

Clinical significance of expression of hsa-mir-1247 and hsa-mir-1269a in ectopic pregnancy due to salpingitis

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Abstract. The clinical significance of hsa-mir-1247 and hsa-mir-1269a expression in ectopic pregnancy due to salpingitis was investigated. Eighty patients with ectopic pregnancy diagnosed by ultrasonography who were admitted to Jinan Maternity and Child Care Hospital from January 2012 to May 2012 were enrolled in this study. To the observation group were assigned 35 patients whose ectopic pregnancy was due to salpingitis. The remaining 45 patients whose ectopic pregnancy was due to reasons other than endometriosis were assigned to the control group. Moreover, 32 healthy pregnant women were enrolled in this study at the same time as the healthy control group. Compared with the healthy control group, hsa-mir-1247 and hsa-mir-1269a were downregulated and upregulated, respectively, in patients with ectopic pregnancy ($P < 0.05$). The difference was even more marked in patients with ectopic pregnancy due to salpingitis ($P < 0.05$). The expression levels of hsa-mir-1247 and hsa-mir-1269a were negatively correlated, and the correlation coefficient r and P -value was -0.667 and 0.006 , respectively. Abnormal expression of hsa-mir-1247 and hsa-mir-1269a may be risk factors for ectopic pregnancy. Abnormal expression of hsa-mir-1247 and hsa-mir-1269a found in patients with ectopic pregnancy due to salpingitis may be used as biomarkers of ectopic pregnancy.

Introduction

Ectopic pregnancy, also known as tubal pregnancy, happens when the fertilized egg attaches itself in a place other than inside the uterus. An ectopic pregnancy can occur in fallopian tube, ovary and abdominal organs. The incidence is approximately 1-2% and the clinical manifestations are mainly acute abdominal symptoms (1,2). The incidence of ectopic pregnancy has risen in recent years, which may be partly attributed to increases in cases

of salpingitis. The most common site for ectopic pregnancy is the fallopian tubes, accounting for 90 to 95% (3,4). The popularity of cesarean section in modern society has also increased the incidence of ectopic pregnancy in a previous cesarean section scar (5). Ectopic pregnancy is a serious medical condition, which may pose a significant threat to the health and life of affected women, if not diagnosed and treated early. Heavy internal bleeding caused by fallopian tubal rupture due to tubal pregnancy is the leading cause of maternal death (6,7).

MicroRNAs (miRNAs) are widely expressed in eukaryotic cells, involving in various pathophysiological processes by regulating cell proliferation, differentiation and apoptosis (7,8). Abnormal expression of hsa-mir-1247 was reported in gynecologic cancers such as cervical cancer, in which it was downregulated (9). hsa-mir-1269a was downregulated in placental tissue of gestational diabetes mellitus (10). Therefore, it is likely that hsa-mir-1247 and hsa-mir-1269a play a role in ectopic pregnancy. In the present study, experiments were designed to explore the clinical significance of hsa-mir-1247 and hsa-mir-1269a in ectopic pregnancy. Eighty patients with histologically proven ectopic pregnancy who received treatment in Jinan Maternity and Child Care Hospital (Jinan, China) from January 2012 to May 2012 were enrolled in the present study. The expression of hsa-mir-1247 and hsa-mir-1269a were evaluated for a glimpse of their roles in ectopic pregnancy.

Patients and methods

Subjects and clinical materials. Eighty patients with ectopic pregnancy confirmed by ultrasonography who received treatment in Jinan Maternity and Child Care Hospital from January 2012 to May 2012 were enrolled in this study. Assigned to observation group were 35 patients whose ectopic pregnancy was due to salpingitis. The remaining 45 patients whose ectopic pregnancy was due to reasons other than endometriosis were assigned to the control group. Moreover, 32 healthy pregnant women were enrolled in this study at the same time as the healthy control group. The expression levels of hsa-mir-1247 and hsa-mir-1269a in serum of patients were determined by reverse transcription-polymerase chain reaction (RT-PCR) assay. The following medical conditions were excluded in enrolling pregnant women as subjects: Local inflammation/systemic infection other than salpingitis, hypertension, diabetes, preeclampsia, previous surgical treatment, mental disorders and communication disorders. This study was approved by the Ethics Committee

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Table I. Medical records of the two patient groups and the healthy control group.

Items in the records	Observation group	Control group	Healthy control group	P-value
Case no.	35	45	32	
Age (years)	30.6±4.2	31.9±4.7	29.3±5.1	0.725
Menopause time (days)	54.2±7.4	55.6±6.9	57.2±8.8	0.833
Parity, n (%)				
Primipara	12 (34.29)	14 (31.11)	10 (31.25)	0.857
Multipara	23 (65.71)	31 (68.89)	22 (68.75)	
Smoking history, n (%)				
Yes	3 (8.57)	5 (11.11)	2 (6.25)	0.813
No	32 (91.43)	40 (88.89)	30 (93.75)	
Residence, n (%)				
Urban	17 (48.57)	21 (46.67)	15 (46.88)	0.792
Rural	18 (51.43)	24 (53.33)	17 (53.12)	
Ethnicity, n (%)				
Han	33 (94.29)	41 (91.11)	29 (90.63)	0.815
Minority	2 (5.71)	4 (8.89)	3 (9.37)	
Type of ectopic pregnancy, n (%)				
Fallopian tube	29 (82.86)	41 (91.11)		0.653
Ovary	2 (5.71)	3 (6.67)		
Cesarean section scar	1 (2.86)	1 (2.22)		
Others	3 (8.57)	0 (0.00)		

of Jinan Maternity and Child Care Hospital, and the patients or their family signed the informed consent form.

Instruments and reagents. The instruments and reagents were purchased from the following manufacturers: PCR instrument was from Applied Biosystems (Foster City, CA, USA); TRIzol reagent from Invitrogen (Carlsbad, CA, USA); TaqMan[®] MicroRNA reverse transcription kit and agarose were from Thermo Fisher Scientific Co., Ltd. (Shanghai, China); and U6 reference and primers from Guangzhou Shangeng Biotechnology Co., Ltd. (Guangzhou, China).

RNA extraction. Total RNA was extracted from serum with TRIzol reagent following the manufacturer's instructions. RNA concentration and purity were determined by using a UV spectrophotometer. RNA integrity was evaluated by 3% agarose gel electrophoresis.

cDNA synthesis. The complementary DNA (cDNA) was synthesized via reverse transcription of the single stranded RNA. The reaction was carried out at 37°C for 45 min and 95°C for 5 min in accordance with the kit manufacturer's instructions. The product was stored at -20°C.

RT-PCR. Total volume of the PCR reaction system was 25 µl. The PCR reaction was performed as follows: Pre-denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 sec (denaturation), 60°C for 45 sec (anneal), and 72°C for 3 min (extension), followed by extension at 72°C for 5 min at the end of the cycles. PCR products were stored at 4°C. The PCR assay was

performed by using an ABI Prism 7900 PCR instrument. Specific primer sequences are as follows: For hsa-mir-1247 the forward sequence is 5'-ACACTCCAGCTGGGACCCGTCCTTCGTCC-3', and the reverse sequence is 5'-CTCAACTGGTGTCGTGGA-3'; for hsa-mir-1269a the forward sequence is 5'-CUGGACUGAGCCGUGCUACUGG-3', and the reverse sequence is 5'-CTCAACTGGTGTCTCGTGA-3'. U6 was used as the reference gene. All samples were run in triplicate. The results were analyzed by using the 2^{-ΔΔC_q} method.

Statistical analysis. Experimental data were statistically analyzed by using SPSS 19.0 software (IBM SPSS, Armonk, NY, USA). The chi-square test was used for counting data. Measurement data are expressed as mean ± SD. The independent sample Student's t-test was used for comparison of data between two groups, and analysis of variance was used for comparison of data between multiple groups. Correlation between hsa-mir-1247 and hsa-mir-1269a expression was analyzed by using logistics regression. Correlation of hsa-mir-1247/hsa-mir-1269a and ectopic pregnancy was evaluated by using univariate COX regression. The receiver operating characteristic curve (ROC curve) was plotted to assess the diagnostic value of serum hsa-mir-1247 and hsa-mir-1269a for patients with ectopic pregnancy. A P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of subjects in different groups. There were no statistically significant differences in age, menopause time and maternal condition between the three groups (P>0.05).

Table II. Expression levels of hsa-mir-1247 and hsa-mir-1269a.

Groups	hsa-mir-1247	hsa-mir-1269a
Observation	0.119±0.021	0.242±0.021
Control	0.697±0.104	0.091±0.001
Healthy control	1.740±0.642	0.018±0.001
P-value		
Between 3 groups	0.035	0.041
Observation vs. control	0.024	0.031
Observation vs. healthy control	0.017	0.012
Control vs. healthy control	0.031	0.042

Table III. Univariate COX regression analysis on patients with ectopic pregnancy.

Variables	Univariate analysis		
	HR	95% CI	P-value
hsa-mir-1247 (low vs. high)	1.389	1.042-1.852	0.037
hsa-mir-1269a (low vs. high)	1.463	1.064-2.013	0.031
Menopause time (<52 vs. ≥52 days)	0.468	0.212-1.034	0.064
Primipara (yes vs. no)	0.893	0.274-2.452	0.779
Age (years) (<31 vs. ≥31)	0.832	0.376-1.864	0.672
Smoking history (yes vs. no)	1.064	0.911-1.243	0.181
Combined salpingitis (yes vs. no)	0.674	0.489-0.930	0.029

HR, hazard ratio; CI, confidence interval.

The proportion of patients with smoking history in the three groups was ~10%, and there was no significant difference in the analysis of variance among the groups ($P>0.05$). The sites of implantation for ectopic pregnancy were mostly fallopian tube for patients in the observation group and the control group. There was one case of ectopic pregnancy in a previous cesarean section scar in each patient group. The difference was not statistically significance ($P>0.05$) (Table I).

RT-PCR amplification of serum hsa-mir-1247 and hsa-mir-1269a for subjects in the three groups. RT-PCR amplification of hsa-mir-1247 and hsa-mir-1269a in serum was performed for the patients with ectopic pregnancy and healthy pregnant women. The results of the analysis of variance indicated that differences of the expression levels of hsa-mir-1247 and hsa-mir-1269a between the three groups were statistically significant ($P=0.035$ and $P=0.041$, respectively). The differences were also significant in the pairwise comparison ($P<0.05$). The expression level of hsa-mir-1247 in the

observation group was lower than that in the control group (0.119 ± 0.021 vs. 0.697 ± 0.104 , $P=0.024$), whereas the level in the control group was lower than that in the healthy control group (0.697 ± 0.104 vs. 1.740 ± 0.642 , $P=0.031$). The above results demonstrated that the expression level of hsa-mir-1247 was downregulated in patients with ectopic pregnancy, and the downregulation was even more obvious in patients with ectopic pregnancy due to salpingitis. The expression level of hsa-mir-1269a in the observation group was higher than that in the control group (0.242 ± 0.021 vs. 0.091 ± 0.001 , $P=0.031$), whereas the level in the control group was higher than that in the healthy control group (0.091 ± 0.001 vs. 0.018 ± 0.001 , $P=0.042$). These results demonstrated that the expression level of hsa-mir-1269a was upregulated in patients with ectopic pregnancy, and the upregulation was even more pronounced in patients with ectopic pregnancy due to salpingitis (Table II).

Logistics regression analysis. The expression levels of hsa-mir-1247 and hsa-mir-1269a were negatively correlated, and the correlation coefficient r and P -value were -0.667 and 0.006 , respectively.

Univariate COX regression analysis. COX regression analysis was conducted on patients with ectopic pregnancy. The median expression levels of hsa-mir-1247 and hsa-mir-1269a in all the 80 patients with ectopic pregnancy were 0.412 and 0.167, respectively. Patients were divided into high expression group and low expression group with the median value as the cut-off point. The median menopause time and median age of all the 80 patients were 52 days and 31 years, respectively. The COX regression analysis showed that abnormal expression levels of hsa-mir-1247 and hsa-mir-1269a, as well as salpingitis, may be risk factors for ectopic pregnancy. The detailed results of the analysis are shown in Table III.

Discussion

The ectopic pregnancy symptoms vary greatly from pregnancy to pregnancy. Some patients may experience no symptoms, whereas others may experience severe pelvic pain. Ectopic pregnancy that implants in the fallopian tube can lead to fallopian tube rupture. In this case, heavy internal bleeding may occur, leading to shock or even death of the patient (11,12). Ectopic pregnancy occurs in the first trimester of pregnancy with high morbidity and mortality, accounting for approximately 6% of maternal deaths (13,14). Etiological factors of ectopic pregnancy are complicated. The roles and mechanism of action of these factors are not yet clear. Risk factors cannot predict occurrence of ectopic pregnancy in approximately 50% of cases (15,16). Early diagnosis and active treatment have a positive and important impact on health and life of women with ectopic pregnancy. In this study, the roles of hsa-mir-1247 and hsa-mir-1269a in ectopic pregnancy were explored by measuring their expression levels in serum of 80 patients with ectopic pregnancy, aiming to provide a reference for clinical diagnosis and treatment of ectopic pregnancy.

In this study, sera of 80 patients with ectopic pregnancy confirmed by ultrasound and 32 healthy pregnant women were collected according to a strict protocol and storage requirements. The expression levels of hsa-mir-1247 and hsa-mir-1269a in the

serum were determined by RT-PCR assay. In ectopic pregnancy hsa-mir-1247 was downregulated, whereas hsa-mir-1269a was upregulated. Out of 80 patients with ectopic pregnancy, 35 experienced salpingitis. The expression levels of hsa-mir-1247 and hsa-mir-1269a in these patients were further analyzed statistically. It was found that the expression level of hsa-mir-1247 in patients who also experienced salpingitis was lower than those in other patients, whereas the expression level of hsa-mir-1269a was higher in the corresponding comparison. Therefore, it was speculated that ectopic pregnancy triggered abnormally high expression of hsa-mir-1269a, which in turn mediated down-regulation of hsa-mir-1247. It appeared that salpingitis promoted this process. Logistics regression analysis also confirmed that expression of hsa-mir-1247 and hsa-mir-1269a was negatively correlated. The above evidence suggested that hsa-mir-1247 and hsa-mir-1269a could be therapeutic targets for ectopic pregnancy. Non-invasive potential treatment represents a trend in the development of modern medicine, which is also the preferred patient care with high patient acceptance and satisfaction (17). Therefore, this speculation is of great significance and we will explore it further in future research. Dominguez *et al* (18) reported that four miRNAs were differentially downregulated (hsa-mir-196b, hsa-mir-30a, hsa-mir-873 and hsa-mir-337-3p) and three upregulated (hsa-mir-1288, hsa-mir-451 and hsa-mir-223) in ectopic pregnancy compared to control tissue samples. In our future research these findings can be confirmed by using patient serum. Treatment options for ectopic pregnancy are mainly surgery or medication to end the pregnancy. Among drugs for treatment of ectopic pregnancy, methotrexate exhibits excellent safety and efficacy profiles. It was reported that methotrexate was safe with virtually no adverse effects on women's reproductive function (19,20).

In the present study, univariate Cox regression analysis was conducted to evaluate the roles of hsa-mir-1247 and hsa-mir-1269a in ectopic pregnancy, aiming to identify risk factors that can be used to predict occurrence of ectopic pregnancy. The analysis showed that hsa-mir-1247 and hsa-mir-1269a, as a potential risk factor for ectopic pregnancy, had a hazard ratio (HR) of 1.389 [95% confidence interval (CI), 1.042-1.852] and 1.463 (95% CI, 1.064-2.013), respectively. Although this result suggested that hsa-mir-1247 and hsa-mir-1269a may be risk factors for ectopic pregnancy, further research and a larger number of samples are needed to verify whether they are independent risk factors for ectopic pregnancy. Currently known risk factors include assisted reproductive technology such as *in vitro* fertilization (21), as well as cesarean section and hormone stimulation (22).

In summary, the abnormal expression of hsa-mir-1247 and hsa-mir-1269a was found in patients with ectopic pregnancy and enhanced in patients with combined salpingitis. These two miRNAs may serve as biomarkers for prediction and treatment of ectopic pregnancy.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SZ conceived and designed the study. QS, XJ and FG collected, analyzed and interpreted the patient data. SZ wrote the manuscript. FG revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Jinan Maternity and Child Care Hospital (Jinan, China). Signed written informed consents were obtained from the patients and/or guardians.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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