Research Article

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Clinical value of serum homocysteine, folate, and ultrasonography detection of yolk sac in predicting the outcome of threatened abortion

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Abstract: Background: To investigate the clinical value of serum homocysteine (Hcy), folate, and ultrasonography detection of yolk sac in predicting the outcome of threatened abortion.

Methods: Ninety-eight pregnant women within 12 weeks of gestation were included in the study. Of these subjects, 29 presented with threatened abortion and were considered the case group (n=29). The other 69 subjects showed as a normal pregnancy were the control group (n=69). The case group was followed up with at 28 weeks of gestation, and further divided into inevitable abortion group (IA, n=9) and successful fetus protection group (SFP, n=20). The yolk sacs of the included cases were measured by ultrasonography. The serum Hcy and folate levels of the included cases were measured by an enzyme cycling assay and chemiluminescent immunoassay, respectively. The yolk sac visualization rate and serum Hcy and folate levels were compared between the two case groups and used as a predicting reference for the outcome of threatened abortion.

Results: The yolk sac visualization rates detected by ultrasonography were 97.1% (67/69) for the control group and 69.0% (20/29) for the threatened abortion group, with a statistical difference (p<0.05). For the IA and SFP groups, the visualization rates were 22.2% (2/9) and 90.0% (18/20) respectively, with a statistical difference (p<0.05). The serum Hcy levels in the threatened abortion group were significantly decreased compared with the control group (15.5±4.2 nmol/L vs 19.1±4.7 nmol/L), with a statistical difference (p<0.05). The serum levels of Hcy and folate were 7.5±1.7 μmol/L and 12.7±4.2 nmol/L for the IA group, which were statistically different compared to the SFP group (p<0.05). The predictive sensitivity for the outcome of threatened abortion based on serum Hcy and folate levels were 85.0% and 75.0%, with a predictive specificity of 66.7% and 88.9% respectively.

Conclusion: Serum Hcy and folate levels were quite different in threatened abortion pregnant woman compared to the control group. Serum Hcy and folate level detection combined with ultrasonography detection of yolk sac can provide useful information for threatened abortions.

Keywords: homocysteine; folate; ultrasonography; yolk sac; threatened abortion.

Background

Threatened abortion refers to a small amount of vaginal bleeding within 12 weeks of pregnancy with or without lower abdominal pain and no pregnancy discharge [1, 2]. Risk factors relevant to threatened abortions include advanced age, previous abortion, exposure to tobacco smoke, obesity, diabetes, thyroid problems, and drug or alcohol use [3]. About 80% of abortions occur in the first 12 weeks of pregnancy [4]. The threatened abortion rate is reported to be about 20% for all pregnant women, with 35% of those threatened abortion cases developing into inevitable abortions [5]. Early identification of high risk inevitable abortion patients and giving them proper treatment is important for the outcome of threatened abortions. Many studies have evaluated the serological markers for predicting the outcome of threatened abortion, such as serum CA125 [6-8], ß-HCG, and E2 [9]. Recently, several studies have evaluated the serum Hcy...
and folate levels between normal pregnant women and threatened abortion cases. They found that the serum Hcy and folate levels were quite different between the normal and threatened abortion cases [10, 11]. In our present study, we investigate the clinical values of serum Hcy and folate, and ultrasonography detection of the yolk sac in predicting the outcome of threatened abortions.

**Material and methods**

**Patients**

Ninety-eight pregnant women within 12 weeks of gestation were included in the study. Of these cases, 29 subjects presented with threatened abortion and were considered as the case group (n=29). The other 69 subjects with normal pregnancy made up the control group (n=69). The threatened abortion case group was followed up with at 28 weeks of gestation, and further divided into inevitable abortion group (IA, n=9) and successful fetus protection group (SFP, n=20).

**Informed consent**: Informed consent has been obtained from all individuals included in this study.

**Ethical approval**: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and was approved by the Medical Ethics Committee of Zhangqiu Maternal and Child Health Care Hospital of Jinan City.

**Ultrasonography detection of yolk sac**

The color Doppler ultrasound diagnostic system was purchased from General Electric Company (VOLUSON E10). An ultrasonic probe with 5 to 9MHz was used. The pregnant women were asked to lie flat on the examination bed and take the bladder lithotomy position. Coupling agent was evenly applied to the ultrasonic probe, a condom was put on the probe, and the probe was placed in the posterior fornix of the vagina. The uterus and bilateral appendages were scanned by ultrasound to observe the yolk sac and gestational sac, Figure 1.

![Figure 1: Ultrasonography detection of yolk sac in pregnant women within 12 weeks of gestation. A: The yolk sac was observed under ultrasonography at 5 weeks of gestation. B, C: The yolk sac was observed under ultrasonography at 9 weeks of gestation. D: The yolk sac was not clearly showed under ultrasonography at 11 weeks of gestation. E: The yolk sac was not clearly showed under ultrasonography at 6 weeks of gestation indicated embryo damage; F: The yolk sac is large with normal embryo undeveloped. G: The yolk sac was not clearly showed under ultrasonography at 6 weeks of gestation indicated embryo damage.](image-url)
Serum Hcy and folate detection

Three mL of peripheral venous blood was taken from the included pregnant women under fasting conditions. The blood was centrifuged for 10 min to obtain the serum, and then stored in a refrigerator for later use. The serum Hcy levels were determined by an enzyme cycling assay. A kit was purchased from Becton Dickinson, United States. The serum folate was examined by a chemiluminescence assay (Siemens, Co Ltd., ADVIA centaurXP).

Statistical analysis

Measured data (serum Hcy and folate levels) were recorded as \( \bar{x} \pm s \), and comparisons were made by \( t \)-test. Counting data was showed by \( n(\%) \), and comparisons were made by chi-square test. Statistical significance was determined when \( p<0.05 \).

Results

Yolk sac detection rate by ultrasonography

The yolk sac visualization rates detected by ultrasonography were 97.1% (67/69) and 69.0% (20/29) in control and case groups respectively, with a statistical difference (\( p<0.05 \)), Table 1. For the IA and SFP groups, the visualization rates were 22.2% (2/9) and 90.0% (18/20) respectively, with a statistical difference (\( p<0.05 \)), Table 2.

Serum Hcy and folate detection

The serum Hcy levels in the threatened abortion group were significantly elevated compared with the control group (6.1±2.0 μmol/L vs 4.5±1.3 μmol/L), with a statistical difference (\( p<0.05 \)), Figure 2. However, the serum levels of folate in the threatened abortion group were significant decreased compared with the control group (15.5±4.2 nmol/L vs 19.1±4.7 nmol/L), with a statistical difference (\( p<0.05 \)), Table 3. The serum levels of Hcy and folate were 7.5±1.7 μmol/L and 12.7±4.2 nmol/L for the IA group, which were statistical different compared to the SFP group (\( p<0.05 \)), Table 4.

Prediction value of serum Hcy and folate levels for the outcome of threatened abortion

The serum Hcy and folate levels were used to predict the outcomes of threatened abortion, Figure 3. The predictive sensitivity for the outcome of threatened abortion by serum Hcy and folate levels were 85.0% and 75.0%, with a predictive specificity of 66.7% and 88.9% respectively, Table 5.

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**Table 1:** Yolk sac detection rate by ultrasonography between control and case groups [n(%)].

| Group          | Yolk sac detection |
|----------------|--------------------|
|                | Positive | Negative |
| Control group  | 67(97.1)  | 2(2.9)   |
| Case group     | 20(69.0)  | 9(31.0)  |

**Table 2:** Yolk sac detection rate by ultrasonography between IA and SFP groups [n(%)].

| Group          | Yolk sac detection |
|----------------|--------------------|
|                | Positive | Negative |
| IA group       | 2(22.2)  | 7(77.8)  |
| SFP group      | 18(90.0) | 2(10.0)  |

**Table 3:** Serum Hcy and folate comparison between control and case group (\( \bar{x} \pm s \)).

| Group          | Hcy (μmol/L) | Folate (nmol/L) |
|----------------|--------------|-----------------|
| Control group  | 4.5±1.3      | 19.1±4.72       |
| Case group     | 6.15±2.0     | 15.51±4.2       |
| \( t \)         | 4.8          | 3.6             |
| \( p \)         | <0.05        | <0.05           |

**Table 4:** Serum Hcy and folate comparison between control and case group (\( \bar{x} \pm s \)).

| Group          | Hcy (μmol/L) | Folate (nmol/L) |
|----------------|--------------|-----------------|
| IA group       | 7.5±1.7      | 12.7±4.2        |
| SFP group      | 5.5±1.8      | 16.8±3.6        |
| \( t \)         | 2.8          | 2.7             |
| \( p \)         | <0.05        | <0.05           |

**Table 5:**
Discussion

Epidemiology studies indicated that 20% of women are affected by threatened abortion during the pregnancy [12]. The outcome of threatened abortion is very concerning. Therefore, identifying high risk inevitable abortion patients and giving them proper treatment can improve the prognosis of threatened abortion. Due to its convenience and non-invasive nature, ultrasonography has become an important examination method in the diagnosis of early pregnancy [13-15]. The yolk sac is often used for evaluating the embryonic developmental status [16, 17]. The yolk sac usually forms around 5 weeks of gestation, and then increases in size during the gestational weeks. The yolk sac is easily affected by teratogenic substances in the early stages of pregnancy. Abnormal development of the yolk sac usually manifests through a larger diameter and shape changes, which eventually leads to abortion. Xie et al. [18] evaluate the ability to predict pregnancy outcomes through the combination of ultrasound scanning of yolk sacs and serum CA125 examination in early threatened abortion patients. The authors found that this combination was of great value for predicting pregnancy outcomes. In our present study, we found that yolk sac visualization rates detected by ultrasonography were 22.2% (2/9) and 90.0% (18/20) for the IA and SFP groups respectively, with a statistical difference (p<0.05). The results indicated that the visualization rate in the inevitable abortion group was significantly lower than that of successful fetus protection group.

Hcy is a non-proteinogenic α-amino acid and considered as a risk factor related to heart attacks and strokes [19]. It has also been reported that hyperhomocysteinemia can also be associated with early...
threatened-abortion and neural-tube defects [20]. Nasiri et al. have evaluated the serum levels of Hcy in threatened abortion cases, and found that the serum concentration of Hcy was significantly elevated and correlated with the poor-pregnancy prognosis [21]. In our study, we found that the serum levels of Hcy and folate were $7.5 \pm 1.7$ μmol/L and $12.7 \pm 4.2$ nmol/L respectively for the IA group, which were statistically different compared to the SFP group ($p<0.05$). The serum Hcy levels were used as an outcome for threatened-abortion prediction reference. The predictive sensitivity and specificity for the outcome of threatened abortion were 85.0%, 66.7% under the cut-off value of 7.0 μmol/L. These results indicated that the greater serum Hcy levels the pregnant women had, the greater the possibility of development of an inevitable abortion.

During pregnancy, insufficient intake of folate and vitamin B12 can lead to elevated blood Hcy levels. Hypercysteinemia in early pregnancy can inhibit the formation of chorion, reducing the number of chorionic vessels, affecting the blood perfusion of embryos, leading to poor implantation of embryos, and eventually leading to poor pregnancy outcomes. In our results, the serum folate levels in the IA group were significantly lower than the SFP group and further analysis indicated that serum folate levels can be used as an outcome predicting serological marker with the relative high sensitivity and specificity (75.0% and 88.9%).

In conclusion, serum homocysteine and folate were quite different in threatened-abortion pregnant women. Serum Hcy and folate detection combined with ultrasonography detection of yolk-sac visualization can provide useful information in threatened abortions. Serum Hcy and folate can be used as serological biomarkers for predicting the outcomes of the pregnant women with threatened abortions.

Conflict of interest: Authors state no conflict of interest

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Availability of data and materials: Please contact the corresponding author for data requests.

Authors’ contributions: Wenhai Wu performed the ultrasonography detection of yolk sac; Qinlin Ji, Baoli Guo collected the data; Jinfen Yu, Jiewei Wang did the data analysis Yezhu Wang, Juan Xu, and Wenhai Wu wrote the manuscript. All authors have read and approved the final manuscript.

References
1. Wilken, H, Schrodt U, El-Zoobi A. [Diagnosis, treatment and prognosis of threatened abortion]. Zentralblatt fur Gynakologie. 1976;98:577-86.
2. Soiva K. [Threatened abortion and its treatment]. Duodecim; laakietieteellinen aikakauskirja. 1974;90:267-75.
3. Weiss JL, Malone FD, Vidaver J, Ball RH, Nyberg DA, Comstock CH, Hankins GD, et al. Threatened abortion: A risk factor for poor pregnancy outcome, a population-based screening study. Am J Obstet Gynecol. 2004;190:745-50.
4. Szabo I, Szilagyi A. Management of threatened abortion. Early pregnancy : biology and medicine : the official journal of the Society for the Investigation of Early Pregnancy. 1996;2:233-40.
5. Petriglia G, Palaia I, Musella A, Marchetti C, Antonilli M, Brunelli R, et al. Threatened abortion and late-pregnancy complications: a case-control study and review of literature. Minerva ginecologica. 2015;67:491-7.
6. Leylek OA, Senocak F, Erselcan T. The prognostic predictive value of maternal serum CA125 levels in threatened abortion. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 1997;59:147-9.

7. Ocer F, Bese T, Saridogan E, Aydilini K, Atasu T. The prognostic significance of maternal serum CA125 measurement in threatened abortion. Eur J Obstet Gynecol Reprod Biol. 1992;46:137-42.

8. Yamane Y, Takahashi K, Kita M. Prognostic potential of serum CA125 and pregnant markers in threatened abortion. Nihon Sanka Fujinka Gakkai zasshi. 1989;41:1999-2004.

9. Bersinger NA, Keller PJ, Naie M, Fischer M, Schneider H. Pregnancy-specific and pregnancy-associated proteins in threatened abortion. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 1987;1:379-84.

10. Xie YH, Chen MX, Liao XM. Relationship of Serum Homocysteine, Vitamin B12, Coagulation Function with Threatened Abortion and Pregnancy Outcome. Clinical Medicine & Engineering. 2018;25:503-504.

11. Ye GC, Wang DE, Cheng LZ, Yuan CL, Peng JM. Study on correlation between serum homocystine and methylenetetrahydrofolate reductase with threatened abortion. Laboratory Medicine and Clinic. 2016;13:443-445.

12. Vigoureux S. [Epidemiology of induced abortion in France]. Journal de gynecologie, obstetrique et biologie de la reproduction. 2016;45:1462-1476.

13. Papaioannou GI, Syngelaki A, Maiz N, Ross JA, Nicolaides KH. Ultrasonographic prediction of early miscarriage. Human reproduction (Oxford, England). 2011;26:1685-92.

14. Schouwink MH, Fong BF, Mol BW, van der Veen F. Ultrasonographic criteria for non-viability of first trimester intrauterine pregnancy. Early pregnancy (Online). 2000;4:203-13.

15. Klejewski A, Urbaniak T, Brazert J, Drews K. [Ultrasonographic evaluation of yolk sac in early pregnancy]. Ginekologia polska. 1994;65:426-9.

16. Schmidt P, Hornmansdorfer C, Elsasser M, Scharf A, Hillemans P, von KCS. Pitfalls of ultrasonographic yolk sac measurement. Ultraschall in der Medizin (Stuttgart, Germany : 1980). 2011;32 Suppl 2:E147-50.

17. Crooij MJ, Westhuis M, Schoemaker J, Exalto N. Ultrasonographic measurement of the yolk sac. British journal of obstetrics and gynaecology. 1982;89:931-4.

18. Xie YJ, Chen M, Chen SJ. Prediction of pregnancy outcomes with combined ultrasound scanning of yolk sacs and serum CA125 determinations in early threatened abortion. Clin Exp Obstet Gynecol. 2014;41:186-9.

19. Blom HJ, Smulders Y. Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. J Inherit Metab Dis. 2011;34:75-81.

20. van der Put NM, van Straaten HW, Trijbels FJ, Blom HJ. Folate, homocysteine and neural tube defects: an overview. Experimental biology and medicine (Maywood, N.J.). 2001;226:243-70.

21. Nasiri M, Arsanjani SA, Sadeghi O, Bagheri BM. The Relationship between Homocysteine Levels and Spontaneous Abortion in Iranian Women with Migraine. Iran J Public Health. 2017;46:1149-1151.