Original Article

Expression of miR-518b and miR-30d in patients with missed abortion and clinical significance

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Received October 7, 2019; Accepted December 10, 2019; Epub February 15, 2020; Published February 28, 2020

Abstract: Objective: To investigate the expression of miR-518b and miR-30d in placental villi tissue of patients with missed abortion and its clinical significance. Methods: 45 patients with missed abortion were enrolled in the study group (SG). 45 healthy induced abortion women in the same period were included in the control group (CG). The expression of miR-518b and miR-30d in placental villi tissue and peripheral blood were tested with PCR method. The predictive value of miR-518b and miR-30d in peripheral blood in missed abortion were analyzed with ROC. Results: The expression levels of miR-518b and miR-30d in placental villi tissue and peripheral blood of the study group were increased (P<0.001). The expression of miR-518b and miR-30d in placental villus was positively correlated with the expression in peripheral blood (P<0.001). Peripheral blood miR-518b and miR-30d had a good performance on predicting missed abortion (P<0.001). miR-518b was closely related to the number of gestational weeks and times of abortions (P<0.050) while miR-30d was closely related to age (P<0.001). Conclusions: The miR-518b and miR-30d are highly expressed in placental villi tissue of patients with missed abortion. It is expected to be an excellent measure for diagnosis and treatment of missed abortion.

Keywords: miR-518b, miR-30d, missed abortion, placental villi tissue, peripheral blood

Introduction

Missed abortion is also known as prolonged abortion or missed labor. It means that the embryo in the uterine cavity is not naturally excreted 2 months after cessation of development [1]. In recent years, the incidence of missed abortion is increasing. Missed abortion has become a common disease clinically [2]. The pathogenesis of missed abortion is unknown. Some studies have suggested that genetic factors, infectious disease factors and immune dysfunction may lead to missed abortion [3-5]. Uterus damage and a series of reproductive organs and systemic diseases are easily caused by missed abortion. Furthermore, the chance of second pregnancy may be lost [6]. Uterine curettage is usually given to the patients with missed abortion clinically. It is a medical treatment. However, the cervix needs to be dilated to the maximum during the surgery. It is very easy to cause the cervical laceration. Meanwhile, the uterus is more likely to be perforated [7]. Therefore, to prevent the occurrence of missed abortion, it is very important to find a sensitive and effective clinical measure.

With the deepening of the study, more and more research scholars think that MicroRNA (miRNA) plays an important role in pregnancy and childbirth [8-10]. miRNA is a kind of non-coding single-stranded RNA molecules with the length of 20–24 nt. It is common in animals and plants. Meanwhile, miRNA has some regulatory effect on cells and tissues [11]. It has been proved that miR-30d has important effect in reproductive tumors [12]. The study of Vilella, et al. [13] has demonstrated that the increased expression of miR-30d can effectively promote the adhesion ability of mouse embryo. MiR-518b is a placental specific miRNA. Study has shown that the abnormal increase of miR-518b in placenta has important functions in pregnancy [14]. The study on miR-518b and miR-30d in missed abortion has not been reported at home and abroad. The effect of miR-518b and miR-30d on missed abortion is unclear. It is suspected that the test of miR-518b and miR-30d expression in placenta is of great clinical significance for the missed abortion. Therefore, the experiment analysis was carried out. Thus, the effective reference and guidance was pro-
Expression of miR-518b and miR-30d

<table>
<thead>
<tr>
<th>Table 1. Primer sequence</th>
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<tr>
<td><strong>Upstream</strong></td>
</tr>
<tr>
<td>miR-518b</td>
</tr>
<tr>
<td>miR-30d</td>
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<tr>
<td>U6</td>
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</table>

vided for the future diagnosis and treatment of missed abortion.

Materials and methods

45 women with missed abortion in our hospital from August 2016 to January 2019 were included in the study group (SG). They were aged 22~38 and 26.85±5.04 in average. 45 women with healthy induced abortion in the same period were enrolled in the control group (CG). They were 23~37 years old and 27.05±4.87 in average. The study was approved by the Ethics Committee of the hospital. All the subjects above signed informed consent form.

Inclusion/exclusion criteria

The inclusion criteria were as follows: the pregnant women were diagnosed with missed abortion in our hospital. The patients underwent induced abortion. The patients were aged 20~40. The patients agreed to cooperate with the medical staffs. The exclusion criteria were shown below: the pregnant women with tumor; the patients with pregnancy-induced hypertension; the pregnant women with autoimmune disease; the pregnant women with other cardiovascular and cerebrovascular disease; the pregnant women with organ failure; the pregnant women with liver and kidney dysfunction; the pregnant women reproductive organ disease; the physically disabled pregnant women unable to take care of themselves. The inclusion criteria in control group were as following: the subjects were 20~40 years old. The routine test had no any abnormalities. The subjects agreed to cooperate with the medical staffs. The patients were given induced abortion in our hospital.

Methods

**Sampling:** Negative uterine aspiration was performed under intravenous anesthesia. The placental villi tissue was collected. It was rinsed with normal saline. 1 cm³ of villi tissue was placed in cryogenic tube (stored in refrigerator at -80°C). Meanwhile, 4 mL of peripheral blood was collected under fasting conditions. After being stored at room temperature for 30 min, the blood was centrifuged for 10 min (4000 rpm/min). The serum in the upper layer was separated and stored in the refrigerator at -80°C to be tested.

**PCR test:** The total RNA was extracted with EasyPure miRNA Kit (Transgen Company, ER601-01). The purity, concentration and integrity of total RNA was determined by ultraviolet spectrophotometer and agarose gel electrophoresis. The reverse transcription was performed with TransScript Green miRNA Two-Step qRT-PCR SuperMix (Transgen Company, AQ202-01). The kit instruction was followed. The cDNA was collected for PCR amplification experiment. The primer sequence was shown in **Table 1**. qPCR amplification system was as follows: cDNA 1 μL, upstream and downstream 0.4 μL respectively, 2× TransTaq Tip Green qPCR SuperMix 10 μL, Passive Reference Dye (50×) 0.4 μL. Finally, ddH₂O was added till the system reached 20 μL. qPCR amplification conditions were shown below: pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing extending at 60°C for 30 s, 40 cycles in total. 3 repeated wells were set for each sample. The experiment was repeated 3 times. U6 was taken as the internal reference in the study. The data were analyzed with 2⁻ΔΔct.

**Outcome measures:** The expression of miR-518b and miR-30d in placental villi tissue, peripheral blood. The correlation between placental villi tissue and peripheral blood. The predictive value in missed abortion. The relationship with clinical characteristics in SG.

**Statistical methods:** The experimental results were statistically calculated with SPSS24.0 (Beijing NDtimes Science and Technology Co., Ltd.). The figures were plotted with Graphpad 8 (Shenzhen Softhead Inc.), Second checking calculation was performed. The enumeration data, such as the patient’s gender, delivery times, were expressed with rate. Chi-square test was used for comparison between the groups. The measurement data, such as the expression levels of miR-518b, miR-30d, were represented...
Expression of miR-518b and miR-30d

...with mean ± standard deviation. T test was adopted for comparison between the groups. Pearson correlation coefficient was introduced for correlation analysis. The diagnostic value was analyzed with ROC. *P*<0.050 implied the significant difference.

**Results**

**Comparison of general information**

There was no difference in age, BMI, gestational weeks, pregnancy times, abortion times, living conditions, smoking, drinking habit, family history and exercise habit between the two groups (*P*>0.050). The patients were comparable (Table 2).

**Comparison of miR-518b and miR-30d expression**

The expression levels of miR-518b in placental villi tissue and peripheral in SG were respectively 7.54±1.52 and 4.05±1.14. They were higher than 2.14±0.63 and 2.27±0.72 in CG (*P*<0.001). The expression levels of miR-30d in CG were accordingly 1.12±0.09 and 1.24±0.12. They were lower than 2.64±0.27 and 1.94±0.38 in SG (*P*<0.001) (Figure 1).

**Correlation of miR-518b and miR-30d expression between placental villi tissue and peripheral blood in SG**

The Pearson correlation coefficient analysis showed that the expression of miR-518b in placental villi tissue was positively correlated with that in peripheral blood (*r*=0.840, *P*<0.001). Similarly, the expression of miR-30d also showed a positive correlation with that in peripheral blood (*r*=0.776, *P*<0.001) (Figure 2; Table 3).

**Predictive value of miR-518b and miR-30d in missed abortion**

The ROC analysis showed that the AUC, sensitivity and specificity of miR-518b for diagnosis of missed abortion were respectively 0.804, 64.44% and 88.89% when the cut-off value was 2.991. The values were correspondingly 0.840, 77.78% and 88.89% if cut-off value was 1.514 (Figure 3; Table 4).

**Relationship between miR-518b, miR-30d in peripheral blood and clinical characteristics in SG**

There was no relationship between the age, BMI, pregnancy times, genetic history and the expression of miR-518b in peripheral blood in SG (*P*>0.050). However, the expression increased in the patients with longer gestational weeks and non-first-time induced abortion (*P*<0.050). The expression of miR-30d had no relationship with the BMI, gestational weeks, pregnancy times, genetic history, induced abortion times and family history (*P*>0.050). It elevated with increasing age (*P*<0.001) (Table 5).

**Discussion**

Missed abortion is usually accompanied by threatened abortion in the early stage. Once
missed abortion occurs, the uterus is no longer dilated but gradually shrinked. Meanwhile, the flexibility significantly decreases [15]. At this time, the organized placenta is closely connected with the inner wall of the uterus [16]. The dead placenta cannot be excreted and dissolved in the maternal organism. The produced hemolytic enzyme enters the blood circulation. It reacts violently with the coagulation factors.

**Table 3.** Correlation of miR-518b, miR-30d expression between placental villi tissue and peripheral blood in SG

<table>
<thead>
<tr>
<th></th>
<th>miR-518b</th>
<th>miR-30d</th>
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<tr>
<td>$r$</td>
<td>0.776</td>
<td>0.776</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.726~0.910</td>
<td>0.625~0.871</td>
</tr>
<tr>
<td>R square</td>
<td>0.706</td>
<td>0.602</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
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</table>
As a result, the microvascular coagulation disorder is caused. The incidence of the disease increases remarkably with the increase of the uterine cavity retention time [17]. With the development of modern medical technology, the development of fetus has been effectively judged by B-ultrasonography. However, the missed abortion has no significant clinical symptoms. It is not paid attention to by patients [18]. Meanwhile, there are difficulties in the implementation of B-ultrasonography during abortion. Therefore, it is of great importance to find a convenient test method for the diagnosis and treatment of missed abortion in the future. At present, study has demonstrated that T lymphocyte subsets and multiple cytokines of FoxP3 play a role in missed abortion [19]. The study of Chen, et al. [20] has shown that GRIM-19 may be related to the pathogenesis of missed abortion.

miRNA is a class of endogenous non-coding small molecule RNA. It only accounts for 1~3% of human genome sequence. Its length is only equivalent to that of 17-25 nucleotides [21]. Incomplete pairing occurs in the non-coding region of its target gene 3'UTR end. The mRNA translation is blocked. The apoptosis, proliferation, metastasis, differentiation and other vital activities of cells involved in the regulation occur. As a cancer-promoting or tumor-suppressor gene, miRNA is proved to have critical effect in various tumor diseases [22, 23]. Due to the characteristics of miRNA, more and more studies on diseases have found new diagnosis and treatment methods through miRNA. However, the study on missed abortion and miRAN has just started. Therefore, the expression of miR-518b and miR-30d in patients with missed abortion was investigated in this study. Thus, a convenient, fast and accurate measure for clinical diagnosis and treatment of missed abortion was provided.

The study results showed that the expression levels of miR-518b and miR-30d in SG were remarkably higher than those in CG. It indicated that miR-518b and miR-30d may be involved in the occurrence and development of missed abortion. MiR-518b is derived from human chromosome 19. It is mainly expressed in human reproductive system and placenta [24]. Liang, et al. [25] have found that miR-518b is specific to human placental tissue by gene chip technology. This study showed that miR-518b was highly expressed in placental villi tissue. Similarly, high expression occurred in peripheral blood. The reason was speculated that miR-518b was mainly secreted by trophoblast cells in placental villi tissue. However, the trophoblast cells also produce exosomes [26]. Therefore, miR-518b may enter the peripheral blood circulation through the accompanying exosomes. Likewise, the study of Miura, et al. [27] also has found that the miR-518b expression in peripheral blood significantly increases during pregnancy and reduces after delivery. This is
Expression of miR-518b and miR-30d

Table 5. Relationship between miR-518b, miR-30d and clinical characteristics in placental villi of SG

<table>
<thead>
<tr>
<th></th>
<th>miR-518b t</th>
<th>P</th>
<th>miR-30d t</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;26 (12)</td>
<td>7.66±1.63</td>
<td>0.152 0.880</td>
<td>7.322 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>≥26 (33)</td>
<td>7.58±1.54</td>
<td>2.02±0.14</td>
<td>2.85±0.53</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>0.146 0.885</td>
<td>0.219 0.828</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;29 (16)</td>
<td>7.68±1.64</td>
<td>2.58±0.28</td>
<td>2.60±0.30</td>
<td></td>
</tr>
<tr>
<td>≥29 (29)</td>
<td>7.60±1.83</td>
<td>2.46±0.35</td>
<td>2.38±0.26</td>
<td></td>
</tr>
<tr>
<td>Gestational weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 (18)</td>
<td>7.12±1.07</td>
<td>0.112 0.911</td>
<td>0.599 0.552</td>
<td></td>
</tr>
<tr>
<td>≥10 (27)</td>
<td>7.95±1.34</td>
<td>2.60±0.43</td>
<td>2.38±0.26</td>
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<tr>
<td>Pregnancy times</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-time (34)</td>
<td>7.52±1.27</td>
<td>2.52±0.37</td>
<td>2.60±0.43</td>
<td></td>
</tr>
<tr>
<td>Non first-time (11)</td>
<td>7.47±1.33</td>
<td>2.60±0.43</td>
<td>2.38±0.26</td>
<td></td>
</tr>
<tr>
<td>Induced abortion times</td>
<td>2.085 0.043</td>
<td>0.358 0.722</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-time (39)</td>
<td>7.09±1.14</td>
<td>2.28±0.46</td>
<td>2.35±0.32</td>
<td></td>
</tr>
<tr>
<td>Non first-time (6)</td>
<td>8.12±1.02</td>
<td>2.37±0.20</td>
<td>2.42±0.31</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (3)</td>
<td>7.51±1.19</td>
<td>0.047 0.963</td>
<td>0.273 0.786</td>
<td></td>
</tr>
<tr>
<td>None (42)</td>
<td>7.48±1.06</td>
<td>2.37±0.20</td>
<td>2.42±0.31</td>
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</table>

also an evidence for the study results. The further analysis showed that the expression of miR-518b was higher in patients with longer gestational weeks and non first-time induced abortion. As a key place to provide nutrition for embryo development, placenta affects the normal development of fetus, maternal pregnancy health, delivery results, etc. The longer the gestational weeks are, the more the capacity and nutrition from placenta are. Meanwhile, more trophoblast cells grow and the expression of miR-518b increases. However, the women with non first-time induced abortion have undergone uterine currettage. Some damages are caused to uterus endothelium and cervix opening. The ability to get pregnant decreases naturally. The trophoblast cells are more likely to have abnormal function. It not only increases the expression of miR-518b, but also increases the possibility of missed abortion.

MiR-30d is a member of miR-30 family. It is located in the 8q24 region of chromosome. Study has shown that miR-30d is involved in the occurrence and development of colon cancer [28]. MiR-30d was highly expressed in patients of SG. It also implied that miR-30d may be closely related to the missed abortion. It is speculated that the mechanism of miR-30d in missed abortion may be similar to that of miR-518b. Namely, miR-30d also has the regulatory effect through trophoblast cells. Trophoblast cells have strong absorption and translocation ability. Meanwhile, they can secrete massive substances. These substances affect the development of embryo [29]. However, the increased miR-30d may lead to the abnormal activity of trophoblast cells. As a result, embryo damage is caused. Furthermore, missed abortion may occur. The study result of Cai et al. [30] on expression of miR-30d in endometrial carcinoma is consistent with that in this study. The expression of miR-30d increased significantly with increasing age. It may be related to the body function. The obvious decline of body function during pregnancy is more likely to affect the metabolism and immune function. Therefore, the negative effect of miR-30d on trophoblast cells is more easily reflected.

The correlation analysis showed that the expression of miR-518b and miR-30d in placental villi tissue of patients with missed abortion was correlated with that in peripheral blood. Therefore, the predictive value of miR-518b and miR-30d in peripheral blood in missed abortion was determined with ROC. The results showed that both of them had good sensitivity and specificity. Therefore, the expression in peripheral blood can be detected to prevent the occurrence of missed abortion.

This study was intended to investigate the relationship between missed abortion and miR-518b, miR-30d. However, there are some deficiencies due to the limited experimental conditions. For example, the study of miR-518b and miR-30d in missed abortion is fewer. The mechanism is exactly known. The effect of miR-518b and miR-30d has not been confirmed by further experiment in this study. The domestic and foreign research scholars are hoped to improve this point. In addition, the sample size
Expression of miR-518b and miR-30d

is smaller. The statistical analysis on big data cannot be performed. The contingency of experimental results cannot be excluded. The subjects in this study will be followed up for a longer time. The experimental design will be constantly improved. The effect of miR-518b and miR-30d will be further analyzed to obtain the optimal experiment results.

In summary, both miR-518b and miR-30d are highly expressed in placental villi tissue of patients with missed abortion. They are expected to become an excellent measure for diagnosis and treatment of missed abortion.

Disclosure of conflict of interest

None.

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