RESEARCH ARTICLE

Evaluation of serum level of MicroRNA323-3p, MicroRNA517a and MicroRNA519d in Ectopic pregnancy: a comparative study

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Abstract

Aim: To assess the role of serum micro ribonucleic acids 323-3p, 517a and 519d in the diagnosis of ectopic pregnancy.

Method: The case-control study was conducted at the Gynaecology Department of Al-Yarmouk Teaching Hospital, Baghdad, Iraq, from June 1, 2020, to May 1, 2021, and comprised women with ectopic pregnancy in group A, and those with a viable intrauterine pregnancy in group B. Serum samples were taken for beta human chorionic gonadotropin measurement and for micro ribonucleic acids 323-3p, 517a and 519d testing using polymerase chain reaction. Data was analysed using SPSS 24.

Results: Of the 100 women with mean age 26.8±5.29 years, 50(50%) were in each of the 2 groups. There was no significant difference between the groups in terms of age, gravida, parity, abortion, and gestational age ($p>0.05$). The mean beta human chorionic gonadotropin in group B was significantly greater than group A ($p=0.001$). In group A, micro ribonucleic acid 323-3p expression was significantly higher, mean micro ribonucleic acid 517a expression was significantly lower and mean micro ribonucleic acid 519d expression was significantly lower compared to group B ($p<0.05$). At micro ribonucleic acid 323-3p cut-off point 2.415, the test accurately distinguished between ectopic and viable pregnancy with 92% sensitivity, 100% specificity and total area under the curve 0.994 ($p=0.001$).

Conclusion: Serum micro ribonucleic acid 323-3p, 517a and 519d might be helpful biomarkers in the diagnosis and follow-up of patients with ectopic pregnancy.

Key Words: Pregnancy, Gestational, Gynecology, Ectopic, Chorionic Gonadotropin, Polymerase Chain Reaction, MicroRNAs. (JPMA 74: S52 (Supple-8); 2024) **DOI: https://doi.org/10.47391/JPMA-BAGH-16-13**

Introduction

Ectopic pregnancy (EP) remains the leading cause of early-pregnancy death, accounting for 2% incidence of confirmed pregnancy and 9-10% incidence of all maternal fatalities[1,2]. Therefore, early EP diagnosis remains a significant challenge. Women with EP usually present with abdominal pain with or without vaginal bleeding. However, these symptoms are nonspecific and frequently associated with miscarriage [3]. Therefore, the diagnosis of EP mainly depends on transvaginal ultrasound (TVU) and serial serum beta human chorionic gonadotropin (βhCG) measurement[4]. According to the Royal College of Obstetricians and Gynaecologists (RCOG), TVU is the most critical approach for diagnosing tubal EP, and laparoscopy is no longer the gold standard procedure[5]. However, when an EP is not present, the TVU features have low sensitivity, while having high specificity for identifying EP[6].

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The use of βhCG discriminatory levels (1000-2000IU/L) to determine an intrauterine pregnancy (IUP) on TVU has its pitfalls, as, unfortunately, many EPs will not reach a level of 2000IU/L and might even rupture before attaining that level[3]. Therefore, utilising a serial serum βhCG measurement puts EP women at danger of rupture while awaiting the next test[7]. Thus, identifying precise serum biomarkers with high sensitivity and specifically for early EP detection would be of great clinical importance to overcome life-threatening complications or unnecessary surgical and medical treatment while waiting for subsequent βhCG measurements. In women at risk of EP or with a pregnancy of unknown origin, numerous promising biomarkers may be employed, including specific micro ribonucleic acids (miRNAs)[8]. Many miRNAs are involved in implantation and embryonic development. They are expressed in the placenta and maternal serum, and recent studies have shown that these miRNAs may have a promising role in diagnosing tubal EP[9].

The current study was planned to examine miRNAs 323-3p, 517a and 519d in the serum of women with EP, and to compare it with those of women with viable IUP to assess their accuracy in EP diagnosis.

Patients and Methods

The case-control study was conducted at the Gynaecology Department of Al-Yarmouk Teaching Hospital, Baghdad, Iraq, from June 1, 2020 to May 1, 2021, after approval from the ethics review committee of the College of Medicine, Mustansiriyah University, Baghdad.

The study comprised outpatients and inpatients with EP aged 18 years or older and gestational age of 10 weeks or less. They were placed in study group A. IUP women matched for age and gestational age who were in their first trimester attending regular antenatal care were enrolled as control group B. Women with multiple pregnancies or those who had a previous EP history were excluded. All participants provided written informed consent, and they were assigned identification codes.

Adequate blood sample was taken from all women for baseline tests, including blood group, complete blood count (CBC) and βhCG. Subsequently, TUG was done to confirm an IUP. In addition, sufficient serum volume sample was obtained from all the participants, which was labelled and frozen at −70°C for miRNA 323-3p, 519-d and 519d testing using quantitative real-time polymerase chain reaction (qRT-PCR) The miRNA was extracted from the blood sample using the ReliaPrep miRNA miniprep system (Promega, Madison, United States) where approximately 1x10 4 cells were lysed using a special lysis buffer, followed by frequent steps of wash. They were eventually rehydrated in 15µl of nuclease-free water. Then, 4µl RNA was mixed with 5Mm of each miRNA-specific reverse stem-loop primer. The 18S ribosomal RNA (rRNA) was used as an internal control with reverse primer into a 10µL multiplex reverse transcription reaction (Reverse Transcription System, Promega, Madison, US) following the manufacturer's instructions. Then, 5µl of the reverse-transcribed product was generated as a template for 25µl of initial PCR reaction composed of 18 cycles containing 5Mm of the universal reverse primer, 50Mm of each forward primer for the specific miRNAs, and 50Mm of forward and reverse primers for 18S rRNA. Pre-PCR product was then diluted and used as a template for a subsequent TaqMan RT-PCR reaction, which contained 0.5Mm of both forward and universal reverse primer and 0.1Mm of TaqMan probe. The PCR run conditions were 55°C for 2min, 95°C for 10min, and 40 cycles of 95°C for 15s and 55°C for 1min.

Data was analysed using SPSS 24. Descriptive statistics were presented as mean ± standard deviation and frequencies and percentages, as appropriate. Kolmogorov-Smirnov test was used to verify data normality. Chi square test was used for categorical variables, and t-test was used to compare between two means. Receiver operating characteristic (ROC) curve analysis was used to clarify validity tests. P<0.05 was taken as the level of significance.

Results

Of the 100 women with mean age 26.8±5.29, 50(50%) were in each of the 2 groups. There was no significant difference between the groups in terms of age, gravida, parity, abortion, and gestational age (Table 1).

Table-1: Clinical and demographic characteristics of women with ectopic pregnancy and women with intrauterine pregnancy (IUP).

Variables	N	Ectopic Mean ±SD	IUP Mean ±SD	P value
Age	50	26.16± 5.11	27.44±5.43	0.228
Gestational age	50	5.92±1.34	6.8±1.92	0.084
Gravida	50	3.56±2.08	3.36±2.14	0.636
Para	50	2.04±1.82	1.84±1.82	0.584
Abortion	50	.52±0.71	.52±0.76	1

SD: Standard deviation

The mean βHCG level in group B was significantly greater than that of group A (p=0.001). In group A, mRNA323-3p expression was significantly higher, while mean miRNA517a and miRNA519d expressions were significantly lower compared to group B (Table 2).

Table-2: Inter-group comparison of numerical variables.

Variables	N	Ectopic Mean ±SD	IUP Mean ±SD	P value
βHCG titer	50	871±304	1806±1095	0.001
MicroRNA323-3p	50	6.248±3.64	0.627±0.598	0.001
MicroRNA517a	50	0.015±0.021	0.026±0.021	0.013
MicroRNA519d	50	0.020±0.028	0.172±0.158	0.001

SD: Standard deviation, βHCG: Beta human chorionic gonadotropin RNA: Ribonucleic acid.

There was significant moderate negative correlation between miRNA323-3p and miRNA519d (r=-0.386, p=0.001). There was a significant moderate negative correlation between miRNA323-3p and βHCG titre (r=-0.306, p=0.002). There was a significant weak positive correlation between miRNA517a and miRNA519d (r=0.281, p=0.005). There was a significant weak positive correlation between miRNA517a and βHCG titer (r=-0.214, p=0.032), and there was a significant moderate positive correlation between miRNA519d and βHCG titer (r=0.314, p=0.001).

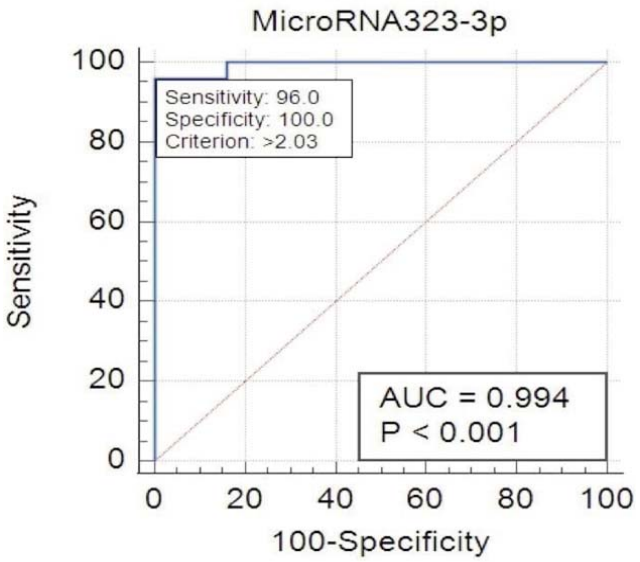

Figure: Micro Ribonucleic acid (RNA) 323-3p sensitivity and specificity..

ROC curve showed that at βHCG level ≤1350mUI/mL, the test differentiated correctly between EP and IUP with 100% sensitivity, 60% specificity and total area under the curve (AUC) 0.734 (p=0.004).

At miRNA323-3p cut-off point 2.415, the test accurately distinguished between EP and IUP with 92% sensitivity, 100% specificity and total AUC 0.994 (p=0.001) (Figure).

The AUC of miRNA323-3p and βHCG titer showed that miRNA323-3p was significantly more capable of detecting EP (95% confidence interval [CI]: 0.917-1.000) compared to βHCG (95% CI: 0.590-0.849).

ROC curve for miRNA517a showed that at cut-off point 1900, the test differentiated correctly between EP and IUP with 72% sensitivity, 60% specificity and total AUC 0.675 (p=0.003). Finally, ROC for miRNA519d showed that at cut-off point 0.052, the test differentiated correctly between EP and IUP with 72% sensitivity, 60% specificity, and total AUC 0.675 (p=0.001)

Discussion

The current study showed that the mean βHCG titer of IUP women was significantly higher than EP women, which was consistent with literature[10,11].

Serum miRNA323-3p was significantly higher in EP women than in IUP women with 92% sensitivity and 100% specificity, making it an excellent diagnostic marker. The finding is consistent with Zhen Zhao et al., who found a significant increase in serum miRNA323-3p in EP women when compared to women with spontaneous abortion and viable IUP with 37% sensitivity and 90% specificity, and that MicroRNA323-3p was the only miRNA that demonstrated a significant difference between EP and spontaneous abortion[11]. The variation in sensitivity and specificity values between the two studies is likely due to sample size differences and the inclusion of the spontaneous abortion group.

Zhen Zhao et al. integrated the assessment of miRNA323-3p, βhCG and progesterone to raise the sensitivity level to 77.8%[11]. In the current study, miRNA323-3p had 92% sensitivity and 100% specificity compared to 100% sensitivity and 60% specificity for βhCG. Adding the two together would give 100% sensitivity and 100% specificity.

Qi Lu et al. also showed that miRNA323-3p was significantly higher in EP subjects than IUP and spontaneous abortion subjects, and the combined assessment of miRNA323-3p, βhCG and progesterone showed a sensitivity of 67.65% for EP detection at a fixed specificity of 90%[12].

The current study showed that serum concentrations of miRNA517a and miRNA519d were significantly lower in EP than IUP subjects, which agrees with Zhen Zhao et al.[11]

Kiyonori et al. evaluated plasma concentrations of cell-free pregnancy-associated miRNAs 323-3p, 515-3p, 517a, 517c and 518b, and confirmed statistically significant difference in EP women compared to IUP and spontaneous abortion subjects[13]. Correlation coefficient analysis showed no relationship between them and serum βhCG levels. Besides, plasma concentrations of miRNA323-3p and mRNA-517a could distinguish EP from spontaneous group, yielding AUC 0.7454 (95% CI: 0.5558-0.9349) and 0.9654 (95% CI: 0.9172-1.0)[13].

The current study revealed that ROC cut-off point 2.415 correctly differentiated between EP and IUP with 92%sensitivity and 100% specificity. With this high sensitivity and specificity, miRN323-3p measurement may be added to βhCG and TVU to significantly improve diagnostic utility with the most heightened sensitivity as also displayed by the study by Ghafouri-Fard et.al.[14]. In addition, further studies with larger sample size is recommended.

Limitation: The sample size was not calculated in the current study which could have decreased the power of the study.

Conclusion

Serum miRNA323-3p was significantly higher in EP women with a cut-off level of 2.415. It could correctly

differentiate between EP and viable IUP with an excellent diagnostic performance compared to miRNA517a and miRNA519d.

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Conflict of Interest: None.

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