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Original Article

Diagnostic value of the plasmatic ADM level for early ectopic pregnancy

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Abstract: Objective: To analyze the plasmatic ADM level in early pregnancy and to investigate the diagnostic value of ADM in early ectopic pregnancy (EP). Methods: 70 patients with EP who had menopause for 5~8 weeks were included as study group, while 155 women with normal intrauterine pregnancy were also included as control group. The correlation between ADM level and menopause weeks was statistically analyzed and ROC curve was used to identify the diagnostic value of ADM. Results: (1) In 155 cases of normal intrauterine pregnancy, the plasmatic ADM level was increased with menopause weeks in linear relationship, and the correlation coefficient (R) was 0.991 ($P<0.05$). In 70 patients with EP, no significant increase was found with menopause weeks and no linear relationship can be found between ADM level and menopause weeks in EP group. The correlation coefficient (R) was 0.744 ($P>0.05$). (2) The multiple of median of plasmatic ADM level in EP group of menopause for 8 weeks was obviously lower than the intrauterine control group ($P<0.01$). (3) ROC curve was used to analyze the cut-off value of ADM level in the diagnosis of EP, and the area under the ROC curve was 0.523 ($P>0.05$) regardless of menopause weeks, however, the area under the ROC curve was 0.702 ($P<0.05$) at 8 weeks after menopause with sensitivity of 53.50% and specificity of 85.00%. Conclusions: Different from normal intrauterine pregnancy, plasmatic ADM level in early EP was relatively lower and no significant increase was found with menopause weeks; further studies are still needed for plasmatic ADM level as an indicator in the early diagnosis of EP.

Keywords: Adrenomedullin, early pregnancy, ectopic pregnancy

Introduction

As a very common acute abdominal condition, ectopic pregnancy (EP) was regarded as a primary cause of death during early pregnancy. However, mysteries still remain on the early recognition and diagnosis of EP in the necessity of reducing mortality. Adrenomedullin (ADM) belongs to calcitonin gene related peptide (CGRP) family, is one of the vascular active peptides, which was isolated from human pheochromocytoma by Japanese scholars in 1993. Some study suggested that the plasmatic level of ADM can be used to predict the occurrence of EP [1]. Our study here intended to do the analysis of plasmatic ADM level during early pregnancy, to investigate the relationship between plasmatic ADM level and menopause weeks, and to evaluate the diagnostic value of ADM in early EP.

Materials and methods

Subjects

70 cases of EP patients who had menopause for 5~8 weeks which were confirmed by postoperative pathology via laparotomy or laparoscopy or diagnostic curettage combined by serum β-HCG, progesterone and ultrasound diagnosis were included as study group, 155 cases of normal intrauterine pregnancy women who had menopause for 5~8 weeks which were confirmed by ultrasound were also included as control group, during March 2014 to December 2014 from *The International Peace Maternity and Child Health Hospital affiliated to Shanghai Jiaotong University* and *Jiangwan Hospital of Shanghai Hongkou District*.

Table 1. Comparison of general information

	N	Age (years)	Gravid (times)	Para (times)	Menopause wks
Control group	155	27.94±4.40	1.72±0.97	0.35±0.54	6.38±1.04
EP group	70	28.83±5.47	2.36±1.02	0.83±0.93	6.61±1.15

Table 2. Plasmatic ADM level of different menopause weeks of study and control groups (μg/L)

	N	5~wks	6~wks	7~wks	8~wks
Control group	155	24.58	45.92	66.40	77.78
EP group	70	24.41	36.89	38.25	36.29
P-value		0.948	0.786	0.170	0.043

Inclusion and exclusion criteria

Singleton pregnancy women aged from 20 to 40 years old without any complications were included. Women who were smoking, having history of diabetes, hypertension and heart disease, and undergoing hormone treatment within 3 months were excluded.

Sample collection

Intravenous blood sample of study and control group was collected respectively. 3 ml of intravenous blood was placed in a separation gel vacuum tube and centrifuged for 3500 r/min. Plasma was then separated and preserved in -20°C for experiments for only once.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was used for detecting the plasmatic level of ADM. ELISA kit was bought from Lengton Bio. Co. Ltd., Shanghai and the experiment procedures were strictly followed with kit protocol.

Statistical analysis

Software SPSS 18.0 was used for data analysis. Age, gravida and para were described as mean ± SD, and U-test was used in comparison between groups. Median number was used in data description of menopause weeks of two groups, and rank-sum test was used in ADM level analysis between two groups. Correlation analysis between ADM level and menopause weeks was performed. ROC curve was used to evaluate the diagnostic value of ADM in early EP.

Results

Comparison of general information

225 women were included in this study of which 155 cases of normal intrauterine pregnancies and 70 cases of EP. The result of U-test was: age (U=1.198, P=0.231), gravida (U=4.423, P<0.001), para (U=4.022, P<0.001), and menopause weeks (U=1.198430, P=0.153); the results showed that there was no statistical difference in age and menopause weeks between the two groups (P>0.05); however, there was statistical difference in gravida and para between the two groups (P<0.001), suggesting gravida and para in the ectopic pregnancy group was higher than normal control group (**Table 1**).

Concentrations of maternal serum ADM in two groups at different gestational weeks

ADM level of every menopause weeks in different groups was shown in **Table 2**. There was no statistical difference in ADM level between normal intrauterine group and EP group at menopause weeks 5-7 (P>0.05); however, there was statistical difference in ADM level between normal intrauterine group and EP group at menopause weeks 8 (P<0.05) with ADM level in intrauterine pregnancy group greater than that in EP group (**Table 2**).

Correlation between median of plasmatic ADM level and menopause weeks during normal intrauterine pregnancy and ectopic pregnancy

As shown in **Figure 1**, plasmatic level of ADM increased with menopause weeks during normal intrauterine pregnancy. Correlation analysis suggested a very good linear relationship showing r=0.991, P=0.009. As shown in **Figure 2**, in EP group, plasmatic ADM level from women who had menopause for 5-6 weeks increased with menopause weeks. However, the increase of ADM level was not obvious, and a plateau can be found during 7 weeks and a decrease can be found during 8 weeks as shown in **Figure 2**. And correlation analysis suggested a poor linear relationship showing r=0.744, P=0.256.

Cut-off value of plasmatic ADM level in the early diagnosis of ectopic pregnancy

In this study, we focused on the early diagnostic value of plasmatic ADM level, and ROC curve

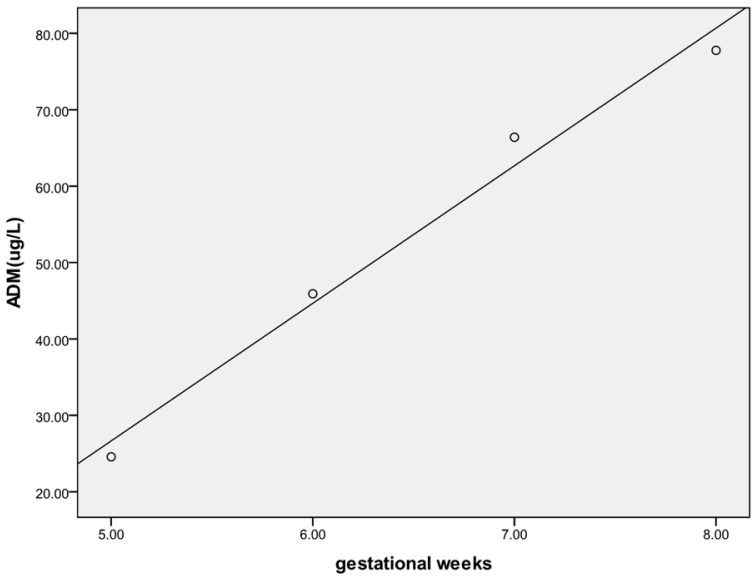

Figure 1. Correlation of the median of maternal serum ADM with gestational weeks in intrauterine pregnancy group. Plasmatic level of ADM increased with menopause weeks during normal intrauterine pregnancy. Correlation analysis suggested a very good linear relationship showing r=0.991, P=0.009.

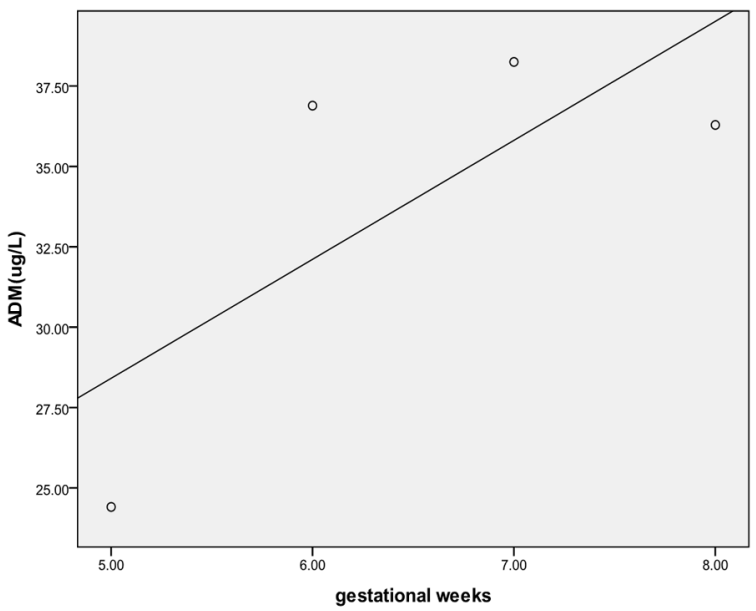

Figure 2. Correlation of the median of maternal serum ADM with gestational weeks in ectopic pregnancy group. In EP group, plasmatic ADM level from women who had menopause for 5-6 weeks increased with menopause weeks. However, the increase of ADM level was not obvious, and a plateau can be found during 7 weeks and a decrease can be found during 8 weeks as shown in **Figure 2**. And correlation analysis suggested a poor linear relationship showing r=0.744, P=0.256.

was used in distinguishing the cut-off value of plasmatic ADM. As shown in **Figure 3A**, the area under the ROC curve of all the gestational weeks (5-8 weeks) was 0.523, P=0.585, suggesting no statistical significance. The *P*-value of area under the ROC curve of 5 weeks was 0.948, suggesting no statistical significance (**Figure 3B**). The *P*-value of area under the ROC curve of 6 weeks was 0.786, sugges ting no statistical significance (**Figure 3C**). The *P*-value of area under the ROC curve of 7 weeks was 0.170, suggesting no statistical significance (**Figure 3D**). However, as shown in **Figure 3E**, the area under the ROC curve of 8 weeks was 0.702, P=0.043, suggesting significant statistical difference. The cut-off value was determined as 72.505 according to Youden index principle with sensitivity of 53.50% and specificity of 85.00%.

Discussion

EP is a very common gynecological emergency, which is often regarded as the primary cause of death during early pregnancy. In United States, approximately 6% of maternal deaths were associated with EP [2-4]. However, mysteries still remain in the early diagnosis of EP. Currently, laparoscopy is regarded as a gold standard in the diagnosis of EP since its accuracy can reach up to 99% [5]. However, clinically laparoscopy is never a first option simply because it costs more and can be invasive. Moreover, in tubal pregnancy abortion or EP with a small gestational sac, diagnosis can be easily missed via laparoscopic examination; sometimes even oviduct can be mistakenly cut off [6]. Clinical signs, lab results such as serum blood hCG, progesterone and ultrasound are

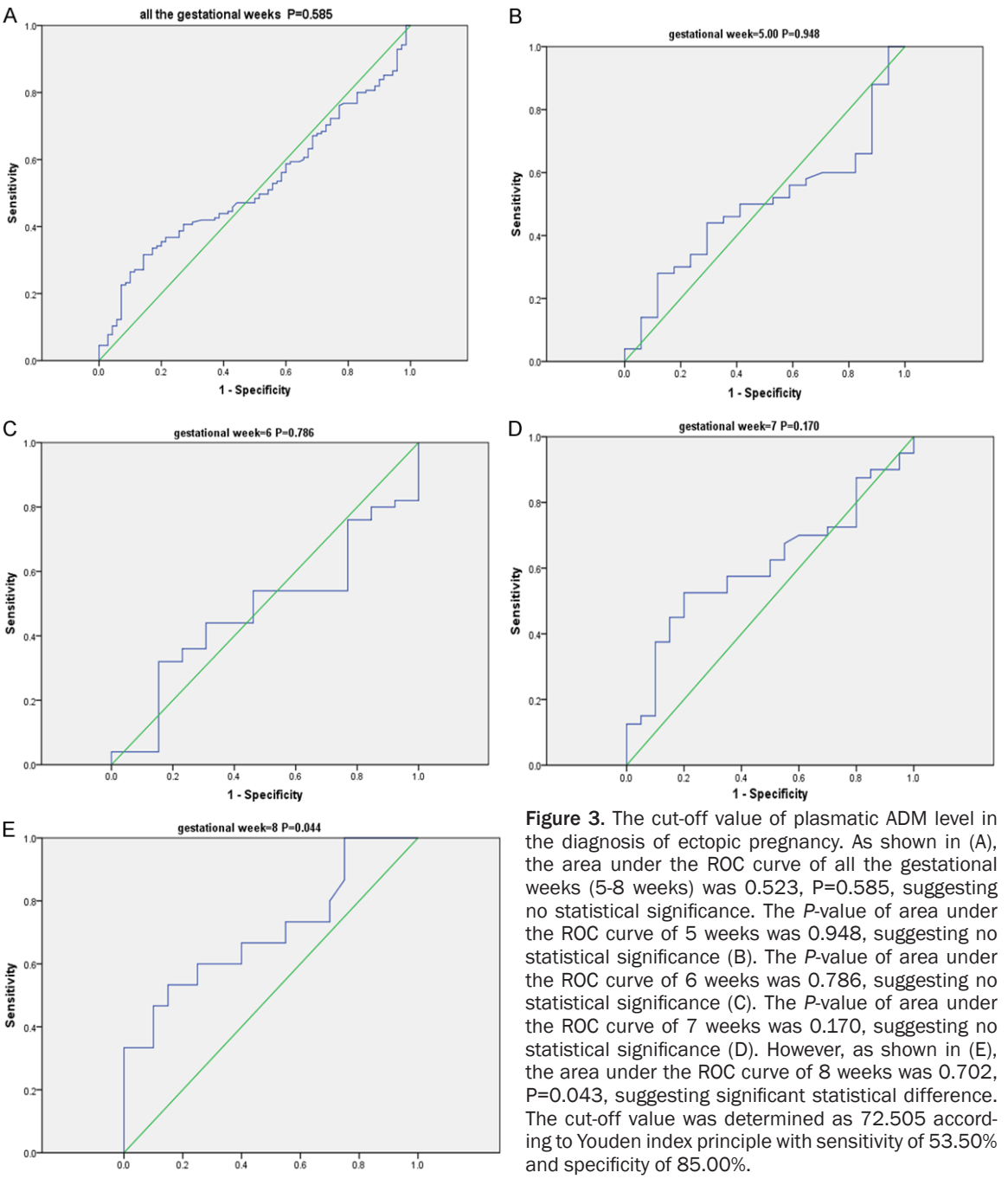

Figure 3. The cut-off value of plasmatic ADM level in the diagnosis of ectopic pregnancy. As shown in (A), the area under the ROC curve of all the gestational weeks (5-8 weeks) was 0.523, P=0.585, suggesting no statistical significance. The *P*-value of area under the ROC curve of 5 weeks was 0.948, suggesting no statistical significance (B). The *P*-value of area under the ROC curve of 6 weeks was 0.786, suggesting no statistical significance (C). The *P*-value of area under the ROC curve of 7 weeks was 0.170, suggesting no statistical significance (D). However, as shown in (E), the area under the ROC curve of 8 weeks was 0.702, P=0.043, suggesting significant statistical difference. The cut-off value was determined as 72.505 according to Youden index principle with sensitivity of 53.50% and specificity of 85.00%.

not accurate enough [7]. Serum βHCG level alone cannot distinguish symptomatic intrauterine pregnancy from EP, while ultrasound alone cannot confirm intrauterine pregnancy either [8]. So it is a necessity to establish a safe diagnostic method of EP more accurately and more rapidly.

The original studies of ADM which was found in 1993 by a Japanese scholar Kitamura focused mainly on vasodilatation and anti-hypertension. Subsequently, ADM was found widely distributed in various human tissues and organs, and was closely associated with normal pregnancy. During normal pregnancy, trophoblast cells and decidual cells secrete ADM, and plasmatic ADM level can be 4-5 folds higher during late pregnancy compared with non-pregnant women [9]. Decrease of plasmatic ADM was quite rare and may only associate with EP [10]. Liao et al. first-

ly began to study the plasmatic ADM level and the pathogenesis of EP [1], and their results showed that the plasmatic ADM level was relatively lower in EP compared with normal pregnancies, for lower level of ADM impaired the process of implantation due to decrease of oscillation frequency of tubal cilia. Based on this, Wai et al. proposed an indirect diagnostic method for EP by testing the ADM level of nasal mucous membrane cells and oscillation frequency of nasal mucosa cilia in order to establish a noninvasive diagnostic method for EP more rapidly, simply and effectively [10].

Despite its promising prospect above, the theory of ADM in diagnosis had never been clinically applied. Here we tried to apply the plasmatic ADM level as an indicator for early diagnosis of EP clinically by comparing the plasmatic ADM level between normal intrauterine pregnancy and EP, and the dynamic variation between menopause weeks was also observed. In consistent with previous reports [1, 10], our results showed that plasmatic ADM level of different menopause weeks in normal intrauterine pregnancy increase with menopause weeks ($r= 0.991$, $P<0.05$). Thus, as a natural way of ADM expression, linear relationship can be found between plasmatic ADM level and menopause weeks during normal pregnancy [11]. However, such linear relationship cannot be found during EP groups ($r=0.744$, $P>0.05$).

In our study, ROC curve was used to find out the cut-off value of ADM. Our results here suggested normal intrauterine pregnancy and EP cannot be distinguished within 5-7 menopause weeks ($P>0.05$). However, normal intrauterine pregnancy and EP can be distinguished at 8 weeks after menopause ($P<0.05$) yet with low sensitivity of 53.50% and low specificity of 85.00%. The reasons might be as follows: firstly, as ADM was secreted by trophoblasts and deciduas in normal pregnancy [9], we studied plasmatic ADM level only between 5-8 weeks of gestational age. There might be statistical difference from 9 weeks of gestational age on yet with limited early diagnostic value, thus in our further studies, larger samples of ADM combined with other serum indicators might be taken into account; secondly, besides ADM, EP might be associated with many other factors such as infection, smoking, surgical history, EP history and assisted reproduction which could impair implantation due to inflammatory infiltration or the damage of fallopian tube homeostasis [12, 13].

In summary, different from normal intrauterine pregnancy, plasmatic ADM level in early EP was relatively lower. However, further studies are still needed for ADM as a clinical indicator in early diagnosis of EP.

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Disclosure of conflict of interest

None.

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