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Original Article

miRNA-145 and IGF-1R in hydrosalpinx-induced infertility

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Abstract: Objective: To explore the expression of miRNA-145 and insulin-like growth factor 1 receptor (IGF-1R) in endometrial tissues of patients with hydrosalpinx-induced infertility and their influencing mechanism. Methods: A total of 37 patients with hydrosalpinx infertility (hydrosalpinx group) who visited our hospital from January to September 2019 were retrospectively analyzed. In the same period, 30 patients with endometriosis (EMs) and 28 normal women underwent abortion operation were selected as the EMs group and the control group, respectively. Meanwhile, patients in the hydrosalpinx group were divided into mild group, moderate group and severe group based on the degree of hydrosalpinx. Endometrial tissues were obtained from all study subjects. Real-time fluorescence quantitative PCR was used to determine the expression level of miRNA-145 in endometrial tissues, and IGF-1R expression level was measured by enzyme-linked immunosorbent assay. The expression levels of miRNA-145 and IGF-1R in endometrial tissues of the three groups of study subjects and patients with different degrees of hydrosalpinx were compared, and their correlations with hydrosalpinx were analyzed. Results: The age, parity and the number of cesarean sections in the hydrosalpinx group and EMs group were significantly higher than those in the control group (all $P < 0.05$), while there was no significant difference between hydrosalpinx group and EMs group (all $P > 0.05$). The expression level of miRNA-145 in the endometrial tissues of the hydrosalpinx group was significantly higher than that in the EMs group and control group (all $P < 0.001$), while the expression level of IGF-1R was significantly lower than that in the EMs group and control group (all $P < 0.001$). With the aggravation of hydrosalpinx, miRNA-145 expression showed an increasing trend and IGF-1R showed a decreasing trend in endometrial tissues of hydrosalpinx patients, and there were significant differences among the three groups (all $P < 0.001$). Pearson correlation analysis showed that there was a significant negative correlation between miRNA-145 expression level and IGF-1R in endometrial tissues of patients with hydrosalpinx infertility ($r = -0.652$, $P < 0.01$), and multiple linear regression analysis showed that there was a significant correlation between the severity of hydrosalpinx and miRNA-145 and IGF-1R expression levels ($P < 0.001$). Conclusion: The abnormal expression of miRNA-145 and IGF-1R may be an important mechanism affecting infertility caused by hydrosalpinx.

Keywords: Hydrosalpinx, infertility, miRNA-145, insulin-like growth factor 1 receptor, influencing mechanism

Introduction

Hydrosalpinx refers to abnormal hydrops in the lumen of fallopian tube, which is a common gynecological disease [1]. Studies have shown that 30.0-40.0% of female infertility is caused by tubal factors, of which hydrosalpinx accounts for 10.0-30.0% [2]. Hydrosalpinx can damage both tubal function and the internal environment of embryo implantation, resulting in a reduction of about 50.0% in embryo implantation rate and pregnancy rate, while causing a more than 2-fold increase in the rate of spontaneous abortion and the risk of ectopic pregnancy. With the aggravation of hydrosalpinx, it can aggravate the degree of damage and increase the risk of infertility [3].

Identifying the specific pathogenesis and pathological mechanism of hydrosalpinx infertility is essential to improve the outcome of clinical treatment. At present, there are many speculations on the mechanism of infertility caused by hydrosalpinx, including mechanical scouring

theory, embryo or gamete toxicity theory and endometrial receptivity theory; while it has not been clearly concluded, endometrial receptivity has gradually become a hot direction of clinical research [4]. Studies have pointed out that impaired endometrial receptivity is closely related to the pathogenesis of female infertility, and hydrosalpinx can damage endometrial receptivity through a variety of mechanisms, including a variety of cytokines, enzymes and genes, which in turn leads to infertility. miRNA-145 and insulin-like growth factor 1 receptor (IGF-1R) are the current research directions, and have been studied in malignant tumors and other diseases, of which miRNA-145 is considered to promote endometrial cell growth, proliferation, adhesion, migration, invasion, etc. [5]. Besides, it has been found that the expression in the endometrium of endometriosis (EMs) infertility patients is significantly higher than that in non-infertility patients, which is closely related to the pathogenesis of EMs and the occurrence of infertility [6]. Studies have found that estrogen and progesterone can mediate endometrial cell growth and differentiation by promoting the expression of insulin-like growth factor 1 (IGF-1) and other growth factors in endometrial tissues, while IGF-1 and IGF-1R can interact to regulate follicular development, endometrial cell proliferation and blastocyst growth, and their decreased expressions may be involved in infertility [7]. However, there are few reports on miRNA-145 and IGF-1R in endometrial receptivity, so this study tried to elucidate their effects on the pathogenesis of hydrosalpinx infertility by exploring their relationships with endometrial receptivity.

Materials and methods

General data

A total of 37 patients with hydrosalpinx infertility (hydrosalpinx group) who visited Guangdong Women and Children Hospital from January to September 2019 were retrospectively analyzed. All patients met the diagnostic criteria for hydrosalpinx infertility. In the same period, 30 patients with EMs were selected and met the diagnostic criteria for EMs. At the same time, 28 patients with normal endometrial tissues that underwent abortion operation were selected as the control group. None of the three groups had severe cardiovascular and cerebrovascular diseases, substantial organ tissue diseases in liver, kidney and lung etc., as well as severe diseases of immune system, endocrine system and metabolic system. Other infertility factors, such as ovulatory dysfunction, immunity, and causes such as tubal tuberculosis, were excluded in patients with hydrosalpinx infertility, and the patients did not receive endocrine or hormonal therapy in the past 6 months.

Endometrial tissues were obtained by sterile endometrial curettage, and the specimens were immediately placed in Enpendorf tubes and frozen in a -80°C freezer within 15 min to facilitate preservation and extraction of protein and RNA. All study subjects were informed and signed the informed consent form, and this study was reviewed and approved by the Ethics Committee of Guangdong Women and Children Hospital. There were no significant differences in baseline data of body mass index (BMI), menstrual cycle, bad living habits (smoking, drinking, high fat diet) and complications (hypertension, diabetes, hyperlipidemia) etc. among the three groups (all $P > 0.05$). The age, parity and the number of cesarean sections of the hydrosalpinx group and EMs group were significantly higher than those of the control group respectively (all $P < 0.05$), while there were no significant differences between the hydrosalpinx group and EMs group (all $P > 0.05$). See **Table 1** for details.

Reagents and instruments

ABI 7000 fluorescence quantitative PCR instrument (Shanghai Langfu Industrial Co., Ltd.) was used; TaqMan miRNA analysis kit was purchased from Haiji Biotechnology Co., Ltd.; Reverse transcription kit was provided by Shanghai Haifang Biotechnology Co., Ltd.; Trizol reagent was provided by Beijing Pulley (APPLYGEN) Gene Technology Co., Ltd.; ELISA detection kit was provided by Beijing Ovia Biotechnology Co., Ltd.; miRNA primers and housekeeping genes were provided by Asia-Pacific Hengxin Biotechnology (Beijing) Co., Ltd.; Multiskan Sky UV spectrophotometer; Microplate reader.

Test methods

The miRNA expression level in endometrial tissues was detected by real-time quantitative RT-PCR. The total RNA in the tissues was

Table 1. Comparison of baseline data among three groups of study subjects

Group	Hydrosalpinx group (n = 37)	EMs group (n = 30)	Control group (n = 28)	F value	Z value	*P* value
Age (years)	34.1±7.2*	36.2±8.4*	25.6±5.2	4.252		0.014
BMI (kg/m^2)	23.53±2.32	22.91±2.18	23.05±2.47	0.427		0.682
Parity (numbers)	1.23±0.36*	1.42±0.43*	0.27±0.16	3.906		0.027
Numbers of cesarean section (numbers)	0.53±0.33*	0.67±0.21*	0.15±0.04	3.549		0.033
Menstrual cycle (d)	29.64±3.57	28.20±3.15	28.73±3.36	1.272		0.336
Bad habits					0.937	0.772
Smoking	7 (18.92)	6 (20.00)	4 (14.29)			
Alcohol consumption	11 (29.73)	9 (30.00)	6 (21.43)			
High fat diet	16 (43.24)	12 (40.00)	10 (35.71)			
Comorbidities					0.412	0.529
Hypertension	9 (24.32)	9 (30.00)	6 (21.43)			
Diabetes	5 (13.51)	4 (13.33)	2 (7.14)			
Hyperlipidemia	4 (10.81)	3 (10.00)	1 (3.57)			

Note: Compared with control group, *P < 0.05. EMs: endometriosis.

extracted by Trizol method and mixed with 3 μL reverse transcriptase. The reaction conditions were as follows: annealing at 16°C for 30 min, extension at 42°C for 30 min, and denaturation at 85°C for 30 min. The synthesized cDNA was collected and 1 μL of cDNA was selected as the template. 2 μL Taq Man primer was mixed with it for PCR amplification. miRNA-145 forward primer: 5'-GTCCAGTTTTCCCATCCCCCT-3', reverse primer: 5'-GCTGTCAACATACGCTAA-CG-3'. U6 was used as the housekeeping gene, housekeeping primer sequence: 5'-CCCATCTA-TGAGGACGC-3', reverse primer: 5'-TTTAATGT-CACCGATTTC-3'. Reaction step: First 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. The final results were processed using ABI Prism 7300 SDS software, amplification kinetics curves were plotted, and miR-145 miRNA expression level was calculated by the $2^{-\Delta\Delta}$Ct method.

IGF-1R expression level in endometrial tissues was measured by ELISA, and the tissues were mashed with an appropriate amount of saline and centrifuged at 3,000 rpm for 10 minutes to obtain the supernatant. The standard solution was added into the reaction well after diluting by a coefficient, and at the same time 50 μL biotin-labeled antibody was added. The antibody was purchased from Detai Biotechnology (Nanjing) Co., Ltd. Finally, the microtiter plate was taken out and 50 μL stop solution was added. The OD value obtained at the wavelength of 450 nm and the dilution of the standard were used as the abscissa and ordinate respectively to draw the standard curve, calculate the linear regression equation, and plug the OD value into the equation to obtain the standard concentration and the actual concentration before dilution.

Outcome measures

The expression levels of miRNA-145 and IGF-1R in endometrial tissues of the three groups were compared.

The patients were divided into mild, moderate and severe groups based on the severity of hydrosalpinx, and the expression levels of miRNA-145 and IGF-1R in endometrial tissues of patients with different degrees of hydrosalpinx were compared.

Pearson correlation analysis was performed to analyze the correlation between miRNA-145 and IGF-1R expression levels in endometrial tissues of patients with hydrosalpinx infertility.

Using miRNA-145 and IGF-1R as independent variables, multiple linear regression analysis was performed to analyze the correlation between miRNA-145 and IGF-1R expression levels in endometrial tissues and the hydrosalpinx severity of hydrosalpinx infertility.

Statistical methods

SPSS 21.0 statistical software was used to process all the collected data in this study, of which all measurement data parameters were expressed in the form of mean ± standard deviation (x ± sd). One-way analysis of variance

Table 2. Comparison of miRNA-145 and IGF-1R expression levels among the three groups (x ± sd)

Group	miRNA-145	IGF-1R (μg/L)
Hydrosalpinx group (n = 37)	0.21±0.05***,###	3.51±1.32***,###
EMs group (n = 30)	0.10±0.03***	6.23±0.97***
Control group (n = 28)	0.06±0.01	7.86±0.83
F value	11.376	8.359
P value	< 0.001	< 0.001

Note: Compared with the control group, ***P < 0.001; compared with the EMs group, ###P < 0.001. IGF-1R: insulin-like growth factor 1 receptor; EMs: endometriosis.

combined with post-hoc Bonferroni test was used for comparison among the three groups. Enumeration data were expressed in the form of case/percentage. Chi-square test and chi-square segmentation test were used for comparison among the three groups. Pearson correlation was used to analyze the correlation between miRNA-145 and IGF-1R expression levels. Multiple linear stepwise regressions were used to analyze the influencing factors of hydrosalpinx severity. Two-tailed test was employed with test level set at $\alpha = 0.05$. $P < 0.05$ indicated statistically significant differences.

Results

Comparison of miRNA-145 and IGF-1R expression levels among three groups of study subjects

The expression levels of miRNA-145 and IGF-1R in the endometrium of the three groups were statistically different (all P < 0.001), and the expression levels of miRNA-145 in the hydrosalpinx group were significantly higher than those in the EMs group and control group (all P < 0.001), and those in the EMs group were significantly higher than those in the control group (P < 0.001). The expression levels of IGF-1R in the hydrosalpinx group were significantly lower than those in the EMs group and control group (all P < 0.001), and those in the EMs group were significantly lower than those in the control group (P < 0.001). See **Table 2** and **Figure 1**.

Comparison of miRNA-145 and IGF-1R expression levels in patients with different degrees of hydrosalpinx

With the aggravation of hydrosalpinx, the expression of miRNA-145 showed an increasing trend (all P < 0.001) and IGF-1R concentration showed a decreasing trend (all P < 0.001), as shown in **Table 3** and **Figure 2**.

Pearson correlation analysis of miRNA-145 and IGF-1R expression levels in patients with hydrosalpinx infertility

Pearson correlation analysis showed that there was a significant negative correlation between miRNA-145 expression levels and IGF-1R in endometrial tissues of infertile patients with hydrosalpinx (r = -0.652, P < 0.01), as shown in **Figure 3**.

Multiple linear regression analysis of influencing factors of hydrosalpinx severity in hydrosalpinx infertility

Multiple linear regression analysis showed that hydrosalpinx infertility was not significantly correlated with age, BMI, parity, cesarean section, menstrual cycle, poor living habits and complications (all P > 0.05), but it was significantly correlated with miRNA-145 and IGF-1R expression levels (P < 0.001). See **Tables 4**, **5** for details.

Discussion

The theory of endometrial receptivity is considered to be an important mechanism of hydrosalpinx infertility. Endometrial receptivity refers to the receptivity of the endometrium to embryo sac implantation. Embryo sac implantation requires a high endometrial environment and there are only a few days per month suitable for ideal implantation of embryos, also known as the embryo "implantation period", at which endometrial receptivity also reaches the highest level during this period, and this is a key factor for successful pregnancy [8]. Endometrial receptivity regulation involves a complex signaling network, including cytokines, enzymes, hormones, adhesion factors, genes, miRNA, etc., which together regulate endometrial receptivity status to achieve the ability to receive embryo implantation [9]. Current studies have confirmed that hydrosalpinx can affect endometrial receptivity by a variety of mechanisms, such as decreasing endometrial integrin αvβ3 expression, affecting its own differentiation and decidualization, and improving blastocyst placement ability, which is a sensitive marker for evaluating endometrial receptivity;

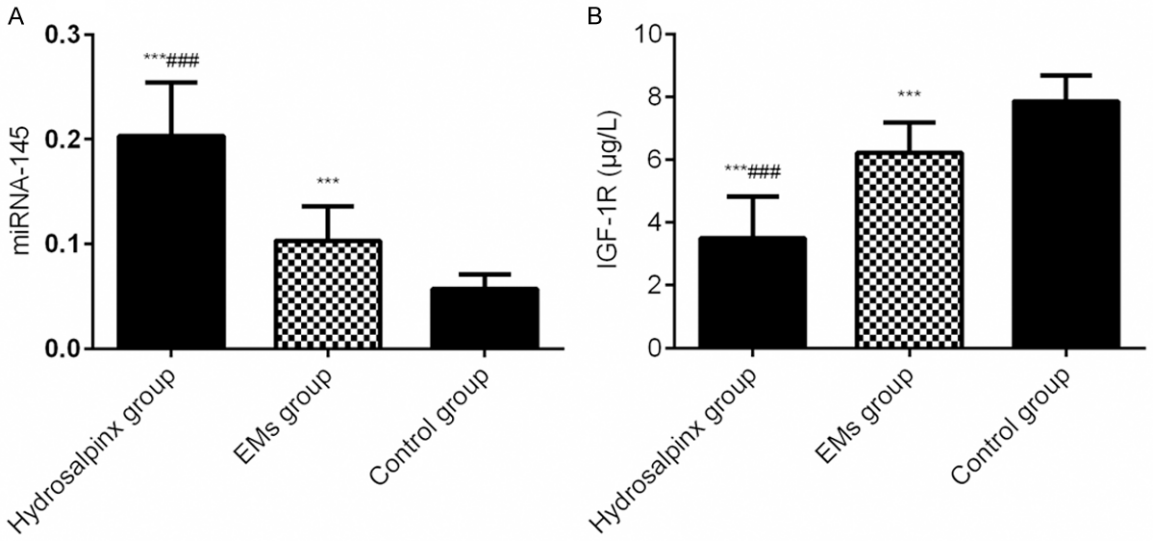

Figure 1. Comparison of miRNA-145 and IGF-1R expression levels among the three groups of study subjects. A. Comparison of miRNA-145 in the three groups. B. Comparison of IGF-1R in the three groups. Comparison with the control group, ***P < 0.001; comparison with the EMs group, ###P < 0.001. IGF-1R: insulin-like growth factor 1 receptor; EMs: endometriosis.

Table 3. Comparison of miRNA-145 and IGF-1R expression levels in patients with different degrees of hydrosalpinx (x ± sd)

Group	miRNA-145	IGF-1R (μg/L)
Mild group (n = 16)	0.14±0.05	5.25±1.34
Moderate group (n = 11)	0.21±0.05***	3.63±1.09***
Severe group (n = 10)	0.34±0.06***,###	2.08±0.95***,###
F value	6.350	9.463
P value	< 0.001	< 0.001

Note: Compared with mild group, ***P < 0.001; compared with moderate group, ###P < 0.001. IGF-1R: insulin-like growth factor 1 receptor.

in the meanwhile, hydrosalpinx can also inhibit the expression of LIF and HOXA10 genes to reduce endometrial receptivity [10].

miRNA is an important molecule in the regulation of post-transcriptional gene expression *in vivo*, and recent studies have confirmed that miRNA can participate in the establishment of the "implantation period" window by regulating physiological processes such as endometrial epithelial cell proliferation and differentiation, as well as endometrial demembranation and embryo sac placement. Thus, clarifying its mechanism of action is expected to provide a brand-new theoretical guidance for the regulation of reproductive processes [11-14]. miRNA-145 locates in human chromosome 5 and was first discovered in mouse heart tissues, mostly expressed in germ layer tissues such as testis, ovary, and uterus. The latest study has confirmed that it is a tumor suppressor gene that can regulate tumor signal transduction pathway targets, including growth factor receptors such as IGF, mucin-1 and other targets, thereby inhibiting tumor cell proliferation, metastasis and invasion [15]. Down-regulation of miRNA-145 expression was detected in patients with malignant tumors such as ovarian cancer, breast cancer and endometrial cancer. Kim et al. showed that miRNA-145 can indirectly affect embryonic stem cell differentiation by regulating the expression of proteins such as OCT3, KLF4 and SOX2, which is closely related to the occurrence of a variety of gynecological diseases [16]. In addition, Wu et al. found that miRNA-145 can also down-regulate the expression of plasminogen activator inhibitor-1 (PAI-1) and some cell scaffold proteins and inhibit the proliferation and differentiation process of ectopic endometrium [17]. However, there is no definite evidence on whether miRNA-145 leads to hydrosalpinx infertility development by regulating endometrial receptivity. The results of this study showed that the expression level of miRNA-145 in endometrial tissues of patients with hydrosalpinx was significantly higher than

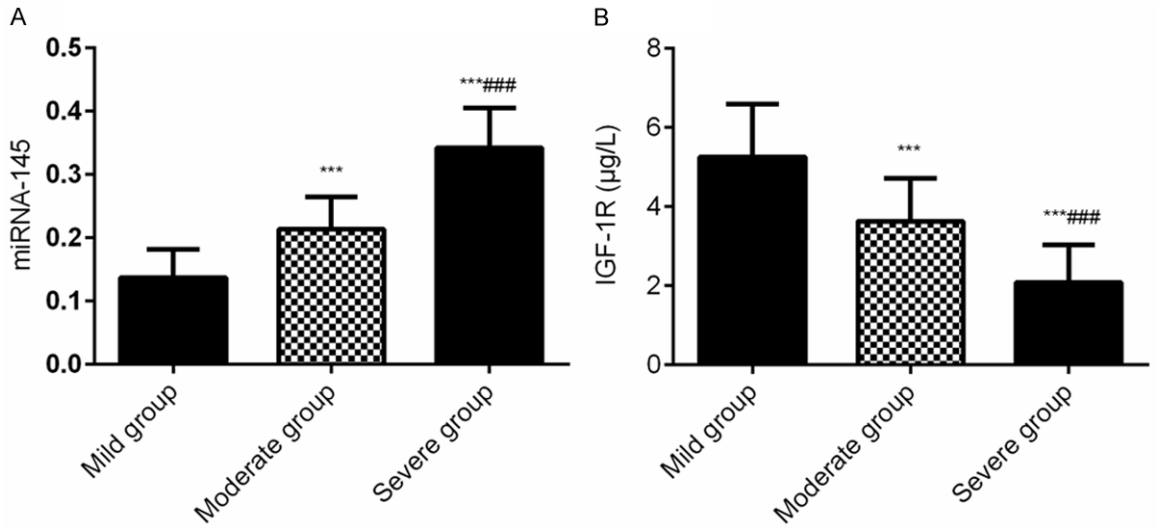

Figure 2. Comparison of miRNA-145 and IGF-1R expression levels in patients with different degrees of hydrosalpinx. A. Comparison of miRNA-145 in the three groups. B. Comparison of IGF-1R in the three groups. Comparison with the mild group, ***P < 0.001; comparison with the moderate group, ###P < 0.001. IGF-1R: insulin-like growth factor 1 receptor.

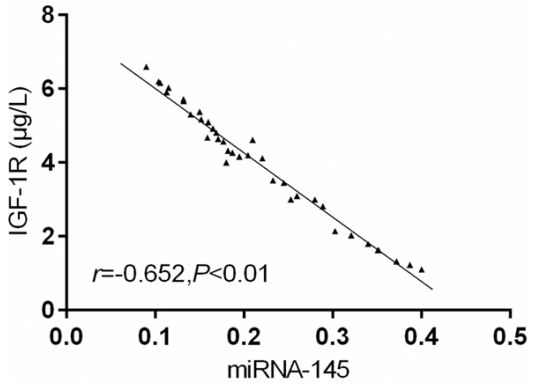

Figure 3. Pearson correlation analysis of miRNA-145 and IGF-1R expression levels in patients with hydrosalpinx infertility. IGF-1R: insulin-like growth factor 1 receptor.

that of EMs group and control group. Severer hydrographic accumulation in the fallopian tubes is associated with higher miRNA-145 expression. These results indicate that the abnormally high expression of miRNA-145 may promote the pathogenesis of hydrosalpinx infertility.

IGF-1R is a tyrosine kinase receptor on the cell surface and a specific receptor on the cell surface of IGF-1. Studies have confirmed that the interaction between IGF-1R and IGF-1 can activate PI3K/AKT and Ras/MAPK signaling pathways, promote the expression of fibronectin in the surface of embryo sac, enhance its adhesion to the endometrium, and promote follicular development and maturation, endometrial cell proliferation and blastocyst growth, and participate in the formation and regulation of endometrial receptivity [18]. However, there are few studies on its specific mechanism. Ghazal et al. found that H19 molecule expression was downregulated and let-7 molecule activity was significantly increased in endometrial tissues of EMs patients; it had a significant inhibitory effect on IGF-1R expression, resulting in a significant decrease in its expression compared with normal endometrium, which hinders the normal proliferation and development of the endometrium, thus impairing endometrial receptivity and causing infertility [19]. Di Pietro et al. found that both growth factor and apoptotic protein expression on the endometrium of infertile patients were significantly changed due to chronic metritis, in which IGF-1R expression was decreased up to 4.7-fold compared with normal, leading to impaired endometrial receptivity by affecting normal decidualization of the endometrium [20]. In addition, it has been found that IGF-1R can also improve granulosa cell sensitivity to sex hormones, promote follicular cell development and maturation, and increase the rate of early embryo and embryo sac formation [21]. IGF-1R is considered to be an essential condition for maintaining endometrial receptivity, and its down-

Table 4. Independent variable value assignment table for multiple linear regression analysis

Variable	Value Assignment
Age	0: < 35 years old; 1: ≥ 35 years old
BMI	0: < 25.00 kg/m^2, 1: 25.00~30.00 kg/m^2, 2: > 30.00 kg/m^2
Parity	0: 0 number; 1: ≥ 1 numbers
Numbers of cesarean section	0: 0 number; 1: ≥ 1 numbers
Menstrual cycle	0: 28-30 days; 1: < 28 days or > 30 days
Comorbidities	0: None; 1: Yes
miRNA-145	0: < 0.10, 1: 0.10~0.20, 2: 0.20~0.30, 3: > 0.30
IGF-1R	0: > 8.00 μg/L, 1: 6.00~8.00 μg/L, 2: 4.00~6.00 μg/L; 3: 2.00~4.00 μg/L; 4: < 2.00 μg/L

Note: BMI: body mass index; IGF-1R: insulin-like growth factor 1 receptor.

Table 5. Multiple linear regression analysis of influencing factors of severity of hydrosalpinx infertility

Variable	Partial regression coefficient	Standard error	Standardized regression coefficient	t	P
Age	0.042	0.195	0.617	0.583	0.446
BMI	0.117	0.356	0.280	1.692	0.121
Parity	0.474	1.738	0.526	1.451	0.136
Numbers of cesarean section	1.026	1.350	0.908	1.924	0.093
Menstrual cycle	0.670	0.805	0.692	1.337	0.149
Bad habits	0.027	0.242	0.836	0.814	0.230
Comorbidities	0.058	0.791	0.374	1.127	0.163
miRNA-145	4.284	2.046	0.447	6.356	0.000
IGF-1R	6.068	3.132	0.368	8.076	0.000

Note: BMI: body mass index; IGF-1R: insulin-like growth factor 1 receptor.

regulation is an important cause of infertility, but its mechanism involved in the pathogenesis of hydrosalpinx infertility is not clear at present. The results of this study showed that the expression of IGF-1R in the endometrium of patients with hydrosalpinx was significantly lower than that of patients in EMs group and control group. With the increase in severity of hydrosalpinx, the expression of IGF-1R in the endometrial tissues showed a significant decrease, suggesting that the decreased expression of IGF-1R may damage endometrial receptivity and cause infertility. At the same time, multiple-variate linear regression analysis in this study showed that miRNA-145 and IGF-1R were significantly associated with the occurrence of hydrosalpinx, which could damage endometrial receptivity to cause infertility. Analysis of the reason suggested that it may be related to the fact that abnormally high expression of miRNA-145 can inhibit IGF-1R expression, interfere with various biological processes such as embryo adhesion and implantation, reduce endometrial receptivity, and participate in the pathogenesis of hydrosalpinx infertility [22].

The molecular mechanisms affecting endometrial receptivity are complicated, and there is a significant bidirectional regulation mechanism, rather than regulation in isolation. The sample size in this study is small, and there is a lack of direct evidence to prove the specific mechanism of action of miRNA-145 and IGF-1R. Which mechanism affects endometrial receptivity and promotes the development of hydrosalpinx infertility still needs further study. Elucidating its specific molecular mechanism and gene expression will provide an important reference for the auxiliary diagnosis and treatment selection for patients, which has important application prospects. Thus, its molecular mechanism still needs to be further explored.

In summary, the abnormal expression of miRNA-145 and IGF-1R may be an important mechanism affecting female infertility caused by hydrosalpinx and may be involved in its occurrence and development process, which is expected to provide guidance for the early diagnosis and treatment selection for hydrosalpinx infertility.

Disclosure of conflict of interest

None.

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