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 hypoxia 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 hypoxia condition determines for successful placentation. Hypoxia-induced production of HIF-1α has role in cell metabolic adaptation to environment lacking oxygen [7]. Hypoxia 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 miR-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 microplate 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
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 SD: 6.49  | Mean: 28.60 SD: 4.23    | 0.051     |
| Gestational age (weeks)        | Mean: 8.2 SD: 1.53    | Mean: 7.8 SD: 2.16      | 0.453     |
| Gravidity                      | Primigravida: 8 (32%) | Primigravida: 8 (32%)   | 1.00      |
|                                | Multigravidas: 17 (68%) | Multigravidas: 17 (68%) |           |

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

| 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     | 0.027     |
| miR-210 (copies/ng)     | EA               | 43.55   | 1.09–61.63    | 0.027     |
|                         | NP               | 17.85   | 0.86–52.68    | 0.001     |
| ROS (ng/mL)             | EA               | 1.36    | 1.02–26.30    | 0.003     |
|                         | NP               | 1.20    | 0.43–2.75     | 0.001     |

Table 2 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   | 0.287     |
| HIF-1α and ROS          | 0.18   | 0.207     |
| miR-210 and ROS         | 0.46   | 0.001     |

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.
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.

**Figure 1**: Mechanism of hypoxic-induced early abortion

**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.

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