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CLINICAL ARTICLE

Diagnostic value of amniotic fluid inflammatory biomarkers for subclinical chorioamnionitis

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ABSTRACT

Objective: To determine the value of measuring amniotic fluid inflammatory biomarkers for diagnosis of subclinical chorioamnionitis. *Methods:* A prospective study was conducted among pregnant women with cervical dilation, preterm premature rupture of membranes, threatened late abortion, or threatened premature labor who attended a tertiary care hospital in Guangzhou, China, between June 1, 2012, and January 31, 2014. Participants were divided into two groups according to the presence or absence of subclinical chorioamnionitis. Surface-enhanced laser desorption/ionization time-of-flight mass spectroscopy (SELDI-TOF-MS) was used to detect human neutrophil defensins (HNP-1 and HNP-2), calgranulins A (S100 A8), and calgranulins C (S100 A12) in amniocentesis samples. *Results:* Overall, 22 patients had subclinical chorioamnionitis and 17 patients did not. Positive test results for HNP-2 were noted for more patients with subclinical chorioamnionitis than for those without for HNP-2 (19 [86%] vs 2 [12%]; $P < 0.001$), HNP-1 (19 [86%] vs 5 [29%]; $P = 0.001$), S100 A12 (20 [91%] vs 9 [53%]; $P = 0.011$), and S100 A8 (12 [55%] vs 0; $P < 0.001$). When three or four of these biomarkers were present, the accuracy for a diagnosis of subclinical chorioamnionitis was 89.7%. The sensitivity, specificity, positive predictive value, and negative predictive value were 81.8%, 100.0%, 100.0%, and 81.0%, respectively. *Conclusion:* Detection of inflammatory biomarkers in the amniotic fluid by SELDI-TOF-MS exhibited high diagnostic accuracy for subclinical chorioamnionitis.

1. Introduction

Worldwide, chorioamnionitis occurs in 1%–4% of all pregnancies, with an incidence of 40%–70% among premature deliveries and 1%–13% among full-term deliveries [1]. This condition is a major cause of spontaneous abortion and preterm birth during the third trimester [2]. Chorioamnionitis has been found in 70% of preterm births at 28 weeks of pregnancy, 40% at 28–32 weeks, and 16% at 32–36 weeks [3].

Chorioamnionitis typically presents as fever, abdominal pain, and premature uterine contractions; by contrast, the signs and symptoms of subclinical chorioamnionitis are difficult to discern and laboratory findings might be nonspecific [1,2]. Microbiological culture of amniotic fluid can be used to diagnose subclinical chorioamnionitis; however, this method is time-consuming and exhibits a high false-negative rate [4]. Gram staining of amniotic fluid shows reasonable specificity for the diagnosis of intrauterine infection, but use of this approach is limited by poor agreement with the findings of pathological examination and failure to identify frequent causes of chorioamnionitis [5]. Some studies have suggested that the presence of cytokines in the amniotic fluid (e.g. interleukin-1 [IL-1]), could be diagnostic of subclinical chorioamnionitis; however, the levels of these proteins are also increased in other complications of pregnancy, including pre-eclampsia [6,7].

Proteomic techniques can be used to identify differentially expressed proteins to further understanding of the pathophysiology of human disease [8]. In the field of obstetrics and gynecology, proteomics is mainly used to screen for biomarkers of gynecologic cancer [8]. Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS; also known as protein fingerprinting) employs a protein microarray and mass spectrometry to measure proteomic profiles [8]. In 2004, Gravett et al. [9] used SELDI-TOF-MS in rhesus monkeys and found that a 10–20 kDa polypeptide exhibited high expression in the amniotic fluid and might reflect amniotic infection. A subsequent study [10] found that human neutrophil defensins (HNP-1 and HNP-2), calgranulins A (S100 A8), and calgranulins C (S100 A12) were associated with preterm birth, histological evidence of chorioamnionitis, and early onset of neonatal sepsis.

The aim of the present study was to examine the clinical utility of measuring HNP-1, HNP-2, S100 A8, and S100 A12 in amniotic fluid by SELDI-TOF-MS for diagnosis of subclinical chorioamnionitis.

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2. Materials and methods

A prospective study was conducted at Sun. Yat-sen Memorial Hospital, Guangzhou, China, between June 1, 2012, and January 31, 2014. The participants provided informed consent for all procedures performed and for inclusion in the present study. The protocol was approved by the institutional review board of Sun. Yat-sen Memorial Hospital.

Eligible participants were women with a singleton pregnancy who were hospitalized owing to painless cervical dilation, threatened premature labor, or premature rupture of the fetal membranes. Patients with high-risk pregnancies, concomitant complications of pregnancy, or concomitant systemic infectious diseases (e.g. viral hepatitis, syphilis, HIV, and diseases in the TORCH complex [toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus-2]) were excluded. The participants were divided into two groups according to the presence or absence of subclinical chorioamnionitis. In all cases, the clinicians proposed recommendations regarding courses of action and then discussed them with the patients and their relatives.

Among patients with regular menstrual cycles, gestational age was determined by the first day of the last menstrual cycle. Among patients undergoing in vitro fertilization-embryo transfer, gestational age was calculated from 17 days before the date of embryo implantation. Ultrasonography was performed during early pregnancy to measure the diameter of the gestational sac and the size of the embryo for the diagnosis of pregnancy. At a gestational age of 10–13 weeks, ultrasonography was performed to measure the crown–rump length, which was used to either estimate or confirm the gestational age. If the last menstrual cycle was irregular or unknown, ultrasonography was performed in middle or late pregnancy to measure the biparietal diameter, head circumference, and femur length to determine gestational age.

Premature rupture of fetal membranes was diagnosed on the basis of alkaline fluid in the posterior fornix or flowing from the cervix, with leaf-like crystals present on microscopic examination. Painless cervical dilatation was defined as cervical dilatation of at least 1 cm, with the amniotic sac observable through the cervix on speculum examination. Chorioamnionitis was defined as a maternal temperature of at least 37.8 °C and the presence of at least two of the following signs: maternal heart rate greater 100 beats per minute; fetal heat rate greater than 160 beats per minute; uterine tenderness; foul-smelling amniotic fluid; and maternal leukocytosis (>15,000 cells per mm^3) [11]. Subclinical chorioamnionitis was defined as an amniotic fluid culture that tested positive for a pathogen and/or postnatal pathological examination of the placenta, fetal membranes, and umbilical cord showing histological chorioamnionitis or inflammation of the umbilical cord [12], with or without clinical signs of chorioamnionitis, but not meeting the definition of chorioamnionitis [11,13]. In all cases, placental pathological examination was performed to exclude or diagnose chorioamnionitis.

All patients underwent transabdominal amniocentesis, which was performed under ultrasonographic guidance. This procedure was generally conducted before administration of antibiotics during hospitalization. The initial 3–5 mL of amniotic fluid drawn was discarded; another 10-mL syringe was subsequently connected and 15 mL of amniotic fluid collected for analysis. An aliquot of amniotic fluid was used to determine the white blood cell (WBC) count and to measure the level of C-reactive protein (CRP) using the BN ProSpec System (Siemens Healthcare, Munich, Germany). This sample was also used for Gram staining, bacterial culture, and mycoplasma culture. The remaining amniotic fluid was centrifuged at 4000 rpm for 5 min; the supernatant was collected and stored at −80 °C. Maternal blood samples were also collected for determination of WBC count, CRP level, and IL-6 level.

Amniotic fluid levels of the inflammatory biomarkers were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits. The levels of IL-6 were assessed using an R&D Systems kit (Minneapolis, MN, USA), whereas the levels of S100 A8, S100 A12, HNP-1, HNP-2, and HNP-3 were measured using kits manufactured by CUSABIO (Wuhan, China). In addition, amniotic fluid levels of HNP-1, HNP-2, S100 A8, and S100 A12 were determined by SELDI-TOF-MS on the basis of weak cation-exchange magnetic nanobeads using a PBS IIC time-of-flight mass spectrometer (Ciphergen Biosystems, Fremont, CA, USA). Details of the method are provided in Supplementary Material S1.

The data were analyzed using SPSS version 22 (IBM, Armonk, NY, USA). Owing to the small sample size, all continuous data were presented as median (range) and all comparisons between two independent groups were examined with the Mann–Whitney test. Categorical data were presented as number (percentage) and compared using the Fisher exact test. Receiver operating characteristic analysis was performed and the area under the curve calculated to evaluate the diagnostic value of the inflammatory markers. The optimized cu-off point was determined by the Youden index (defined as the maximum of sensitivity + specificity−1). Accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to determine the diagnostic value of the inflammatory biomarkers. $P < 0.05$ was considered statistically significant.

3. Results

The characteristics of the two groups are shown in Table 1. The subclinical chorioamnionitis group had a shorter amniocentesis-to-delivery interval than the non-subclinical chorioamnionitis group (median 1.0 vs 15.0 weeks; $P = 0.006$) and also delivered earlier (median 26.0 vs 33.4 weeks; $P = 0.005$). The number of fetal deaths was 16 (73%) in the subclinical chorioamnionitis group and 5 (29%) in the non-subclinical chorioamnionitis group ($P = 0.011$). In the subclinical

Table 1
Characteristics of participants (n = 39).[a]

Characteristic	Without subclinical chorioamnionitis (n = 17)	With subclinical chorioamnionitis (n = 22)	*P* value[b]
Maternal age, y	29.0 (23.0–39.0)	31.5 (24.0–41.0)	0.306
Gravidity	2.0 (1.0–5.0)	2.5 (1.0–7.0)	0.846
Parity	0 (0–1)	0 (0–2)	0.183
Late abortion history	5 (29)	6 (27)	>0.99
Gestational age at amniocentesis, wk	26.0 (18.6–33.9)	25.1 (19.6–31.3)	0.246
Gestational age at delivery, wk	33.4 (19.0–38.0)	26.0 (20.0–31.4)	0.005
Amniocentesis-to-delivery interval, wk	15.0 (0.0–142.0)	1.0 (0.0–32.0)	0.006
Delivery type			
Vaginal	10 (59)	19 (86)	0.071
Cesarean	7 (41)	3 (14)	
Fetal death			
Yes	5 (29)	16 (73)	0.011
No	12 (71)	6 (27)	
Microorganisms identified in amniotic fluid culture			
Ureaplasma urealyticum	0	6 (27)	0.027
Mycoplasma hominis	0	5 (23)	0.056
Escherichia coli	0	1 (5)	>0.99
Streptococcus agalactiae	0	1 (5)	>0.99
Streptococcus mitis	0	1 (5)	>0.99
Microorganisms identified in vaginal fluid culture			
Ureaplasma urealyticum	4 (24)	11 (50)	0.112
Mycoplasma hominis	2 (12)	5 (23)	0.438
Escherichia coli	1 (6)	2 (9)	>0.99
Streptococcus agalactiae	0	3 (14)	0.243
Streptococcus mitis	0	1 (5)	>0.99
Staphylococcus epidermidis	1 (6)	0	0.436
Klebsiella pneumoniae	1 (6)	1 (5)	>0.99
Enterococcus faecalis	0	1 (5)	>0.99
Candida albicans	0	1 (5)	>0.99
Chlamydia trachomatis	0	1 (5)	>0.99

[a] Values are given as median (range) or number (percentage), unless indicated otherwise.
[b] Comparisons between two independent groups were evaluated with the Mann–Whitney test for continuous data and the Fisher exact test for categorical data. $P < 0.05$ indicates a statistically significant between-group difference.

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