European Journal of Obstetrics & Gynecology and Reproductive Biology 210 (2017) 370–375

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

Ratios of biochemical markers in peritoneal fluid to those in venous blood for the diagnosis of ruptured tubal pregnancy: A prospective study

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ARTICLE INFO

Article history:
Received 30 January 2016
Received in revised form 27 December 2016
Accepted 14 January 2017
Available online xxx

Keywords:
Ectopic pregnancy
Diagnosis
Monitoring
Human chorionic gonadotropin
Cancer antigen 125
Creatine kinase
Progesterone
Vascular endothelial growth factor

ABSTRACT

Objectives: Ectopic pregnancies are among the leading causes of maternal morbidity and mortality in both developed and emerging nations, but tests for early, accurate, and convenient detection are lacking.
Study
Design: Between January 2013 and February 2015, 504 women with tubal pregnancy were prospectively recruited, and their clinical characteristics were recorded. Samples of peritoneal fluid were collected by culdocentesis, and venous blood was drawn from the antecubital vein. In samples from each source, levels of the following biochemical markers were measured: cancer antigen 125 (CA125), human chorionic gonadotropin (hCG), progesterone, vascular endothelial growth factor, and creatine kinase.
Results: The ratios of biochemical markers in the peritoneal fluid and in the blood ($R_{p/v}$) were calculated. The median of $R_{p/v}$-CA125 and $R_{p/v}$-hCG were significantly lower in the ruptured ectopic pregnancy group than in the unruptured group. The optimal cutoff value to detect ectopic pregnancy rupture was 401.5 U/mL as the upper limit for peritoneal CA125, with a sensitivity of 93.5% and specificity of 74.2%. The optimal cutoff value was 18.7 as the upper limit in the peritoneal fluid/blood ratio ($R_{p/v}$) of CA125, with a sensitivity of 77.5% and specificity of 68.4%.
Conclusions: In countries with poor access to laparoscopy, culdocentesis is useful. In this study, culdocentesis provided additional information for management of abdominal pain when laparoscopy is not available. The authors propose $R_{p/v}$ cutoff values that can be used conveniently and quickly to diagnose ruptured ectopic pregnancies and bleeding, enabling rapid and appropriate therapeutic responses.

Introduction

Extra-uterine pregnancy (including ectopic pregnancy and pregnancy of unknown location) is a first-trimester disorder that appears in 1.3%–2.4% of all pregnancies, and accounts for up to 6% of pregnancy-associated mortality [1]. Ectopic pregnancy is associated with rupture, hemodynamic instability, hemorrhagic shock/hemodynamic instability, syncope, and death [1]. Early diagnosis enables physicians to intervene before rupture of the ectopic pregnancy, which can both reduce mortality and enable nonsurgical interventions.

During a normal intrauterine pregnancy, serum human chorionic gonadotropin (hCG) is detectable 8–10 days after ovulation (about 23 days into the menstrual cycle), and its concentration increases with gestational age. The doubling time of hCG is 1.2–1.4 days during the first 3 weeks after fertilization, and 3.3–3.5 days during weeks 4–6 after fertilization. In contrast, with an ectopic pregnancy the doubling time of serum hCG is 3–8 days longer than in a normal pregnancy. These differential rates of hCG increase can be used to distinguish between intrauterine and ectopic pregnancies [2]. Guvendag et al. suggested that determining the level of serum hCG should be the first step in diagnosing suspected ectopic pregnancy [3]. Measurement of hCG can be problematic, however: Desai et al. [4] used 7 different test kits used to measure hCG in 80 standardized samples and reported significantly different measured concentrations (reported values between 74 and 6660 IU/L, $p < 0.0001$).

Abbreviations: AUC, area under the curve; CA125, cancer antigen 125; CK, creatine kinase; hCG, human chorionic gonadotropin; IQR, interquartile ranges; P, progesterone; $R_{p/v}$, ratio of peritoneal serum relative to venous serum; ROC, receiver operating characteristic; SD, standard deviation; VEGF, vascular endothelial growth factor.

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http://dx.doi.org/10.1016/j.ejogrb.2017.01.017
0301-2115/

In an ectopic pregnancy, the zygote implants in the oviduct, ovary, or other sites in the peritoneal cavity. The metabolism of hCG in the peritoneal cavity is slower than that in the blood, and with an ectopic pregnancy the hCG content in peritoneal fluid may be higher than that in venous blood. In the case of an intrauterine pregnancy, fluid in the abdomen may be due to luteal rupture, pelvic inflammation, or blood after a miscarriage. Under these conditions, relatively small amounts of HCG are released into the peritoneal cavity, and thus the hCG content of peritoneal fluid is similar to or lower than that of venous blood. Thus, the ratio of hCG in peritoneal fluid to that in venous blood may be useful for the diagnosis of ectopic pregnancy. A previous retrospective study showed that a ratio of >1 had a sensitivity and a specificity of 100% and 100%, respectively, for the diagnosis of ectopic pregnancy [5]. Other study also showed an hCG ratio >1 in peritoneal fluid relative to venous serum ($R_{p/v}$) was useful for diagnosis of an ectopic pregnancy, and a ratio ≤ 1 consistent with an intrauterine pregnancy [2].

Researchers have also examined other serum markers, including progesterone (P), vascular endothelial growth factor (VEGF), cancer antigen 125 (CA125), and creatine kinase (CK) to diagnose ectopic pregnancy. For example, Butler et al. [6] measured total serum hCG, hyperglycosylated hCG, the free β subunit of hCG, P, and CA125 for early diagnosis of ectopic pregnancies, and found that sequential use of total serum hCG and CA125 were promising, but establishing reliable cutoff values among the different measurements required further work.

The purpose of this study was to determine the value of the peritoneal fluid to venous blood ratio of hCG, P, VEGF, CA125, and CK for the diagnosis of an ectopic pregnancy and the likelihood of active bleeding.

Patients and methods

Patients

Between January 2013 and February 2015, a total of 504 women with tubal pregnancies were recruited from the Shanghai 6th People's Hospital of Shanghai Jiaotong University, Jinshan Branch of Shanghai 6th People's Hospital, and Maternal and Child Care Service Centre. The inclusion criteria were: 1) normal menstrual cycle and duration of amenorrhea <90 days; 2) positive urine or blood β-hCG; 3) ultrasound showed pelvic fluid or culdocentesis results were positive; 4) laparotomy or laparoscopy and postoperative pathology indicated a tubal pregnancy; and 5) other diseases, including serious gynecological complications, were excluded. This prospective study included more patients than did our earlier reported work [2]. The study was performed in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of Shanghai Jiaotong University, Shanghai, China, and all patients provided written informed consent.

Methods

Pathological and intraoperative findings, as well as patient clinical characteristics (including age, fertility, duration of amenorrhea, duration of vaginal bleeding, and duration of abdominal pain) were recorded in detail.

Peritoneal fluid (5 mL) was collected by culdocentesis, and venous blood (5 mL) was collected from the antecubital vein and transferred into Vacutainer. If culdocentesis was not performed or failed, peritoneal fluid (5 mL) was collected during surgery. The time interval between blood collection and collection of peritoneal fluid was <2 h. Blood and peritoneal fluid were centrifuged at $1000 \times g$ at 4 °C for 10 min, and the supernatant was collected for immediate biochemical detection or was stored at 4 °C for biochemical detection within 24 h.

Assays were performed with the following kits: CA125 (Elecsys CA125 II Kit, Roche Diagnostics U.S., Indianapolis, IN, USA), β-hCG (Access Total β-hCG Kit, Beckman Coulter, Brea, CA, USA), progesterone (Siemens, Munich, Germany), CK (Cobas Integra Creatine Kinase, Roche Diagnostics U.S., Indianapolis, IN, USA), and VEGF (human VEGF Quantikine ELISA Kit, R&D Systems, Inc., Minneapolis, MN, USA).

Blood levels of biochemical markers were expressed as $CA125_v$, $\beta\text{-}hCG_v$, P_v, CK_v, and $VEGF_v$, and those of the peritoneal fluid as $CA125_p$, $\beta\text{-}hCG_p$, P_p, CK_p, and $VEGF_p$. The ratios of biochemical markers of blood to those of the corresponding peritoneal fluid ($R_{p/v}$) were calculated and expressed as $R_{p/v}$-CA125, $R_{p/v}$-β-hCG, $R_{p/v}$-P, $R_{p/v}$-CK, and $R_{p/v}$-VEGF.

The procedural flow chart is shown in Fig. 1. An example of an information sheet for patients with ectopic pregnancy is provided as Supplemental Table S1.

Statistical analysis

Categorical variables were presented as counts and percentages, and the chi-square test was used for comparisons between the 2 groups. Continuous variables with normal distribution were presented as means and standard deviations (SDs), and the independent *t* test was used for group comparisons. Variables without a normal distribution were presented as median and interquartile ranges (IQRs) and Mann-Whitney *U* test was applied for group comparisons.

Receiver operating characteristics (ROC) curves were determined examine the diagnostic performance of the biomarkers. The optimal cutoff values to distinguish a ruptured from an unruptured ectopic pregnancy were selected by maximization of the Youden index with the formula of sensitivity + specificity −1. A test of the null hypothesis was to set the area under the curve (AUC) equal to 0.5 after performance of the Wilcoxon rank sum test. Using a finding of an AUC ≥ 0.7 with a significant difference as the null hypothesis indicated that a result was better than chance to make a possible prediction.

Statistical analyses were performed with IBM SPSS statistical software version 22 for Windows (IBM Corp., Armonk, New York, USA), and a 2-tailed value of $p < 0.05$ indicated statistical significance.

Results

A total of 504 women were included in this study, 89 with a ruptured ectopic pregnancy and 415 with an unruptured ectopic pregnancy, and their characteristics are summarized in Table 1. Less vaginal bleeding was observed in the ruptured ectopic group than in the unruptured group (58.43% vs. 85.54%, $p < 0.001$). Abdominal pain, active intraperitoneal bleeding, and intraperitoneal blood volume ≥ 500 mL were more common in the ruptured ectopic group than in the unruptured group (abdominal pain: 94.38% vs. 61.93%, $p < 0.001$; active intraperitoneal bleeding: 66.29% vs. 8.92%, $p < 0.001$; intraperitoneal blood volume ≥ 500 mL: 70.79% vs. 10.6%, $p < 0.001$).

Biomarker levels are summarized in Table 2. Median $CA125_p$, $\beta\text{-}HCG_p$, and CK_v levels were significantly lower in the ruptured ectopic group than in the unruptured group, and median $\beta\text{-}HCG_v$, $VEGF_p$, CK_p, P_v, and P_p levels were all significantly higher in the ruptured ectopic group than in the unruptured group. Median $R_{p/v}$-CA125 and $R_{p/v}$-β-hCG ratios were significantly lower in the ruptured ectopic group than in the unruptured group ($R_{p/v}$-CA125: 7.33 vs. 59.51, $p < 0.001$; and $R_{p/v}$-β-hCG: 3.69 vs. 11.77, $p < 0.001$). Median $R_{p/v}$-VEGF, $R_{p/v}$-CK, and $R_{p/v}$-P ratios were significant