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About one-half of early spontaneous preterm deliveries can be identified by a rapid matrix metalloproteinase-8 (MMP-8) bedside test at the time of mid-trimester genetic amniocentesis

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Abstract

Objective—Mid-trimester amniocentesis continues to be used for the prenatal diagnosis of chromosomal anomalies and other genetic disorders. Analysis of amniotic fluid obtained at the time of mid-trimester genetic amniocentesis identifies those patients who are at risk for early spontaneous preterm delivery. This is based on a solid body of evidence that found subclinical intra-amniotic inflammation/infection to be causally linked to early spontaneous preterm birth. Although several biomarkers have been proposed to identify intra-amniotic inflammation, the accumulated data suggest that the determination of amniotic fluid matrix metalloproteinase-8 (MMP-8), or neutrophil collagenase, is a powerful predictor of spontaneous preterm delivery. MMP-8 is released by inflammatory cells in response to microbial products or “danger signals.” A rapid point-of-care test has been developed to determine MMP-8 at the bedside within 20 minutes, and without the requirement of laboratory equipment. The objective of this study was to determine whether an elevation of MMP-8 in the amniotic fluid, measured by a rapid point-of-care test, can

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Declaration: The test described in this article is the subject of a patent by the Seoul National University in Seoul, Republic of Korea. Dr. B.H. Yoon is listed as an inventor and has a financial interest in OBMed Co., Ltd., Seoul, Republic of Korea, the manufacturer of this test. A financial interest is defined as a potential gain or a potential loss derived from the activity of this company.

identify those patients at risk for spontaneous preterm delivery after a mid-trimester genetic amniocentesis.

Study Design—A case-control study was designed to obtain amniotic fluid from asymptomatic singleton pregnant women who underwent mid-trimester genetic amniocentesis. An MMP-8 bedside test was performed to analyze the amniotic fluid of 64 patients with early spontaneous preterm delivery (<30 weeks) and 128 matched controls with normal pregnancy outcomes.

Results—1) The MMP-8 bedside test (Yoon's MMP-8 Check™) was positive in 42.2% (27/64) of patients with spontaneous preterm delivery but in none (0/128) of the control cases ($p<0.001$); 2) the MMP-8 bedside test had a sensitivity of 42.2%, and a specificity of 100% in the prediction of spontaneous preterm delivery (<30 weeks) following a mid-trimester genetic amniocentesis; and 3) among the patients with spontaneous preterm delivery, those with a positive MMP-8 bedside test had a significantly higher rate of spontaneous delivery within 2 weeks and 4 weeks of an amniocentesis [40.7% (11/27) vs. 5.4% (2/37); 63.0% (17/27) vs. 24.3% (9/37)] and a shorter interval-to-delivery period than those with a negative test [interval-to-delivery: median (range), 16 days (0–95 days) vs. 42 days (2–91 days); $p<0.05$ for each].

Conclusion—We conclude that 42% of patients with an early spontaneous preterm delivery (<30 weeks) could be identified by a rapid MMP-8 bedside test at the time of their mid-trimester genetic amniocentesis. The MMP-8 bedside test is a powerful predictor of early spontaneous preterm birth in asymptomatic pregnant women.

Keywords

amniotic fluid; intra-amniotic inflammation/infection; point-of-care (POC) test; preterm parturition

Introduction

Mid-trimester genetic amniocentesis has been widely used for the prenatal diagnosis of fetal chromosomal disorders [1, 2]. With the introduction of and interest in non-invasive prenatal testing (NIPT) [3–13], amniocentesis continues to be used as a final diagnostic test after aneuploidy has been detected by means of NIPT [8, 14–25]. Indeed, Papp has recently proposed that an amniocentesis is preferable to chorionic villous sampling (CVS) in this setting in order to avoid the diagnostic problems related to confined placental mosaicism [26, 27].

A solid body of evidence indicates that subclinical intra-amniotic inflammation or infection is causally linked with spontaneous preterm delivery [28–52] and adverse pregnancy outcome [38, 40, 46, 53–68]. Such pathologic processes can be detected by analyzing amniotic fluid for microorganisms (bacteria or viruses) [69–92] or inflammatory markers [38, 50, 53, 57, 74, 76, 90, 93–160]. We previously reported that amniotic fluid matrix metalloproteinase-8 (MMP-8) [121, 123, 124, 127, 128, 132–134, 136, 161] and interleukin (IL)-6 [53, 74, 76, 95, 103, 105] are powerful biomarkers of intra-amniotic inflammation/infection. These studies were largely conducted using enzyme-linked immunoassays with research reagents. Translation of the findings to clinical practice requires that results be available quickly and easily. Therefore, we have developed a qualitative

immunochromatographic kit that detects the presence of MMP-8 in amniotic fluid at the patient's bedside. In previous studies, we documented the value of the amniotic fluid MMP-8 bedside test for patients with preterm labor [127] as well as those with prelabor rupture of the membranes (PROM) [128]. Moreover, we found that a positive amniotic fluid MMP-8 bedside test is a predictor of funisitis (a marker of fetal systemic inflammation) [129]. The objective of this study was to evaluate the performance of this MMP-8 rapid test in the identification of patients at risk for spontaneous preterm delivery after a mid-trimester genetic amniocentesis.

Materials and Methods

Study design

A case-control study was designed using amniotic fluid from asymptomatic pregnant women who underwent a genetic amniocentesis during the mid-trimester. The study population included patients with singleton gestations who underwent spontaneous preterm delivery before 30 weeks. These patients were matched for maternal age (within 5 years), parity (nulliparous vs. multiparous), gestational age at amniocentesis (within 2 weeks), and year of amniocentesis (within 3 years) with 128 controls who underwent genetic amniocentesis and delivered singleton newborns after 37 weeks of gestation (1:2 matching). Patients with abnormal fetal karyotypes, major fetal anomalies, or any symptom or sign of preterm delivery at the time of genetic amniocentesis were excluded (e.g. suspicion of ruptured or dilated membranes). Written innformed consent was obtained from each patient prior to the procedure. The Institutional Review Board of Seoul National University Hospital approved the collection and use of amniotic fluid samples and clinical information for research purposes.

MMP-8 Rapid Test

Amniotic fluid was processed for fetal karyotyping and the unused fluid was centrifuged at 2,000 rpm for 10 minutes at 4 °C, aliquoted and pipetted, and then stored at −70 °C until assay. After thawing the stored amniotic fluid, the MMP-8 rapid test (Yoon's MMP-8 Check™; OBMed Co., Ltd., Seoul, Republic of Korea) was performed by personnel blinded to the clinical information. Yoon's MMP-8 Check™ is a qualitative immunochromatographic test that detects the presence of MMP-8 in human amniotic fluid with a threshold of 10 ng/ml. The manufacturer recommends the addition of 25 μl of amniotic fluid and 75 μl (3 drops) of buffer to the test window (Figure 1). The results were scored after 20 minutes. When the results were equivocal (very weak bands), the test was repeated using 12.5 μl of amniotic fluid and 75 μl (3 drops) of the buffer.

Statistical analysis

The Mann-Whitney *U* test was used to compare continuous variables, and the Fisher's exact test was used for the comparison of proportions.

Results

The clinical characteristics and pregnancy outcomes of the study population are shown in Table 1. There were no significant differences in the maternal age, frequency of nulliparity, and gestational age at amniocentesis between the two groups.

The MMP-8 bedside test was positive in 42.2% (27/64) of patients with early spontaneous preterm delivery (<30 weeks) but in none (0/128) of the control group (p<0.001). The MMP-8 bedside test had a sensitivity of 42.2%, and a specificity of 100% in the prediction of spontaneous preterm delivery (<30 weeks) after mid-trimester genetic amniocentesis in asymptomatic singleton pregnant women.

Table 2 demonstrates the comparison of clinical characteristics and pregnancy outcomes according to the results of the MMP-8 bedside test among the patients with spontaneous preterm delivery. There were no significant differences in the maternal age, frequency of nulliparity, and gestational age at amniocentesis between the two groups. However, the median interval-to-delivery period after amniocentesis was significantly shorter for those with a positive MMP-8 bedside test than for those with a negative test [median (range), 16 days (0–95 days) vs. 42 days (2–91 days); p=0.011]. Patients with a positive MMP-8 bedside test had a significantly higher rate of delivery within 2 to 4 weeks after an amniocentesis than those with a negative test [40.7% (11/27) vs 5.4% (2/37), p=0.001; 63.0% (17/27) vs 24.3% (9/37), p=0.004].

DISCUSSION

Principal finding of the study

A positive MMP-8 bedside test identified about one-half of the patients who underwent a mid-trimester genetic amniocentesis and subsequently had a spontaneous abortion or an early spontaneous preterm delivery (<30 weeks of gestation).

Clinical implications

The current practice of mid-trimester genetic amniocentesis is specifically focused on the prenatal diagnosis of chromosomal abnormalities; however, amniotic fluid analysis provides a powerful tool to identify patients at risk for spontaneous preterm delivery by determining the presence of intra-amniotic inflammation. An obstacle to the assessment of inflammation has been the availability of a rapid test that can be informative, reliable, and inexpensive.

The MMP-8 bedside test is a simple point-of-care method for the rapid identification of intra-amniotic inflammation without laboratory equipment [127–130, 135, 136]. The test has performed well in the identification of intra-amniotic inflammation/infection in patients with preterm labor [127, 136] and preterm PROM [128, 135]. Moreover, a positive MMP-8 bedside test is a marker for fetal systemic inflammation, presumably because neutrophils found in the amniotic fluid in cases of inflammation are of fetal origin [162]. Indeed, an elevated concentration of MMP-8 in the amniotic fluid is associated with funisitis [129], the hallmark of the fetal inflammatory response syndrome.

We previously reported that the odds ratio of an elevated concentration of MMP-8 (>23 ng/mL) in the amniotic fluid is approximately 68 [122]. In the current study, the odds ratio could not be calculated due to the lack of false-positive results. Once patients are identified as "at risk," the next step is to investigate the etiology of the inflammatory process. A rapid test allows identification of the samples that need to be sent to the microbiologic laboratory for both cultivation and molecular microbiologic studies. A positive culture for microorganisms has been the gold standard for the diagnosis of intra-amniotic infection. However, recent data suggest that the combined use of cultivation and molecular microbiologic methods results in the increased detection of microorganisms: some bacteria are fastidious or cannot be cultured with traditional techniques available in hospital clinical laboratories [75, 78, 81, 82, 85, 86, 163–166]. The performance of an amniotic fluid rapid MMP-8 test is of great value because it allows immediate identification of the samples that need to be worked up for the presence of bacteria or viruses. The immediate results of the amniotic fluid MMP-8 rapid test will prevent the need to retrieve the sample if the laboratory informs the clinician that there is evidence of intra-amniotic inflammation based on conventional methods such as an amniotic fluid white blood cell count, glucose, or other methods [71, 73, 74, 76, 96, 98, 167–175]. The identification of intra-amniotic infection is crucial because antibiotics would be helpful only in patients with infection. Sterile intra-amniotic inflammation has been recently recognized as a major factor in patients with preterm labor [47, 48], preterm PROM [46], and a sonographic short cervix [176]. The identification of danger signals responsible for such inflammatory processes has not been determined [156, 177–186]. However, it is possible that patients with sterile intra-amniotic inflammation may benefit from anti-inflammatory agents rather than antibiotics [187–192]. Sterile intra-amniotic inflammation could be detected when a patient has a positive amniotic fluid MMP-8 rapid test but is negative for bacteria and virus when a combination of cultivation and molecular microbiologic techniques is used. Randomized clinical trials are required to address the optimal treatment in patients with intra-amniotic inflammation and microorganisms and of those with sterile intra-amniotic inflammation. An important observation in this study is that the interval between amniocentesis and delivery was approximately 16 days in patients with intra-amniotic inflammation. Therefore, there is a window of time during which treatment may address the pathologic process responsible for the intra-amniotic inflammatory response.

Strengths and Limitations

The major strengths of this study are the large number of early spontaneous preterm deliveries and that personnel were blinded to the clinical outcome. The limitations are related to its case-control nature. A cohort study would be an ideal way to estimate the predictive values of the MMP-8 rapid test.

Conclusion

A positive MMP-8 rapid test of amniotic fluid obtained at the time of mid-trimester genetic amniocentesis identified about one-half of those patients who were at risk of an early spontaneous preterm delivery (<30 weeks). This information has prognostic value and could be the basis for the design of intervention trials to determine whether anti-inflammatory

agents and/or antibiotics can reduce the rate of preterm delivery in patients with intra-amniotic infection or sterile intra-amniotic inflammation.

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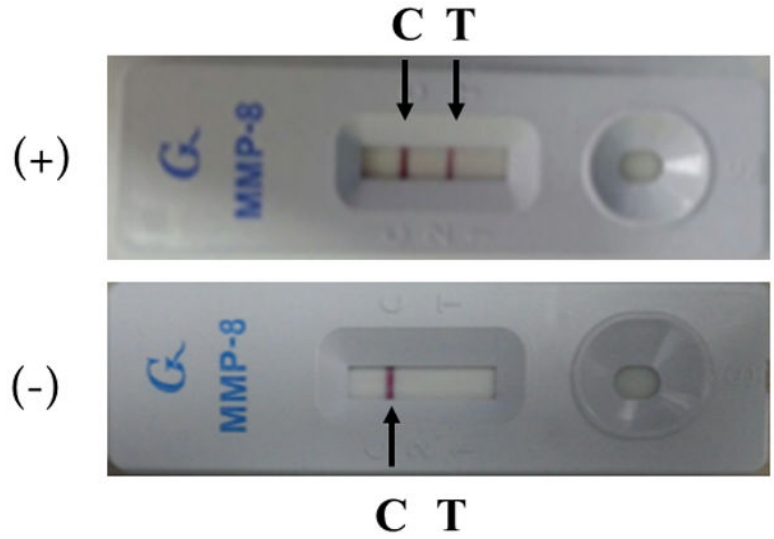

Figure 1.
The MMP-8 rapid test (Yoon's MMP-8 Check™) was considered positive when two bands were visible, corresponding to the control (C) and testing (T) channels of the kit (arrows).

Table 1

Clinical characteristics and pregnancy outcome of the study population

Characteristics	Spontaneous early preterm delivery < 30 weeks (n=64)	Term delivery (n=128)	P-value
Mean maternal age, years (±SD)	34.9 ± 4.5	35.0 ± 4.0	NS
Nulliparity	15 (23.4%)	30 (23.4%)	NS
Previous spontaneous preterm delivery	6 (9.4%)	5 (3.9%)	NS
Median gestational age at amniocentesis, weeks (range)	17.7 (15.6–22.3)	17.6 (15.4–23.0)	NS
Median gestational age at delivery, weeks (range)	23.0 (16.6–29.7)	39.0 (37.0–41.4)	<0.001
Positive MMP-8 rapid kit result	42.2% (27/64)	0.0% (0/128)	<0.001

SD = Standard deviation; NS = Not significant; MMP = Matrix metalloproteinase

Data are presented as median (range) or n (%).

Table 2

Clinical characteristics and pregnancy outcomes of 64 cases with spontaneous early preterm delivery (<30 weeks of gestation) according to results of the MMP-8 bedside test

Characteristic	MMP-8 bedside test Positive (n=27)	MMP-8 bedside test Negative (n=37)	P-value
Mean maternal age, years (±SD)	33.8 ± 4.5	35.6 ± 4.4	NS
Nulliparity	7 (25.9%)	8 (21.6%)	NS
History of spontaneous preterm delivery	1 (3.7%)	5 (13.5%)	NS
Median gestational age at amniocentesis, weeks (range)	18.0 (15.6–22.3)	17.6 (15.6–21.7)	NS
Median gestational age at delivery, weeks (range)	21.0 (16.6–29.6)	24.4 (17.2–29.7)	NS
Indication for delivery			NS
• Preterm labor	10 (37.0%)	17 (45.9%)	
• PROM	15 (55.6%)	17 (45.9%)	
• Cervical insufficiency	0 (0.0%)	3 (8.1%)	
• Spontaneous abortion	2 (7.4%)	0 (0.0%)	
Median amniocentesis-to-delivery interval, day (range)	16 (0–95)	42 (2–91)	0.011
• Time from amniocentesis to delivery ≤1week	6 (22.2%)	2 (5.4%)	NS
• Time from amniocentesis to delivery ≤2weeks	11 (40.7%)	2 (5.4%)	0.001
• Time from amniocentesis to delivery ≤4weeks	17 (63.0%)	9 (24.3%)	0.004

MMP = Matrix metalloproteinase; SD = Standard deviation; NS = Not significant; PROM = Premature rupture of the membranes

Data are presented as median (range) or n (%).