birth at Saiful Anwar Hospital Malang and Iskak Hospital Tulungagung. This sample selection specifically on preeclamptic pregnancies which is marked by an increase in blood pressure >140/90, accompanied by proteinuria >300 mg/24 hour or dipstick +1.

42 samples were taken by researchers with 21 samples group of preeclampsia non IUGR and 21 samples group of preeclampsia IUGR. Primigravida were 7 patients with multigravida 35 patients. Most of 31 patients aged 30-35 years old, while the remaining 20-29 years old. Aterm pregnancies is 31 cases and premature delivery confirmed by balard score is 10 cases. Deliveries are made mostly in 38 patients in sectio cesarea cito while 4 patients in semi elective sectio cesarea, where before childbirth received induction of lung maturation. Type preeclampsia occurs mostly severe preeclampsia were not accompanied by partial hellp syndrome or impending eclampsia is 20 patients. While the partial hellp syndrome of preeclampsia is 2 patients. Preeclampsia with HELLP syndrome were 9 patients. Preeclampsia with impending eclampsia is 3 patients and Preeclampsia with partial HELLP syndrome and impending eclampsia is 8 patients.

This study aims to determine the HIF2α expression on placenta preeclamptic IUGR and preeclamptic non-IUGR, to know relationship between HIF2α expression with birth weight and degree of IUGR.

Placenta samples were taken then fixed and given immunohistochemical staining with HIF2α antibodies. Further examination with HIF2α immunohistochemistry using immunoratio methode. From the

results of preparat found a brown color in the cell nucleus where the reagent antibodies used specifically binds to HIF2α intracellular, does not bind to HIF1α. The counting results obtained into HIF2α expression group of severe preeclampsia IUGR and severe preeclampsia non-IUGR. The average of each group in severe preeclampsia non IUGR is 16.1905 ± 13,9847 and in severe preeclampsia IUGR is 61.1905 ± 16.9606. From the data above obtained 1 results of severe preeclampsia non-IUGR that exceed the average which is 65%. The placenta which taken is severe preeclampsia non-IUGR with partial HELLP syndrome and impending eclampsia which is not found in the other sample placenta in preeclampsia non-IUGR.

From the results of calculation of birthweight obtained an average from severe preeclampsia non IUGR is 2983.714 ± 417.96808 and severe preeclampsia IUGR is 2028.667 ± 388.39585. On severe preeclampsia IUGR then grouped by percentil <10 and <5 based on estimates of fetal weight table according to Cunningham in 2010.

HIF2α and birthweight data then conducted tests of normality and homogenity, the results are not normal distribution. Further calculations with SPSS method using non-parametric analysis with Mann-Whitnet test. In this study significantly HIF2α expression in preeclampsia IUGR have higher expression compared with HIF2α expression in preeclampsia non-IUGR. with the Mann-Whitnet test obtained a significance of 0.000. with the average value of the HIF2α expression in preeclampsia IUGR is 61.1905 ± 16.9606 and the average value of the HIF2α expression in preeclampsia non-IUGR is 16.1905 + 13.8947. from this values it can be seen that the average HIF2α expression in preeclampsia IUGR is higher than the average HIF2α expression in preeclampsia HIF2α non-IUGR.

This study also analyzed the relationship between HIF2α expression and birth weight. Based on the results obtained Spearman r-count value of -0.705 with a significance value is 0.000. It can be concluded that there is a significant correlation between the HIF2α expression and birth weight. The correlation value is negative so that means that the higher HIF2α expression in placenta the lower the birth weight. Conversely the lower HIF2α expression the higher birth weight.

This study also analyzed the relationship between the expression of HIF2α with degree of IUGR. Based on the results obtained Spearman r-count value of 0.705 with a significance value of 0.000. It can be concluded that there is a significant correlation between the HIF2α expression with degree of IUGR. The correlation value is positive so that means that the higher the HIF2α expressin the more severe the degree of IUGR. Conversely the lower HIF2α expression the more mild degree of IUGR. The degree of mild IUGR shown with birth weight below the percentile 10. The heavy degree of IUGR, the birth weight can reach under 5 percentile.

From the research that has been done can be seen that the pregnancies with preeclampsia IUGR have higher HIF2α expression. Increased expression of HIF2α in preeclampsia IUGR shows hypoxia condition inutero. Hypoxia factors HIF2α have specificity in hypoxic conditions were mild and chronic. So it can be concluded that in preeclampsia IUGR occurs a mild and long hypoxic conditions.

This study was supported with research conducted by Pringle in 2010 that HIFα expressed more in the villi of the placenta with preeclampsia than placenta normal. Helske research also stating that HIFα levels increased in pregnancies with preeclampsia and IUGR. Hypoxia Inducible Factor (HIF), which is found in intra cells may appear in low-oxygen conditions and have an important role in cellular and systemic responses. HIF has HIFα heterodimer (1, 2 or 3) and HIFβ or Arnt. Hypoxic conditions resulted in increased expression in trophoblast HIF1α and HIF2α. Once there is no oxygen, HIF will be activated and entrance to the nucleus resulting in the transcription process. One of the active process is a process that occurs in the nucleus of apoptotic trophoblasts. From this research it is known that the aggregate apoptotic nuclei found in trophoblast cells are characterized by increased expression of HIF2α. Trophoblast cells serves transport nutrients, oxygen and hormonal between maternal and fetal. This causes a decrease transport growth in the fetus, which in turn appears as intrauterine growth restriction (IUGR).

From this research may ultimately prove a hypothesis that has been presented previously by researchers, that HIF2α increased in preeclampsia IUGR compared with preeclampsia non-IUGR. The higher expression of HIF2α the lower birth weight, and the higher expression HIF2α the heavier the degree of IUGR. Hope of the researchers that this research could ultimately beneficial to increase the repertoire of knowledge, especially in the mechanism of IUGR, especially HIF2α role in preeclampsia. As well as basic research can be further research to determine the role of the hypoxia transcription factor in IUGR pregnancies.

V. Conclusion

1. Expression of HIF2α is significantly higer in pregnancy with preeclampsia IUGR than pregnancy with preeclampsia non-IUGR
2. The more higher the expression of HIF2α, the more lower the weight of baby born
3. The more higher the expression of HIF2α, the more severe degree of IUGR.

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