How close are we to diagnosing Polycystic ovary syndrome with miRNAs: A Meta-analysis and review

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Abstract

Objective: To investigate the accuracy of miRNA in the diagnosis of patients with Polycystic ovary syndrome (PCOS).

Design: Systematic review and meta-analysis of controlled trials.

Setting: Not applicable.

Patient(s): Individuals with PCOS and control.

Intervention(s): Summary sensitivity and specificity and positive and negative likelihood ratios (LRs) a random effects model were employed.

Main Outcome Measure(s): Accuracy of miRNAs as diagnostic indicators.

Result(s): The analysis showed that miRNA significantly increased the accuracy of the diagnosis of PCOS, which included 9 articles with 1303 patients. The pooled sensitivity and specificity of miRNAs in the diagnosis of PCOS were 0.73 and 0.81, respectively. Multiple RNAs as biomarkers in qRT-PCR (SYBR) method showed higher sensitivity when compared to a single RNA and the Area Under Curve (AUC) was up to 0.88.

Conclusions: MiRNAs may increase the accuracy of diagnosis and short the time required for diagnosis. Multiple miRNAs in SYBR method have good discriminating ability as biomarker for diagnosis.

Background

Polycystic ovary syndrome (PCOS) is a common reproductive-metabolic, psychological and endocrine disorder with lifelong condition, accounting for a large proportion of infertility [1]. The diagnosis of PCOS worldwide is primarily based on the Rotterdam criteria, NIH Criteria and Androgen Excess-PCOS Criteria, but the subjective judgment of the doctor and the diversity of the patients' symptoms affect the accuracy of the diagnosis [2-4]. Specifically, due to the character of PCOS may confound with pubertal development, including irregular menses, and multi-follicular ovary morphology, the PCOS accuracy diagnosis required at least one year [5]. Under-diagnosis and delayed diagnosis interfered prevention and intervention in PCOS health care [6]. PCOS is positively associated with endometrial cancer while early diagnosis and treatment can decrease the PCOS' common manifestation and comorbidities. Therefore, the discovery of biomarkers for the early diagnosis of PCOS is essential [7].

Recently, MicroRNAs (MiRNAs) showed quite advantage potential in diagnosing diseases, while whether or not suitable in PCOS is still unclear [8, 9]. MiRNAs are small non-protein-coding RNAs of approximately 21-25 nucleotides in length that are involved in the post-transcriptional regulation of gene expression [10]. Mounting research confirms that miRNAs play an important role in the pathogenesis of PCOS. Aldakheel et al had confirmed that the overexpression of MicroRNA-21 in human granulosa-like tumor cells inhibits proliferation of ovarian granulosa cell in PCOS patients [11]. Additionally, the level of insulin resistant in PCOS may manipulated by the change of miR-1260a, miR-18b-5p, miR-424-5p, and miR let-7b-3p [12]. Interestingly, miR-424-5p in exosomes of PCOS follicular fluid suppressed cell proliferation [13]. Additionally, The phenotype of PCOS patients of oligo amenorrhea had an over expression of miR-30a-3p, which influenced menstrual dysfunction [14]. However, current evidence indicates a lack of consensus on the effectiveness of miRNAs as biomarkers for PCOS diagnosis. The present study hypothesized that the potential role of miRNAs as novel diagnostic biomarkers for PCOS. To verify the conjecture, we performed threshold effect and pooled sensitivity and specificity of miRNAs in the diagnosis of PCOS.

Methods

Search strategy

This report was subject to the proposals of the preferred reporting items for systematic reviews and meta-analyses protocols (PRISMA-P) guidelines and PRISMA-P 2015 statement [15, 16]. Since it is a systematic review analysis and a network meta-analysis, formal ethical approval was not a necessary factor and a comprehensive electronic document search was performed using the following terms in the Cochrane, Web of Science, Embase, Pubmed, and Science Direct databases as of June 2021 with the keywords of “polycystic ovary syndrome”, “PCOS”, “miRNA”, “microRNA”, “biomarker”, “plasma”, serum”, ‘blood”, “follicular fluid” and “diagnosis”. No restrictions on language were imposed when searching for documents. Reference lists and related comments were also manually searched to avoid missing potential qualified studies.

Inclusion and exclusion criteria

The miRNA as biomarkers for the diagnosis of PCOS was considered an eligible study. The following specific inclusion criteria were used: (1) the studies evaluated the diagnostic accuracy of miRNAs in PCOS; (2) diagnosis of PCOS confirmed by Rotterdam consensus; and (3) the article provided sufficient data to satisfy 2×2 tables, which means the true positive (TP), false positive (FP), false negative (FN), and true negative (TN) can be obtained by calculated with the sensitivity, specificity, and number of patients. The TP is the PCOS group with the monitored outcome present. The FP is the control group with the outcome present. The FN is the PCOS group with the outcome absent. The TN is the control group with the outcome absent. TP equals the number of PCOS patients times the sensitivity. FP equals the number of control times the (1-specificity). FN equals the number of PCOS patients times the (1-sensitivity) and TN equals the number of control times the specificity. The specific details of the exclusion criteria are shown in Fig. 1A and C, which followed the PRISMA 2009 flow chart. Two independent reviewers screened the searched documents. Disagreements were resolved after a full discussion and analysis with a third researcher (Fang Lyu).

Data extraction and quality assessment

Two independent evaluators (Lin Liu and Jie Jyu) extracted the data into an Excel spreadsheet and evaluated the quality of diagnostic accuracy of each included studies and the risk of deviation based on the Quality Assessment of Diagnostic Accuracy Study 2 (QUADAS-2) assessment, including 7 questions in 4 domains, and classified the answers as “low risk”, “high risk” or “unclear risk” bias [17, 18]. For example, patients included in the studies who were diagnosed clearly using the gold standard would be judged as low risk.

Statistical analysis

All statistical analyses were performed using Stata version 15.1 (Stata Corporation, College Station, TX 77845, USA) and Review Manager (RevMan) version 5.3.5 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark). Pooled OR was used to assess the ability of miRNAs to distinguish infertile patients from controls. To test the risk of bias across the included literature, sensitivity analysis was employed by deleting one or two of the included documents. Deeks’ funnel plot asymmetry test was used to detect and assess whether potential publication bias existed across the included studies. Fagan’s nomogram was used to calculate the probability after the test and compare it with previous probabilities, showed the relationship of the pre-test probability, the likelihood ratio and the post-test probability. A post-test probability that is higher than the pre-test probability indicates a more important diagnosis test. The overall performance of miRNAs in the diagnosis of PCOS was obtained from a summary receiver operator characteristic curve. The heterogeneity between studies was measured using the Q-test and I^2 statistics. I^2 was classified into low (25%), moderate (50%) and high (75%) according to the QUADAS. When $P < 0.1$ or $I^2 > 50\%$ indicated significant heterogeneity did existed due to the existence of a non-threshold effect, which should be in derSimonian-Laird (REM) model, and subgroup analysis of the included studies was required to explore the reason of heterogeneity. $P < 0.05$ indicated statistical significance.

Results

Screened inclusion studies, basic characteristics and quality assessment of the included literature

The specific strategy initially searched 911 articles related to the miRNA diagnosis of PCOS with the keywords until June 2021. All the studies identified in the 6 databases were imported into Endnote X7, and 303 duplicate documents were removed. 9 articles with 1303 individuals were included based on the inclusion criteria (Fig. 1A) [18-26]. Most miRNAs were up-regulated in PCOS, and the expression levels of miR-320, miR-140 and let-7b were down-regulated [19, 20]. Most of the study specimens were derived from plasma and serum, and two studies used samples from follicular fluid [20, 21]. One study specimen employed ovarian granulosa cells (GCs) [22]. The results of the final quality assessments of QUADAS-2 were employed to describe the overall quality of the included studies and examine possible sources of potential heterogeneity (Fig. 1B).

Diagnostic threshold effect

The threshold effect is an important source of heterogeneity in diagnostic accuracy meta-analysis. One of the best ways to assess threshold effects is Spearman's sensitivity and specificity correlation coefficients. The results of the Spearman correlation coefficients in this meta-analysis were -0.30 and $P=0.21$, respectively, which means that a threshold effect did not exist. The sensitivity and specificity and positive and negative likelihood ratios (LRs) of the included literature can be pooled and analyzed.

Diagnostic accuracy of MIRNAS for PCOS

The pooled sensitivity and specificity and overall positive and negative LR of this analysis was 0.73 (95% CI: 0.68-0.77), 0.81 (95% CI: 0.74-0.86), 3.79 (95% CI: 2.66-5.40), and 0.34 (95% CI: 0.27-0.42) in random effects model, respectively (Fig. 2A, 4B, 4C and 4D). The heterogeneity of the OR value was high in the pooled results (Fig. 3A). Therefore, sensitivity analysis, subgroup analysis and meta-regression analysis were performed. The ROC curve fitted by the weight of the diagnostic OR of the included studies was used to comprehensively evaluate the accuracy of the diagnostic analysis. The sensitivity and specificity of each study was obtained from the curve (Fig. 3B).

Sensitivity and subgroup analysis

There is no statistically significant change in the results of pooled sensitivity analysis, which means none of the articles would affect the pooled result. Subgroup analysis was performed according to miRNA numbers, miRNA sources, countries and detection methods. The sensitivity of follicular fluid and granulosa cells derived miRNAs as diagnostic markers were higher than serum-derived and plasma (77% vs 68%, and 77% vs 67%, respectively), but also specificity increased comparing with serum and plasma (82% vs 78%, 82% vs 68%, respectively). Area under the ROC curve (AUC) of miRNAs as biomarker within Asia was higher than outside of Asia (78% vs 58%). The higher the DOR value is, the stronger the diagnostic ability will be. The DOR value for qRT-PCR (SYBR) was significantly higher than qRT-PCR (Taqman) (9.62 vs 6.58). The area under the curve was up to 88% when employed the multiple miRNAs as diagnosis marker (Table 2).

Meta-regression analysis and publication bias

Univariate regression analysis demonstrated that the miRNA number ($P=0.27$), the detection method ($P=0.49$) or region ($P=0.93$) did not contribute to the heterogeneity in our sample. However, miRNA source ($P=0.02$) may be an important source of heterogeneity. The slope coefficient of the P value of Deeks' funnel plot asymmetry test was 0.59 (Fig. 4A), which indicated no significant publication bias in this meta-analysis.

Clinical application of Fagan's nomogram

In Fagan's nomogram, it assumed that the pre-test probability of the left column is 20%. If the positive likelihood ratio of the intermediate column is 4, then the probability of the test is 49%. If the negative likelihood ratio of the connected column is 0.34, then the probability after the test is 8%. Therefore, if a patient had a positive result after the related miRNA

examination, the probability of having PCOS was 49%, if a negative result was obtained, the probability of having no corresponding disease was 8% (Fig. 4B).

Discussion

The present study summarizes studies of miRNAs employed as biomarkers for the diagnosis of PCOS succeed or not. To clarify the hypothesize, Stata version 15.1 and Review Manager were employed to explore the potential of miRNA as biomarker. The results showed that multiple miRNAs may be the promising diagnostic biomarker with a high sensitivity, specificity and AUC of 0.76, 0.81 and 0.88, respectively, including miR-182, miR-145, miR-93, miR-223, hsa-miR-3188, hsa-miR-3135b, miR-30a, miR-140, let-7b. Interestingly, one of included researches, the sensitivity and specificity of diagnostic biomarker when employed hsa-miR-3188, hsa-miR-3135b was up to 0.91 and 0.96, which means the great potential ability of miRNAs as biomarker is. Furthermore, the up-expression of hsa-miR-3188 and hsa-miR-3135b were consistent with the research of Hou, who had demonstrated that hsa-miR-3135b had positive correlation with the number of oocytes retrieved, while negative correlation with fertilization and cleavage rates [27]. We also found the down-expression levels of miR-320, miR-140 and let-7b only were observed in PCOS patients, which is in accordance with studies of Cirillo's research. MiR-320 has correlated with insulin concentrations and serum E₂ concentrations [28], which act as the key factor in folliculogenesis and oocyte maturation by regulating mediators downstream the follicle-stimulating hormone (FSH) receptor [29], as well as, regulate steroidogenesis in vitro [30]. The study of non-invasive PCOS markers for early diagnosis is currently one of the most rapidly growing areas in reproductive research, including transvaginal ultrasound, transcriptomic and RNA biomarkers. The diagnostic value of PCOS multiple miRNAs as diagnostic markers was 0.88 in this article, which was higher than any other single biomarker for diagnosis, compared with the ratio of luteinizing hormone (LH)/FSH (0.655), IL-29 (0.727), or testosterone (0.818) [31].

MiRNAs are structurally stable in vitro, and the levels of miRNAs in serum are reproducible and easily extracted from fresh samples. MiRNAs also have a 24-hour half-life at room temperature, which sequences are easily verified using qRT-PCR [32]. Additionally, qRT-PCR (SYBR) showed advantage over TaqMan chemistry for qRT-PCR in diagnosis cost [33]. In this analysis, when qRT-PCR (SYBR) was employed, the DOR reached 9.62, which combines the advantages of positive and negative likelihood ratios. And the AUC of this subgroup results was up to 0.82 using the qRT-PCR (SYBR) method. These results suggest that the multiple miRNAs and in qRT-PCR (SYBR) method will increase the sensitivity, specificity and odds ratios of future large-scale diagnostic marker control experiments.

MiRNA showed quite good AUC in Asian regions of 0.78 when compared with non-Asian with 0.58. The diagnostic heterogeneity was high, which may be related to confounding factors, such as race, illness severity, and the economic situation of patients. Although previous studies demonstrated that miRNAs had certain limitations for diagnosis, a subgroup analysis in the present study was performed based on different diseases, regions, ethnicity and verification methods to attain stronger evidence about the performance of miRNAs increasing the internal validity [34]. In the future, different miRNA combinations may be able to detect different subtypes of PCOS. Additionally, studies demonstrated that miRNA expression had excellent prognostic scores only when discovered in early-stage of cancer [35, 36]. The microRNA signatures may also help to identify these women in early stage and change treatment regimens in timely manner, when women respond poorly to progestin-based therapy leading to low currency of PCOS and hirsutism and acne [37]. Good diagnostic markers should not be affected by the menstrual cycle, age, region, ethnicity, or sample collection time. The evidence suggests an increasing role for miRNAs in PCOS for their use as novel disease biomarkers is still limited [38]. Many researchers are working to identify diagnostic biomarkers for diseases at an early stage, but there is no recognized diagnostic ratio for practical diagnostic biomarkers. The design of large-scale experiments for early diagnostic markers must be improved. The limitations of the present study are that the heterogeneity was high, and there were many confounding factors to consider. Larger sample size studies are needed to further verify the diagnostic use of miRNAs.

Conclusion

The present study is the first systematic review and analysis to find that follicular fluid and granulosa cells than serum and plasma-derived miRNAs were more sensitive for the diagnosis of PCOS. Multiple miRNAs in qRT-PCR will increase the advantage of diagnosis PCOS.

Abbreviations

PCOS: Polycystic ovary syndrome

LR: Likelihood Ratios

qRT-PCR: Realtime Quantitative Polymerase Chain Reaction

ROC: Receiver Operating Characteristics

AUC: Area Under Curve

MiRNAs: MicroRNAs

TP: True Positive

FP: False Positive

FN: False Negative

TN: True Negative

QUADAS: Quality Assessment of Diagnostic Accuracy Study

FSH: Follicle-stimulating Hormone

LH: Luteinizing Hormone

Declarations

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of supporting data

All data generated or analysed during this study are available from the corresponding author.

Competing interests

The authors declare no competing financial interests.

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Authors' contributions

Lin Liu and Fang Lyu collected and extracted the data. Lin Liu, Jie Jyu and Xin Wang participated in statistical analyses and drafting of the manuscript. Fang Lyu and Hong Zhang reviewed the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. The basic elements contained in the 9 literature included in this meta-analysis. “Expression level” is the expression of miRNA in polycystic ovary syndrome patients when compared with the control group. “AUC” is the area under the curve to predict the ability of biomarkers.

Author	Period	disease	microRNA type	Expression level	AUC	Sensitivity	Specificity	TP	FP	FN	TN	N.P	N.C	Cut-off value
Naji	2018	PCOS	miR-182	U	0.73	0.76	0.70	15	6	5	15	20	21	NA
			miR-182↓miR-145	U	0.76	0.76	0.82	15	4	5	17	20	21	NA
	2015	PCOS	miR-93	U	0.72	0.64	0.76	16	6	9	18	25	24	1.1
			miR-223	U	0.60	0.56	0.72	14	7	11	17	25	24	1.2
Sathyapalan			miR-93↓miR-223	U	0.73	0.64	0.76	16	6	9	18	25	24	NA
	2011-2013	PCOS	miR-222	U	0.80	0.73	0.76	53	18	20	55	73	73	1.28
			miR-146a	U	0.71	0.63	0.85	46	11	27	62	73	73	2.53
			miR-30c	U	0.69	0.72	0.64	52	26	21	47	73	73	1.42
Long	-	PCOS	hsa-miR-3188	U	0.90	0.83	0.86	20	3	4	18	24	21	1.38
			hsa-miR-3135b	U	0.76	0.62	0.86	15	3	9	18	24	21	1.40
			hsa-miR-3188,hsa-miR-3135b	U	0.91	0.96	0.76	23	5	1	16	24	21	1.60
Jiang	2013	PCOS	miR-193b,BMI	U	0.75	0.62	0.80	40	22	25	88	65	110	0.54
Rashad	-	PCOS	miR-320	D	0.86	0.80	0.98	48	1	12	39	60	40	0.74
Scalici	-	PCOS	miR-30a(U), miR-140 and let-7b	U/D	0.83	0.70	0.84	21	15	9	76	30	91	NA
Tabrizi	2020	PCOS	miR-27a	U	0.71	0.66	0.66	35	18	18	35	53	53	<0.53
			miR-130b	D	0.77	0.70	0.79	37	11	16	42	53	53	>1.55
			miR-301b	U	0.66	0.66	0.59	35	22	18	31	53	53	<3.33
Deswal	2021	PCOS	miR-29a-5p	U	0.95	0.92	1	108	0	9	72	117	72	-
			miR320a	U	0.74	0.75	0.8	134	31	44	122	178	153	-

Abbreviations: polycystic ovary syndrome(PCOS), down-regulated(D), up-regulated(U), area under the curve(AUC), true positive(TP), false positive(FP), false negative(FN), true negative(TN), Number of control(N.C), Number of patients(N.P), not available(NA).

Table 2. The results of the subgroup analysis.

Subgroups	No.of study	Pooled Sensitivity (95% CI)	Pooled Specificity (95% CI)	Pooled PLR (95% CI)	Pooled NLR (95% CI)	Pooled DOR (95% CI)	AUC
ALL studies	9	0.73(0.68-0.77)	0.81(0.74-0.86)	3.79(2.66-5.40)	0.34(0.27-0.42)	11.19(6.36-19.70)	0.78(0.59-0.94)
miRNA types							
Single	5	0.73(0.70-0.77)	0.79(0.76-0.81)	2.99(2.25-3.98)	0.37(0.30-0.46)	8.23(5.26-12.87)	0.75(0.58-0.94)
multiple	4	0.76(0.66-0.84)	0.81(0.74-0.87)	3.74(2.64-5.28)	0.34(0.20-0.57)	11.75(5.29-26.10)	0.88(0.65-0.97)
Sample types							
Serum	4	0.68(0.63-0.73)	0.78(0.74-0.82)	2.83(2.09-3.83)	0.42(0.35-0.52)	6.85(4.29-109.93)	0.74(0.58-0.88)
Plasma	1	0.67(0.59-0.75)	0.68(0.60-0.75)	2.09(1.40-3.14)	0.48(0.38-0.62)	4.39(2.26-8.53)	0.72(0.61-0.82)
ff and GCs	3	0.77(0.69-0.83)	0.82(0.76-0.87)	3.93(2.87-5.37)	0.32(0.23-0.46)	13.61(7.71-24.02)	0.87(0.66-0.97)
Study location							
Asia	5	0.70(0.66-0.73)	0.75(0.71-0.78)	2.75(2.21-3.43)	0.43(0.37-0.49)	7.02(4.88-10.10)	0.78(0.61-0.91)
Not Asia	3	0.70(0.62-0.77)	0.83(0.77-0.88)	3.31(1.81-6.06)	0.40(0.27-0.60)	8.93(3.29-24.24)	0.58(0.55-0.95)
Method types							
SY	5	0.72(0.67-0.76)	0.77(0.73-0.81)	3.11(2.18-4.42)	0.37(0.29-0.49)	9.62(5.18-17.84)	0.82(0.62-0.94)
TM	3	0.67(0.62-0.72)	0.77(0.72-0.81)	2.78(2.16-3.59)	0.44(0.37-0.52)	6.58(4.68-9.26)	0.76(0.58-0.87)

Abbreviations: polycystic ovary syndrome(PCOS), endometriosis(EMT), follicular fluid(ff), granulosa cell(GCs), Quantitative Real-time Polymerase Chain Reaction for miRNAs (qRT-PCR), qRT-PCR TaqMan (T.M), qRT-PCR SYBR (S.Y).AUC (area under the Receiver Operating Characteristics curve)

Figures

Figure 1

(no caption included)

Figure 2

(no caption included)

Figure 3

(no caption included)

Figure 4

(no caption included)