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Advancing diagnostics: integrating microRNA profiling and protein markers in ectopic pregnancy detection

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ABSTRACT

Introduction Ectopic pregnancy (EP) poses significant health risks, particularly in developing nations, necessitating improved diagnostic methods. This study aimed to explore potential biomarkers for EP diagnosis.

Methods A case–control study was conducted at the Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu. It included 140 EP cases and 140 pregnant controls, aged 19–38 years, attending routine visits. Serum samples were analysed for beta-human chorionic gonadotropin (β-hCG), progesterone, soluble fms-like tyrosine kinase-1 (sFLT-1) and eight microRNAs (miRs).

Results Differential expression of biomarkers was observed in EP cases. Four miRs (hsa-miR-141, hsa-miR-218, hsa-miR-519d and hsa-miR-873) were downregulated, and four miRs (hsa-miR-223, hsa-miR-517a, hsa-miR-523 and hsa-miR-323-3p) were upregulated. Statistically significant expression fold changes were noted ($p<0.05$), except for hsa-miR-141 and hsa-miR-218. miR-519d exhibited promising diagnostic potential with the highest specificity (97.1%) and a sensitivity of 47.1%. sFLT-1, as an individual marker, demonstrated a sensitivity of 98.6% and a specificity of 90%. The combination of sFLT-1 and miR-519d significantly enhanced the sensitivity to 100% with a specificity of 87.1%.

Conclusions The combination of miR-519d and sFLT-1 emerges as a promising diagnostic tool for EP, offering a sensitivity of 100% and a specificity of 87.1%. These findings underscore the potential of biomarker-based approaches in improving EP diagnosis, especially in resource-limited settings. Further validation and clinical implementation studies are warranted to corroborate these findings and enhance EP management strategies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Ectopic pregnancy diagnosis is challenging due to time-consuming and costly procedures, with 40% of cases undiagnosed.
⇒ This study aims to address this by exploring microRNA (miR) profiling combined with protein markers as a novel approach to diagnose ectopic pregnancy with high sensitivity and specificity, preventing unnecessary medical or surgical interventions.

WHAT THIS STUDY ADDS

⇒ This study adds to our understanding by identifying miRs and protein markers as promising biomarkers for diagnosing ectopic pregnancy.
⇒ miR-519d is the most specific and sensitive biomarker among other eight miRs, and combining it with soluble fms-like tyrosine kinase-1 (sFLT-1) enhances its sensitivity and specificity.
⇒ Progesterone and also sFLT-1 show higher sensitivity and specificity as an individual marker, potentially reducing follow-up and surgery time, enhancing decision-making and improving care and outcomes of women experiencing early pregnancy.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study suggests that incorporating the identified biomarkers into routine first-trimester screening could lead to earlier detection of ectopic pregnancy.

INTRODUCTION

Ectopic pregnancy (EP) stands as a grave concern in obstetrics, denoting the implantation of the ovum outside the uterine cavity, predominantly within the fallopian tube. This condition poses a significant threat to maternal health, representing a leading cause of pregnancy-related morbidity and mortality, particularly during the initial trimester.[1] Numerous factors contribute to the likelihood of EP, encompassing maternal age, previous EP occurrences, tubal pathology and a history of infertility, among others.[2] The early manifestations of EP, characterised by symptoms such as vaginal bleeding, abdominal discomfort and menstrual irregularities, often mimic those of a healthy pregnancy, rendering prompt identification challenging.[3] Current diagnostic methodologies, such as serum beta-human chorionic gonadotropin (β-hCG) level testing and transvaginal ultrasonography, play pivotal roles in EP diagnosis.[4] Nonetheless, limitations persist, particularly in detecting small or premature EPs that evade initial ultrasound assessments.[5]

Within this context, the exploration of novel serum biomarkers emerges as a compelling avenue. Notably, β-hCG, maternal progesterone and soluble fms-like tyrosine kinase-1

(sFLT-1) have surfaced as promising candidates. β-hCG, a glycoprotein hormone, contributes to various physiological processes crucial for successful embryo implantation. Conversely, EP and other abnormal intrauterine masses are associated with diminished progesterone levels, underscoring its potential as a diagnostic marker.[6] Additionally, sFLT-1, the soluble form of the vascular endothelial growth factor receptor-1,[7] exhibits altered expression in EP cases, potentially serving as a biomarker reflective of the abnormal angiogenic milieu characteristic of EP.[8]

Moreover, microRNAs (miRs), small non-coding RNA molecules, have garnered attention for their regulatory roles in gene expression and cell signalling.[9] Implicated in embryo implantation processes, circulating miRs offer promise as biomarkers for EP diagnosis.[10] Notably, miR-519d and miR-873 have shown significant associations with EP, warranting further investigation into their diagnostic utility.[11]

In light of these considerations, this study endeavours to explore potential biochemical markers for EP diagnosis, with a focus on parameters conducive to early detection during the critical gestational window of 4–7 weeks. By elucidating the intricate interplay of these biomarkers, we aim to advance the diagnostic landscape of EP, facilitating timelier interventions and improved patient outcomes.

MATERIALS AND METHODS

Study design

This case–control study involved 280 women aged 19–38 at Sri Ramachandra Medical College and Research Institute, Tamil Nadu.

Sample size

The study comprised 140 patients with EP admitted to the obstetrics and gynaecology inpatient department between 4 and 10 weeks of gestation, followed up for diagnosis confirmation via ultrasound or surgical treatment. Additionally, 140 controls were enrolled from pregnant women meeting specific criteria: aged 18 and above, with serum total β-hCG of 5 IU/L, singleton gestation confirmed by ultrasound and gestational age of ≤10 weeks.

Patient and public involvement statement

The study aimed at non-invasive early diagnosis of EP. It involved normal pregnant women aged 18 and above meeting the specified criteria as controls. For cases, patients aged 18 and above admitted with suspected EP, singleton ectopic gestation confirmed by ultrasound/surgical intervention and gestational age of ≤10 weeks were included. Recruitment was conducted by the principal investigator, with all participants providing written informed consent.

Total β-hCG, progesterone and sFLT-1 estimation

Serum samples were collected and stored at −80°C. Quantitative estimation was performed using chemiluminescent immunoassay technique for β-hCG and progesterone, and Quantikine Colorimetric ELISA Kit (catalogue number DVR100B; R&D Systems, Minneapolis, USA) for sFLT-1.

miR quantification

Total miR extraction from serum was done using miRNeasy Serum/Plasma Kit. Reverse transcription PCR (RT-PCR) for miRs was performed using miScript II RT Kit (catalogue number 21816; Agilent Technologies, Delaware, USA), followed by real-time PCR using specific primers in Rotor-Gene Q 5PLEX HRM.

Principles and procedure

The miRNeasy Serum/Plasma Kit (catalogue number 217184; Qiagen, Hilden, Germany) was used for total RNA purification, followed by a two-step RT-PCR protocol using the QuantiTect SYBR Green PCR Kit (catalogue number 218073; Qiagen). *Caenorhabditis elegans* miR 39.1 and RNU6 were used as spike-in and endogenous controls, respectively.

Statistical analysis

SPSS V.16.0 was used for statistical analysis with the significance level set at $p<0.05$. Student's t-tests and Karl Pearson's correlation coefficient were employed to ascertain the statistical differences among the groups. Mean and SD were calculated.

RESULTS

The results indicated that in normal pregnant women the average maternal age was 24.9±3.5 years, with average gestational age of 6.6±1.5 weeks, total β-hCG of 73 808.6±59575 mIU/mL, progesterone of 24.6±8.8 ng/mL and sFLT-1 of 1148.4±1323.5 pg/mL. In EP, the corresponding values were 25.6±3.3 years, 5016.2±10114.6 mIU/mL, 6.4±3.8 ng/mL and 396.7±129.8 pg/mL, respectively. A statistically significant difference ($p<0.001$) was observed between normal pregnancy and EP for total β-hCG, progesterone and sFLT-1. Gestational age showed a positive correlation with sFLT-1 in normal pregnancy and a negative correlation with EP.

The receiver operator characteristic curve analysis (figure 1) revealed that achieving a sensitivity of 95.7% and a specificity of 80% requires a high cut-off value of 25 126 mIU/mL for total β-hCG. However, this value exceeds the clinically used discriminatory zone (typically 1000–2000 mIU/mL for this parameter). Progesterone at a cut-off of 14.4 ng/mL demonstrated a sensitivity of 94.3% and a specificity of 93.6% in distinguishing an EP from a viable

Figure 1 ROC analysis of the different biomarkers: (a) total β-hCG, (b) progesterone, (c) sFLT-1, (d) miR-141, (e) miR-218, (f) miR-223, (g) miR-873, (h) miR-517a, (i) miR-519d, (j) miR-523-3p and (k) miR-323-2p. AUC, area under the curve; β-hCG, beta-human chorionic gonadotropin; miR, microRNA; ROC, receiver operator characteristic; sFLT-1, soluble fms-like tyrosine kinase-1.

Table 1 Fold change in expression of miRNAs (miRs) with statistical significance (p value) using miR-39.1 as a control in *Caenorhabditis elegans*

	Average control Ct value (mean)	SD	Average case Ct value (mean)	SD	Expression fold change ($2^{-\Delta\Delta Ct}$)	SE	Statistical significance (p value)
C. elegans miR-39.1	19.65	1.92	18.24	3.11	1	–	–
hsa-miR-141	20.31	1.32	18.41	1.81	0.72	0.25	0.087
hsa-miR-218	18.52	8.85	16.65	3.58	0.73	0.21	0.069
hsa-miR-223	16.57	0.69	16.20	6.13	2.05	0.21	0.001
hsa-miR-517a	24.32	5.70	23.88	3.31	1.96	0.29	0.010
hsa-miR-519d	15.05	1.84	12.50	3.75	0.45	0.24	0.001
hsa-miR-523	19.58	5.40	18.79	3.54	1.53	0.21	0.021
hsa-miR-873	23.36	2.83	21.20	1.94	0.59	0.29	0.032
hsa-miR-323-3p	20.05	5.88	20.68	4.05	4.13	0.46	0.002

Delta represents the difference between two values; the $2^{-\Delta\Delta Ct}$ method represents fold change in gene expression between samples (control vs case groups).
Ct value, cycle threshold value.

intrauterine pregnancy (VIP). sFLT-1, at a threshold of 634 pg/mL, effectively differentiated between an EP and a normal pregnancy, achieving a sensitivity of 98.6% and a specificity of 90%.

The area under the curve (AUC) values for miRs (miR-141, miR-218, miR-223, miR-323-3p, miR-517a, miR-519d, miR-523-3p, miR-873) ranged from 0.55 to 0.89, indicating varying levels of test accuracy. miR-141 exhibited moderate accuracy with a low sensitivity of 11.4% and a specificity of 25.7%. Similarly, miR-218 shows improved sensitivity (15.7%) and specificity (31.4%). miR-223 yielded a sensitivity of 16.4% and a specificity of 18.6%. miR-323-3p had the lowest AUC of 0.55, with a sensitivity of 15.7% and a low specificity of 1.4%. miR-517a had an AUC of 0.61 but showed no sensitivity and a specificity of 55.7%. miR-523-3p had a sensitivity of 17.1% and a specificity of 11.4%. miR-873 exhibited an AUC of 0.65, with a sensitivity of 38.6% and no specificity. In contrast, miR-519d showed the highest AUC of 0.89, with a specificity of 97.1% and a sensitivity of 47.1%, suggesting its potential as a more reliable biomarker for EP diagnosis compared with the other miRs.

Among the eight miRs assessed in this study, the sera of women with EP revealed differential downregulation of four miRs (hsa-miR-141, hsa-miR-218, hsa-miR-519d and hsa-miR-873), while four miRs were upregulated (hsa-miR-223, hsa-miR-517a, hsa-miR-523 and hsa-miR-323-3p) (tables 1 and 2). The fold change in expression was statistically significant ($p<0.05$) for all miRs, except for hsa-miR-141 and hsa-miR-218.

The investigation evaluated the efficacy of diverse biomarker constellations (table 3), incorporating miRs to ascertain their diagnostic utility. The incorporation of miRs other than miR-519d to the sFLT-1 marker did not significantly enhance specificity. Despite its lower sensitivity at 47.1%, miR-519d exhibited high specificity (97.1%) and a positive predictive value of 94.3%, with only 2.9% false positives. Combining sFLT-1 with miR-519d enhanced sensitivity to 100% while maintaining good specificity at 87.1%. Moreover, the incorporation of total β-hCG failed to augment the diagnostic efficacy.

The commonly used miR target prediction programs miRBase and DIANA - mirPath tools were employed to predict the target genes for the differentially expressed miRs. The gene targets and pathway analysis results are tabulated in table 4.

DISCUSSION

EP is a significant obstetric concern, particularly in developing nations, where ruptured EPs account for 5%–10% of pregnancy-related deaths and contribute to 9%–14% of maternal mortality in the first trimester.[12] Diagnosis of women with a pregnancy of unknown location necessitates multiple visits for blood tests and ultrasound examinations. Delays in diagnosis can lead to ruptured EPs, impaired fertility and life-threatening intra-abdominal haemorrhage, particularly problematic during 4–6 weeks of gestation when ultrasound findings are inconclusive and serial total β-hCG measurements pose risks due to delayed diagnosis. Early detection of EP can prevent morbidity associated with delayed treatment, inappropriate management strategies and adverse effects on future pregnancies.[13]

The current approach to identifying EP involves a combination of ultrasound and total β-hCG measurements, neither of which is the gold standard. Ultrasound can only detect 8%–26% of EPs during the initial 4–7 weeks of gestation, and the total β-hCG discriminatory zone offers limited diagnostic assistance,[14] particularly when ultrasound findings are inconclusive. Although a total β-hCG level below 1500 mIU/mL raises suspicion for EP after 8 weeks of gestation,[15] EPs can occur at any total β-hCG level as this cut-off is not gestational age-dependent.

Table 2 Fold change in expression of miRNAs (miRs) using the housekeeping gene RNU6 as the control

	Average case Ct value for miRs (TE)	Average case Ct value for housekeeping gene (HE)	Average control Ct value for miRs (TC)	Average control Ct value for housekeeping gene (HC)	ΔCt value (case)	ΔCt value (control)	ΔΔCt value	Expression fold change $2^{-\Delta\Delta Ct}$ value
RNUS gene	–	20.05	–	18.63	–	–	–	1.00
hsa-miR-141	20.31	–	18.41	–	0.26	−0.21	0.47	0.72
hsa-miR-218	18.52	–	16.65	–	−1.53	−1.98	0.44	0.73
hsa-miR-223	15.49	–	17.00	–	−4.56	−1.63	−2.93	7.61
hsa-miR-517a	22.83	–	23.88	–	2.78	5.26	−2.48	5.56
hsa-miR-519d	15.05	–	12.50	–	−5.00	−6.12	1.13	0.46
hsa-miR-523	19.58	–	18.79	–	−0.47	0.16	−0.63	1.54
hsa-miR-873	23.36	–	21.20	–	3.31	2.57	0.74	0.60
hsa-miR-323-3p	20.05	–	20.68	–	0.00	2.06	−2.06	4.16

The ΔΔCt value is a method used in quantitative PCR to compare gene expression levels between different groups (control vs case groups); the ΔCTE value represents the difference in Ct values between the target gene (miRs) and the housekeeping gene in the experimental (case) condition as expressed by the equation ΔCTE=TE−HE; the ΔCTC value represents the difference in Ct values between the target gene (miRs) and the housekeeping gene in the control condition as expressed by the equation ΔCTC=TC−HC.
Ct value, cycle threshold value; HC, cycle threshold for the housekeeping gene in the control condition; HE, cycle threshold for the housekeeping gene in the experimental condition; TC, cycle threshold for target gene in the control condition; TE, cycle threshold for target gene in the experimental condition; ΔCTC, change in cycle threshold for control condition; ΔCTE, change in cycle threshold for experimental condition; ΔΔCt value, double delta Ct value.

Protein markers

In this study, we evaluated total β-hCG along with progesterone, sFLT-1 and miRs. Diagnostic confirmation in normal pregnancies relied on ultrasound in all cases, while EP diagnosis was confirmed by ultrasound in 113 cases and laparotomy in 7 cases.

The average total β-hCG in normal pregnancy was 73 809 mIU/mL, compared with 5016 mIU/mL in EP,[4] indicating a significant difference. Conversely, to achieve maximum sensitivity and specificity, it is imperative to establish a

Table 3 Diagnostic accuracies of single markers and of multimarker combinations for predicting EP

Parameter	Sensitivity (%)	Specificity (%)	Positive predictive value	Negative predictive value	False positives (%)	False negatives (%)
Total β-hCG	31.4	100	100	59.3	0	68.6
sFLT-1	98.6	90.0	90.8	98.4	10	1.4
Progesterone	94.3	93.6	93.6	94.2	6.4	5.7
miR-519d	47.1	97.1	94.3	64.8	2.9	52.9
Total β-hCG, progesterone	94.3	93.6	93.6	94.2	6.4	5.7
Total β-hCG, sFLT-1	98.6	90	90.8	98.4	10	1.4
Total β-hCG, progesterone, sFLT-1	100	85	87	100	15	0
Total β-hCG, progesterone, sFLT-1, miR-519d	100	82.1	84.8	100	17.9	0
Total β-hCG, sFLT-1, miR-519d	100	87.1	88.6	100	12.9	0
Progesterone, sFLT-1, miR-519d	100	82.1	84.8	100	17.9	0
sFLT-1, progesterone	100	85	87	100	15	0
Total β-hCG, miR-519d	60.7	97.1	95.5	71.2	2.9	39.3
Total β-hCG, miR-519d, progesterone	97.1	90.7	91.3	96.9	9.3	2.9
Progesterone, miR-519d	97.1	90.7	91.3	96.9	9.3	2.9
sFLT-1, miR-519d	**100**	**87.1**	88.6	100	12.9	0

EP, ectopic pregnancy; miR, microRNA; sFLT-1, soluble fms-like tyrosine kinase-1; β-hCG, beta-human chorionic gonadotropin.

Table 4 miR gene targets and pathway analysis results from the software

KEGG pathway	P value	Number of genes	Number of miRs
Fatty acid biosynthesis	4.26×10^{-19}	2	2
Adherens junction	1.67×10^{-4}	16	4
p53 signalling pathway	3.28×10^{-4}	18	3
Fatty acid metabolism	3.28×10^{-4}	8	4
Viral carcinogenesis	3.28×10^{-4}	31	4
Prostate cancer	3.28×10^{-4}	23	4
Bacterial invasion of epithelial cells	4.50×10^{-4}	18	5
Central carbon metabolism in cancer	8.57×10^{-4}	15	4
Proteoglycans in cancer	9.56×10^{-4}	28	5
Pathways in cancer	1.244×10^{-3}	53	6
Glycosaminoglycan biosynthesis - heparan sulfate/heparin	1.636×10^{-3}	5	2
Shigellosis	1.636×10^{-3}	15	2
FoxO signalling pathway	1.636×10^{-3}	25	4
Chronic myeloid leukaemia	6.075×10^{-3}	15	4
PI3K-Akt signalling pathway	8.009×10^{-3}	47	6
Glioma	9.211×10^{-3}	13	3
Hepatitis B	1.832×10^{-2}	22	4
Prolactin signalling pathway	2.802×10^{-2}	12	3
Oocyte meiosis	2.821×10^{-2}	18	5
Colorectal cancer	2.973×10^{-2}	12	5
Bladder cancer	3.320×10^{-2}	10	4
mTOR signalling pathway	4.282×10^{-2}	12	4

FoxO signalling, Forkhead box O signalling; KEGG Pathway, Kyoto Encyclopedia of Genes and Genomes pathway; miRs, microRNAs; mTOR signalling, mammalian target of rapamycin signalling; PI3K-Akt signalling, phosphatidylinositol 3-kinase/protein kinase B signalling.

significant threshold of 25 126 mIU/mL for the total β-hCG, which surpasses the typically used discriminant level.

In the current investigation, the mean concentration of progesterone during a normal pregnancy was observed to be 24.6±8.8 ng/mL,[16] which markedly differs from the level of 6.4±3.8 ng/mL detected in the EP group,[17] indicating a statistically significant disparity. A 2012 meta-analysis by Verhaegen *et al*[18] showed progesterone's ability to distinguish non-viable pregnancies with 74.6% sensitivity and 98.4% specificity, but not EP or other anomalous intrauterine pregnancies. Al-Bayati *et al* suggested a cut-off of 11.7 ng/mL.[19]

The mean sFLT-1 concentration in normal pregnancy was 1148.4 pg/mL, reduced to 396.7 pg/mL in the EP group,[20] nearly one-third of the normal population. Daponte *et al*[21] reported the mean sFLT-1 levels in normal pregnant women to be 1390.32±655.37 pg/mL and 288.79±375.76 pg/mL for failed pregnancies (including EP and missed abortions). Dominguez *et al* observed lower sFLT-1 levels in normal pregnancy (505 pg/mL) compared with EP (84 pg/mL).[22] Despite fluctuations in mean sFLT-1 levels, there was a 75%–80% decrease in the EP group.

MicroRNAs

In our study, eight miRs showed differential expression, with four downregulated and four upregulated. Although miR-323-3p was previously highlighted for EP detection, its sensitivity in our study was lower.[23] miR-873, proposed as a marker for early EP detection, exhibited reduced sensitivity compared with previous findings.[24] According to Zhao *et al*,[23] miR-323-3p concentration was notably elevated in EP, showing more promising results than miR-517a, miR-519d and miR-525-3p, with a sensitivity rate of 37% when used as a single marker. However, in this study, miR-323-3p, miR-517a and miR-523-3p demonstrated sensitivities of 17.1%, 68.6% and 11.4%, respectively. Miura *et al*[24] also found significant statistical differences in the plasma concentration of cell-free pregnancy-associated miRs—miR-323-3p, miR-515-3p, miR-517a, miR-517c and miR-518b—and the concentration of β-CG among women with spontaneous abortion (SA), EP and normal pregnancies.

In 2017, Lu *et al*[25] proposed miR-873 as a single, non-invasive and stable marker for early EP detection. miR-873 exhibited the highest sensitivity of 61.76% as a single marker at a fixed specificity of 90%. However, in this study,

its sensitivity was only 38.6%, at 90% specificity.[25] Lu *et al*[25] also identified miR-141 and miR-218 to be differentially expressed between EP, VIP and SA groups. Nonetheless, in this study with a larger sample size, miR-141 showed a poor sensitivity of 25% and miR-218 had a sensitivity of 30% in distinguishing EP from a normal pregnancy.[25] Lu *et al*[25] suggested that miR-223 is significantly downregulated in EP compared with SA, which contrasts the findings of Dominguez *et al*,[22] who showed upregulation in EP women. However, the diagnostic potential of miRs was limited, with sensitivities less than 20% at fixed specificities of 90% and 95%.

miR-519d demonstrated promise with high specificity but insufficient sensitivity as a single biomarker for EP detection, highlighting the need for further investigation.

Performance of biomarker combinations

Comparing the diagnostic performance of individual markers, sFLT-1 stands out with high sensitivity (98.6%) and negative predictive value (98.4%), aligning with the National Institute for Health and Care Excellence guidelines that dismiss progesterone as a single biomarker. Combining it with total β-hCG did not alter sensitivity or specificity significantly. However, adding progesterone (<14.4 ng/mL) to sFLT-1 increased sensitivity to 100%, with lowered specificity (85%).

The inclusion of miR-519d with sFLT-1 significantly improved specificity, despite its lower sensitivity (47.1%). Combining sFLT-1 with miR-519d enhanced sensitivity to 100% with good specificity (87.1%).

Further investigation into miR expression in EP and non-viable pregnancies is warranted. Estimating circulating miR levels alongside sFLT-1 could aid in early EP prediction and treatment decisions, given miRs' stability in circulation.

miR gene targets and pathway analysis

miR gene targets and pathway analysis were conducted using miRBase and DIANA - mirPath tools (table 4). For additional information, refer to online supplemental table 1. These tools predict miR target genes and regulatory mechanisms, indicating potential roles in microRNA degradation and protein translation inhibition.

For instance, miR-519d upregulates HOXA10 gene expression, relevant to mullerian duct development at ectopic implantation sites in the fallopian tube. It also facilitates intercellular communication between trophoblast and immune cells via extracellular vesicles.[26] miR-873 and miR-517a regulate the PROKR2 gene, impacting prokineticin dysregulation in the fallopian tube, which is crucial for smooth muscle contractility and embryo tubal transport.[26] miR-141 also modulates PROKR2, influencing prokineticin dysregulation and facilitating intercellular communication between trophoblast and immune cells.[26] miR-218 affects mucin-type O-glycan biosynthesis and the extracellular matrix receptor interaction pathways, playing a role in prokineticin dysregulation via the PROKR2 gene.[26] However, pathways influenced by miR-223, miR-523 and miR-323-3p were not deduced. miR-223, for instance, targets GALNT7, GALNT1 and GALNT13 genes involved in mucin biosynthesis, potentially altering mucin expression in EP tissues.[22]

Limitations

A larger sample size is needed for further validation of miR testing. The performance of these miRs may vary based on the specific condition and the population under study. This study evaluated the diagnostic efficacy of combined markers for EP, regardless of gestational age. However, it did not specifically examine small or premature EPs. Future studies are suggested to investigate the diagnostic utility of this method in detecting small or premature EPs with a focused cohort study. This study focuses on sFLT-1's role in EP, neglecting its application in pre-eclampsia or its diagnostic distinction between the two conditions.

Implications of the study

These miRs hold potential for early routine screening of EP in conjunction with protein biomarkers like progesterone and sFLT-1, enhancing sensitivity and specificity. This approach could offer time-saving, cost-effective and painless testing, ultimately reducing the complications associated with EP.

CONCLUSIONS

The study revealed that progesterone exhibited a sensitivity of 94.3% and a specificity of 93.6%. sFLT-1, as an individual marker, demonstrated a notable sensitivity of 98.6% and a specificity of 90%. Among the eight miRs assessed in EP, miR-519d emerged as a promising potential biomarker, displaying the highest specificity of 97.1% and a sensitivity of 47.1%, with an expression fold change of 0.46. Furthermore, combining sFLT-1 with miR-519d enhanced sensitivity to 100% while maintaining a good specificity of 87.1%.

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