Published in final edited form as:

J Perinat Med. 2015 July ; 43(4): 381–389. doi:10.1515/jpm-2014-0276.

A Transcervical Amniotic Fluid Collector: A New Medical Device for The Assessment of Amniotic Fluid in Patients with Ruptured Membranes

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Abstract

Aim—To describe a new device for the transcervical collection of amniotic fluid (AF) in patients with ruptured membranes, and to compare the concentration of proteins in fluid retrieved by transabdominal amniocentesis and the transcervical AF collector.

Study Design—Paired AF samples were collected in patients with preterm prelabor rupture of membrane (PROM) (n=11) by transabdominal amniocentesis and with the transcervical AF collector. Three proteins known to have high concentrations in AF [alpha-fetoprotein (AFP), β -human chorionic gonadotropin (β -hCG), and prolactin] were measured.

Results—1) There was a significant correlation between the concentrations of analytes in AF obtained by transabdominal amniocentesis and by the transcervical AF collector ($r=0.95$, $p<0.001$ for AFP; $r=0.95$, $p<0.001$ for β -hCG; $r=0.84$, $p<0.005$ for prolactin); 2) Bland-Altman plots showed no evidence of heteroscedasticity between transabdominal and transcervical AF concentrations of these markers.

Conclusions—There was a strong correlation between the concentrations of proteins in AF collected by amniocentesis or with the transcervical device.

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Keywords

premature rupture of membranes; transcervical amniotic fluid collector; α -fetoprotein; β - human chorionic gonadotrophin; prolactin

Introduction

Rupture of the membranes (ROM) occurs spontaneously during the course of labor [76]. Prelabor rupture of membranes (PROM) is defined as chorioamniorrhesis prior to the onset of labor and complicates 10% of all pregnancies [1,10,11,14,19,22,25,32,33,38,51,52,70,72,73,82,84,95,99]; in 2% of the cases, it occurs in preterm gestations [4,9,17,29,31,37,54,59,63,64,74–77,89,100–102]. Preterm PROM accounts for 30–35% of all preterm births [4,29,94].

Amniotic fluid analysis is used to assess the likelihood of intra-amniotic infection, intra-amniotic inflammation [3,6,8,12,13,15,18,23,24,34,35,40,42–46,48,50,53,56–58,60–62,66–69,75,78–81,83,86,90,96,98,103,106] and fetal lung maturity [7,16,21,26,27,30,36,39,41,55,83,85,88,91–93,97] in preterm PROM. Amniotic fluid can be retrieved with amniocentesis; however, this procedure is technically demanding in the context of rupture of membranes due to the decreased volume of amniotic fluid frequently observed in these cases [5,71,102].

We report herein a novel device designed for transcervical collection of amniotic fluid. The purpose of this article is to describe the device and to report the concentration of proteins in amniotic fluid collected transabdominally, as well as that obtained transcervically with the use of the device. For this, we measured Alpha-fetoprotein (AFP), beta-human chorionic gonadotropin (β -hCG), and prolactin, which are known to be present in high concentrations in AF [20,47,49,65,87].

Materials and Methods

Study design

Eleven women with singleton pregnancies admitted to the Seoul National University Hospital, Seoul National University Bundang Hospital, or Dongguk University Hospital, Korea were included in the study. All patients had the diagnosis of preterm PROM ($\leq$ 35 weeks of gestation). Amniotic fluid was collected using the new medical device described herein. Transabdominal amniocentesis was also performed to examine the presence or absence of intra-amniotic infection and/or inflammation and is part of the standard of practice in our institution.

The retrieval of amniotic fluid was performed after obtaining written informed consent from the patients, and the Institutional Review Boards of the Seoul National University Hospital, Seoul National University Bundang Hospital, and Dongguk University Hospital approved the collection and the use of these samples and information for research purposes.

Collection of amniotic fluid

Transcervical amniotic fluid collection was performed with a device developed by the authors and patented (patent number: Korea 10-1170053-0000***). The transcervical amniotic fluid collector was placed against the cervix during a sterile speculum exam. AF which leaked through the cervix was collected into the transcervical AF collector (Figure 1). Transabdominal AF was obtained after transcervical AF collection by amniocentesis under ultrasonographic guidance as previously described [103–105]. AF was centrifuged for 10 minutes, and stored in polypropylene tubes at -70°C until assayed.

Amniotic Fluid analysis

Amniotic fluid was assayed for AFP, β -hCG, and prolactin by immunoradiometric assays (IRMA) with commercially-available kits (AFP: Immunotech, Prague, Czech Republic; β -hCG: Shinjin, Seoul, Korea; Prolactin: Shinjin, Seoul, Korea).

Statistical analysis

The concentrations of amniotic fluid proteins in samples retrieved either by transabdominal amniocentesis or using the transcervical amniotic fluid collector were compared using Spearman's correlation and Passing and Bablok regression analysis. Bland-Altman plots were used to evaluate the differences between protein concentrations obtained from transabdominal amniotic fluid and those retrieved transcervically. We used SPSS, version 20.0 for Windows (SPSS Inc, Chicago, IL, USA) and MedCalc version 12.7.1.0 (MedCalc Software, Mariahherke, Belgium). A p-value <0.05 was considered significant.

Results

The clinical characteristics of patients enrolled in the study are summarized in Table 1. None of the patients developed clinical chorioamnionitis.

Figure 2 shows the distribution of protein concentrations in fluid collected either by transabdominal amniocentesis or the transcervical amniotic fluid collector. The transcervical amniotic fluid collector was effective in collecting amniotic fluid from all patients. There was a significant correlation between the concentrations of analytes in transabdominal and transcervical AF (Spearman correlation coefficients: $r=0.936$, $p<0.001$ for AFP; $r=0.964$, $p<0.001$ for β -hCG; $r=0.718$, $p<0.05$ for prolactin; Figure 3 and Table 2). Bland-Altman plots showed no evidence of heteroscedasticity between transabdominal and transcervical AF concentrations of these markers (Figure 4). Passing-Bablok regression analysis revealed neither constant distance (with intercept including 0 for all of proteins) nor proportional difference (with slope including 1) for all three proteins (Table 2).

Discussion

Principal findings of the study

1) We describe a new device for the transcervical collection of amniotic fluid from patients with ruptured membranes; and 2) a strong and significant relationship was observed between

the concentration of proteins in amniotic fluid obtained by the transabdominal and transcervical method.

Amniotic fluid analysis in the assessment of patients with ruptured membranes

Amniotic fluid analysis has been used to study fetal lung maturity [7,16,21,27,28,30,55,85,88,91,93], karyotype, intra-amniotic inflammation, microorganisms (bacteria and viruses) [3,6,8,12,13,15,18,23,24,34,35,40,42–46,48,50,53,56–58,60–62,66–69,75,78–81,83,86,90,96,98,103,106] in patients with preterm prelabor rupture of membranes. Fluid obtained from the vaginal pool has been employed to assess fetal lung maturity [7,16,21,26–28,30,36,39,41,55,83,85,88,91–93,97]. However, this fluid cannot be used for the assessment of microbial invasion of the amniotic cavity or the assessment intra-amniotic inflammation, because bacteria are normally present in the vagina, and vaginal fluid contains a high concentration of inflammatory mediators [15,42,45,48,56–58,90,96]. Therefore, we devised a method to obtain amniotic fluid noninvasively, by collecting this biological material directly through the cervix before it reached the vaginal canal. Ultrasound images demonstrate that this fluid is present in the cervix of patients who have ruptured membranes, and can be seen with color Doppler in the endocervical canal [2]. Obtaining amniotic fluid noninvasively can allow assessment of patients with severe oligohydramnios in which amniocentesis is not possible. Moreover, it allows serial evaluation of patients with preterm PROM without repeat amniocentesis.

Collecting of amniotic fluid through transcervical amniotic fluid collector

We collected amniotic fluid using a transcervical amniotic fluid collector designed for this specific purpose. This device has the following features: 1) it allows collection at the level of the external cervical os, and thereby reduces the likelihood of dilution of amniotic fluid with vaginal discharge; 2) it prevents leakage of amniotic fluid into the vagina, and therefore, fluid can be obtained even in patients with scant vaginal pooling; and 3) placement of the device for a short period of time (60 minutes) allowed collection of enough fluid for analysis. Patients did not report discomfort during collection of fluid with this device (Figure 1). Importantly, there was an excellent correlation between the concentration of a set of analytes in fluid retrieved by transabdominal amniocentesis and the use of the transcervical amniotic fluid collector, suggesting that this method may be useful in assessing the composition of amniotic fluid in patients with preterm PROM. Future studies are required to determine if this material can be used to assess the likelihood of intra-amniotic inflammation and fetal lung maturity.

This was a feasibility study; therefore, the sample size was small. We measured AFP, PRL, and hCG, all of which are known to be present in high concentrations in amniotic fluid. It would be important to assess other components of amniotic fluid, including “omics” characterization (proteomics, metabolomics, lipidomics, etc.).

Conclusion

We describe a new device for the collection of amniotic fluid from patients with ruptured membranes. The transcervical amniotic fluid collector might be useful in the evaluation of amniotic fluid in patients with ruptured membranes.

Acknowledgments

This research was supported, in part, by the Perinatology Research Branch, Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services (NICHD/NIH); and, in part, with Federal funds from NICHD, NIH under Contract No. HHSN275201300006C and by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (No. HI12C0768).

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Figure 1.

(A) Transcervical amniotic fluid collector (B) Schematic drawing of transcervical amniotic fluid collector placed in vagina

Figure 2.

Protein concentrations in transabdominal amniotic fluid and those in transcervical amniotic fluid, with means and 95% confidence interval presented in solid line. (A) Alphafetoprotein (AFP) (IU/mL) (B) β -human chorionic gonadotrophin (β -hCG) (mIU/mL) (C) Prolactin (ng/mL)

Figure 3.

Scatter plot of correlation between protein concentrations in transabdominal amniotic fluid and those in transcervical amniotic fluid. (A) Alphafetoprotein (AFP) (IU/mL) (B) β -human chorionic gonadotrophin (β -hCG) (mIU/mL) (C) Prolactin (ng/mL)

Figure 4.

Bland-Altman plots of protein concentrations in transabdominal amniotic fluid and those in transcervical amniotic fluid (A) Alpha-fetoprotein (AFP) (IU/mL) (B) β-human chorionic gonadotrophin (β-hCG) (mIU/mL) (C) Prolactin (ng/mL)

Table 1

Clinical characteristics of study population

	N=11
Age [†]	33 (29–36)
Nulliparity	5 (46%)
Gestational age at amniocentesis [†]	30.1 (27.3–34.1)
Positive AF culture	2 (18%)
Intraamniotic infection and/or inflammation	3 (27%)
Gestational age at delivery [†]	32.6 (27.4–34.4)
Cesarean delivery	2 (18%)
Birthweight [†]	1956 (1080–2800)
Histologic chorioamnionitis	6/10 (60%)
Funisitis	3/10 (30%)

AF, amniotic fluid

[†] median, range

Table 2

The protein concentrations in transabdominal amniotic fluid and those in transcervical amniotic fluid

Concentrations [mean (SD)]	Alpha fetoprotein (IU/mL)		β -hCG (mIU/mL)		Prolactin (ng/mL)	
	Transabdominal AF	Transcervical AF	Transabdominal AF	Transcervical AF	Transabdominal AF	Transcervical AF
	2006 (1771)	1997 (1575)	1190 (1122)	1322 (1220)	784 (380)	829 (592)
Wilcoxon signed rank test (p value)	0.93		0.82		1.00	
Spearman correlation coefficients (p value)	0.94 (p<0.001)		0.96 (p<0.001)		0.72 (p<0.05)	
Passing-Bablok regression analysis						
Intercept [95% CI]	176.9 [−415.2 to 629.9]		75.6 [−989.7 to 260.7]		−48.5 [−989.9 to 372.7]	
Slope [95% CI]	0.86 [0.62 to 1.46]		0.89 [0.67 to 2.50]		1.05 [0.39 to 2.26]	

SD: standard deviation; AF: amniotic fluid
AFP: alpha fetoprotein, β hCG: human Chorionic Gonadotropin