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USING 16S rRNA-SPECIFIC PCR ON PREOPERATIVE AMNIOTIC FLUID SAMPLES TO PREDICT SUCCESS OF EMERGENCY CERCLAGE PLACEMENT

ACIL SERKLAJ BAŞARISINI ÖNGÖRMEK IÇIN PREOPERATIF AMNIYOTIK SIVI ÖRNEKLERINDE 16S rRNA-SPESIFIK PCR KULLANIMI

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ABSTRACT

Introduction: The success of emergency cerclage in the presence of incompetent cervix and protruding fetal membrane is a matter of debate. Theories suggest that the microbiome may play an important role in influencing the structural integrity of the cervix. Hence, understanding the microbiome status before cerclage operation may have value in predicting the success rate of the procedure. This study aimed to examine the existence of microbial organisms in amniotic fluid through polymerase chain reaction (PCR) detection of 16S rRNA species and assess its correlation with perinatal outcomes in mid-trimester emergency cerclage cases.

Methods: Nineteen patients were scheduled for amniodrainage and emergency cerclage due to cervical insufficiency and they formed the cerclage group. The control group was formed by 56 patients who were seeking consultation for routine control and underwent karyotyping and physical examination at our clinic.

Results: In the cerclage group, PCR results were negative in all control group patients, whereas nine patients tested positive for PCR ($p<0.001$). Chorioamnionitis was observed in five PCR-positive patients but not in PCR-negative patients ($p<0.001$), and three newborns had sepsis in PCR-positive patients, while none were observed in PCR-negative patients ($p=0.01$). Neonatal mortality was significantly higher in PCR-positive patients compared to PCR-negative patients ($p=0.011$), with all infants born to PCR-positive patients succumbing. Conversely, 60% of the infants of PCR-negative patients in the cerclage group were discharged in good health.

Conclusions: In this study, the presence of a potential link between the microbiome status and the success of emergency cerclage procedures was underlined.

Keywords: Cervical insufficiency, emergency cerclage, perinatal outcome, polymerase chain reaction, prolapsed amniotic membranes.

ÖZET

Giriş: Servikal yetmezlik ve prolabe amniyon membranı varlığında acil serklajın başarısı tartışma konusudur. Teoriler, mikrobiyomun serviksin yapısal bütünlüğünü etkilemede önemli bir rol oynayabileceğini öne sürmektedir. Bu nedenle, serklaj operasyonundan önce mikrobiyom durumunun anlaşılması, prosedürün başarı oranını tahmin etmede değerli olabilir. Bu çalışma, 16S rRNA türlerinin polimeraz zincir reaksiyonu (PCR) tespiti yoluyla amniyon sıvısında mikrobiyal organizmaların varlığını incelemeyi ve orta trimester acil serklaj vakalarında perinatal sonuçlarla korelasyonunu değerlendirmeyi amaçlamaktadır.

Yöntemler: Servikal yetmezlik nedeniyle amniodrenaj ve acil serklaj planlanan 19 hasta serklaj grubunu oluşturdu. Kontrol grubu, rutinde yapılan perinatal testlerde risk saptanması üzerine amniyosentez yapılması planlanan 56 hastadan oluşturuldu.

Bulgular: Tüm kontrol hastalarında PCR negatifti, serklaj grubundaki dokuz hastanın ise PCR sonucu pozitif idi ($p<0,001$). PCR-pozitif 5 hastada koryoamniyonit tespit edilirken PCR-negatif hastalarda tespit edilmedi ($p<0,001$). PCR-pozitif hastalar arasında üç yenidoğanda sepsis gelişti, ancak PCR-negatif hastaların yenidoğanlarında gelişmedi ($p=0,01$). PCR-pozitif hastalarda neonatal mortalite PCR-negatif hastalara kıyasla önemli ölçüde daha yüksekti ($p=0,011$), PCR-pozitif hastalardan doğan tüm bebekler kaybedildi. Serklaj grubundaki PCR-negatif hastaların %60'ının bebekleri ise sağlıklı olarak taburcu edilmiştir.

Sonuç: Bu çalışmada, mikrobiyom durumu ile acil serklaj prosedürlerinin başarısı arasında olası bir bağlantının varlığı vurgulanmıştır.

Anahtar Kelimeler: Servikal yetmezlik, acil serklaj, perinatal sonuçlar, polimeraz zincir reaksiyonu, prolabe amniyon membranı.

INTRODUCTION

Two cases per 1000 births are complicated by amniotic membrane prolapse. The main cause of amniotic membrane prolapse is cervical insufficiency (1). Hassan et al reported that 9% of asymptomatic women with a shortened cervix have microbiologically proven intraamniotic infection, suggesting that these infections may precede the development of acute cervical insufficiency with bulging membranes (2). The clinical value of cerclage has been the subject of several studies (3-5). In addition to dilatation and effacement of the cervix, the fetal membranes are bulging into the vagina, making imminent delivery or cerclage failure more likely. Although the incidence of infection-related complications may be high with emergency cerclage (EC),

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pregnancy can be prolonged in most cases of cervical insufficiency and may improve perinatal outcomes (6,7).

Investigators have shown that many of the infection markers increase in the amniotic fluid (AF) when pregnancies are complicated by preterm delivery (8,9). However, only ~45% of pregnancies with elevated interleukin (IL-6, IL-1α, IL-1β, prostaglandin-E2, tumor necrosis factor (TNF)-α) concentrations have microbial infections in AF cultures (10,11). It is therefore possible that a higher percentage of AF samples may have microbial infection but the infection cannot be detected due to limitations in standard culturing techniques (3). Of the 116 patients, 87 (63%) were classified as infected and 52 (37%) as uninfected. In the infection group, 13 cultures were negative but PCR-positive (12). Mays et al reported that evaluation of AF for infection markers before EC placement may identify patients with subclinical chorioamnionitis who would not benefit from cerclage (13). However, assessment of the levels of these infection markers in AF is time-consuming and costly process creating a need for a simple and quick test i.g., PCR.

PCR allows for the detection of trace amounts of deoxyribonucleic acids. Bacterial DNA sequences e.g., those coding for 16S rRNA, which are not found in mammalian cells are rational targets for the PCR and could potentially be used to detect microbial invasion of the amniotic cavity in women with cervical dilatation and amniotic membrane prolapse. To date, it remains unclear whether it is possible to detect bacterial 16S rRNA in AF samples collected via amniodrainage before EC in improving obstetric and perinatal outcomes for decision-making regarding EC. The purpose of this study was to test the feasibility of using the 16S rRNA PCR to detect bacteria in AF samples and compare perinatal and obstetric outcomes in women undergoing EC for amniotic membrane prolapse.

METHODS

This study was approved by the Kocaeli University Faculty of Medicine Human Ethics Committee (KAEK 2011/160), financed by the Scientific Research Unit (BAP), and was designed and carried out by the Declaration of Helsinki. All subjects signed an informed consent. 138 patients who applied to the Kocaeli University Faculty of Medicine's Department of Gynecology and Obstetrics with a diagnosis of cervical insufficiency between 2010 and 2012 were included in the study. Nineteen patients with prolapsed amniotic membranes from the cervix to a more distal position, beyond the urethral entrance of the bladder were included in the study as the cerclage group (Figure 1). Emergency cerclage was applied between 14+0 and 28+0 weeks of gestation. Informed consent was obtained after providing detailed information to the patients about the pros and cons of amniodrainage and EC. The inclusion criteria for the cerclage group were: 1. Presence of a live intrauterine fetus without detected anomalies, 2. Advanced cervical change and herniated amniotic membranes, are defined as protrusion and prolapse from the cervix to a more distal position than the urethral entrance of the bladder (Figure 1). The exclusion criteria for the cerclage group were: 1. Placental ablation, 2. Vaginal bleeding, 3. Uterine contractions, 4. Amniorrhexis with positive amnisure test results (AmniSure® Qiagen N.V.), 5. Multiple pregnancies, 6. Fetuses with growth retardation, and 7. Clinical findings such as fever and/or the presence of infection markers suggestive of chorioamnionitis.

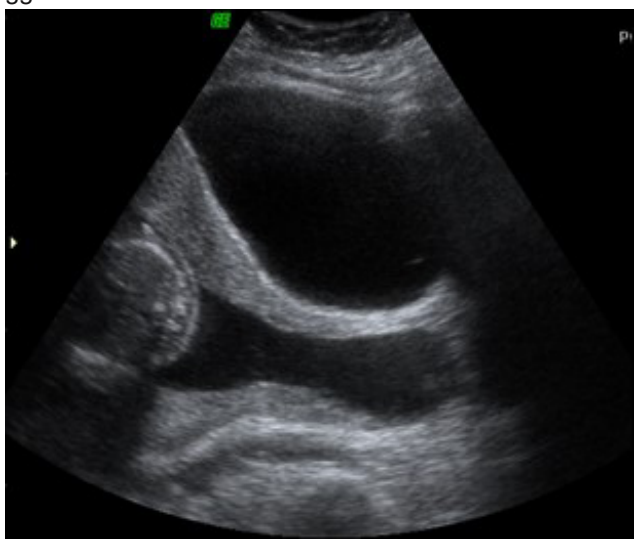

Figure 1. Transabdominal ultrasonography shows that herniated amniotic membranes, defined as advanced cervical change and protrusion, protrude from the cervix more distally than the urethra entrance of the bladder.

Fifty-six women with a high risk for chromosomal abnormalities in a first-trimester screening test and who required amniocentesis between 16-18 gestational weeks were selected as the control group. This group had four times more patients with no signs of infection and inflammation and allowed assessment of highly reliable negative predictive value for the 16S rRNA PCR approach. The diagnosis of advanced cervical change and protrusion of the amniotic membranes was confirmed by speculum and sterile digital examinations and ultrasonography (Figures 1, 2a). Uterine activity was monitored using conventional tocography and vital signs were evaluated. Betamethasone was administered to patients in the cerclage group if the gestational age exceeded 24 weeks. Cervicovaginal, urine, blood, and AF cultures, along with blood samples were collected. Prophylactic tocolysis with indomethacin suppositories was used for all patients selected for cerclage. Calcium channel blockers were preferred in cases where indomethacin could not be administered. Despite the absence of clinical signs of infection in any of the patients in the cerclage group, intravenous antibiotics (1g ampicillin, 2x1, and 500mg aminoglycoside, 2x1) were administered before the cerclage procedure and continued for up to one

week, even if the culture results were negative. Before undergoing EC all patients in the cerclage group underwent amniodrainage. 1–2 cc of AF was sent for PCR evaluation of 16S rRNA.

The PCR results from cultures were not available at the time of EC placement because the collected AF samples were stored at -80oC for later analysis. The PCR and culture results were evaluated afterward to assess any potential association between microbial infection and the need for EC. In cases requiring prompt intervention, patients were monitored for six hours before cerclage placement to ensure that cervical dilatation was not caused by active labor, placental abruption, or clinical signs of infection. The intervention was performed immediately after the necessary preparations for anesthesia were completed.

At the time of amniocentesis or amniodrainage, to prevent skin flora contamination, the skin was cleansed using an antiseptic solution. With an amniocentesis needle guided by an ultrasound device (Voluson®Dawei, PRC), 2 cc of AF was aspirated and immediately discarded to mitigate the risk of contamination. For the control group, 20 cc of AF was collected and sent to the genetics laboratory, while 1–2 cc of AF was sent to the PCR laboratory and stored frozen at -80°C until use (n=56).

In the cerclage group (n=19), varying amounts of fluid, ranging from 110 to 230 cc were extracted through amniodrainage. Following amniodrainage, each patient in the cerclage group received one gram of ampicillin intraamniotically. Sterile aliquots of AF were collected into samples for the BacT/Alert blood culture system (Bactec®Becton Dickinson, USA), Gram staining, and AF culture analysis. Additionally, sterile 1–2ml samples of AF were frozen at -80°C for subsequent PCR analysis. After completion of these procedures, patients underwent EC in the operating room (Figure 2b).

The protruding membranes were gently guided back into the cavity under general anesthesia and in the Trendelenburg position, using sterile moist gauze with gentle pressure. Emergency cerclage was then performed using the McDonald procedure with 5-mm Mersilene tape (Mersilene®Ethicon, USA). A second cerclage suture was placed at the distal end of the Mersilene tape using 1.0 Vicryl (Figure 2c). Postoperative tocolysis was continued for 48 hours. If no complications arose within 72 hours after surgery, patients were discharged with instructions for bed rest, avoiding strenuous activity, and refraining from sexual intercourse.

AF samples stored at -80°C were thawed on ice and centrifuged at 1500 × g for 10 minutes at 4°C. PCR was performed with a long PCR enzyme mix (Fermentas, USA). The sense and antisense primers used were 5'-TGGCTCAGATTGAACGCTGGCGGC and 5'-TACCTTGTTACGACTTCACCCCA, respectively. A 25 µL PCR reaction mixture consisted of 1 ×PCR buffer, 0.2 mM of each dNTP, 0.5 µM of each primer, 1.25 mM MgCl2, 1.5 units of PCR enzyme mix, and 2 µL of AF. An initial 5-minute denaturation at 94°C was followed by 35 cycles of 30 seconds denaturation at 94°C, 1-minute annealing at 57°C, and 1.5 minutes elongation at 72°C. PCR reactions were ended with a 10-minute final elongation at 72°C. PCR products were analyzed by agarose gel electrophoresis, cleaned with a PCR purification kit (Qiagen, USA) and sequenced (Iontek Inc., Istanbul, Turkey).

All statistical analyses were performed using IBM SPSS for Windows version 20.0 (IBM Corp., Armonk, NY, USA). Shapiro-Wilk's test was used to assess the normality assumption. Continuous variables were presented with mean±standard deviation or median (IQR: Interquartile range). Categorical variables were summarized as counts and percentages. Comparisons between groups were carried out using the Mann-Whitney U test. The association between two categorical variables was examined using the Chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS

All control patients tested negative for PCR. PCR results were positive in 9 cases and negative in 10 cases in the cerclage group (p<0.001). The demographic and obstetric data for the cerclage group were compared between PCR-positive and PCR-negative patients (Table 1). In PCR-positive patients, the parity rate and the rate of having live children were lower (p< 0.05).

Table 1. Demographic and obstetric data of the cerclage group between PCR-positive and PCR-negative patients.

Cerclage group (n = 19)	PCR-positive (n = 9)	PCR-negative (n = 10)	p value*
Age (years), mean ±SD	29.6 ± 5.5	30.7 ± 5.4	0.48
BMI† (kg/m²), mean ±SD	26.5 ± 5.5	25.1 ± 3.0	0.09
Smoking, n	2	1	N/A
Gravida, mean ±SD	1.8 ± 1.1	2.4 ± 1.7	0.16
Parity, mean ±SD	0.42 ± 0.9	1.0 ± 1.1	0.03
Miscarriage, mean ±SD	0.37 ± 0.76	0.27 ± 0.67	0.58
Live healthy child, mean ±SD	0.16 ± 0.5	1.0 ± 1.0	< 0.0001
Preterm birth history, n	2	0	N/A
Cerclage at previous pregnancy, n	1	0	N/A
History of intrauterine fetal death, n	1	0	N/A
Infertility treatment at current pregnancy, n	2	1	N/A
Iron supplement, n	5	8	0.350
Multivitamins supplement, n	5	9	0.141
Threatened miscarriage, n	5	2	0.170

†: BMI: Body mass index; *p< 0,05 significant; N/A: not available.

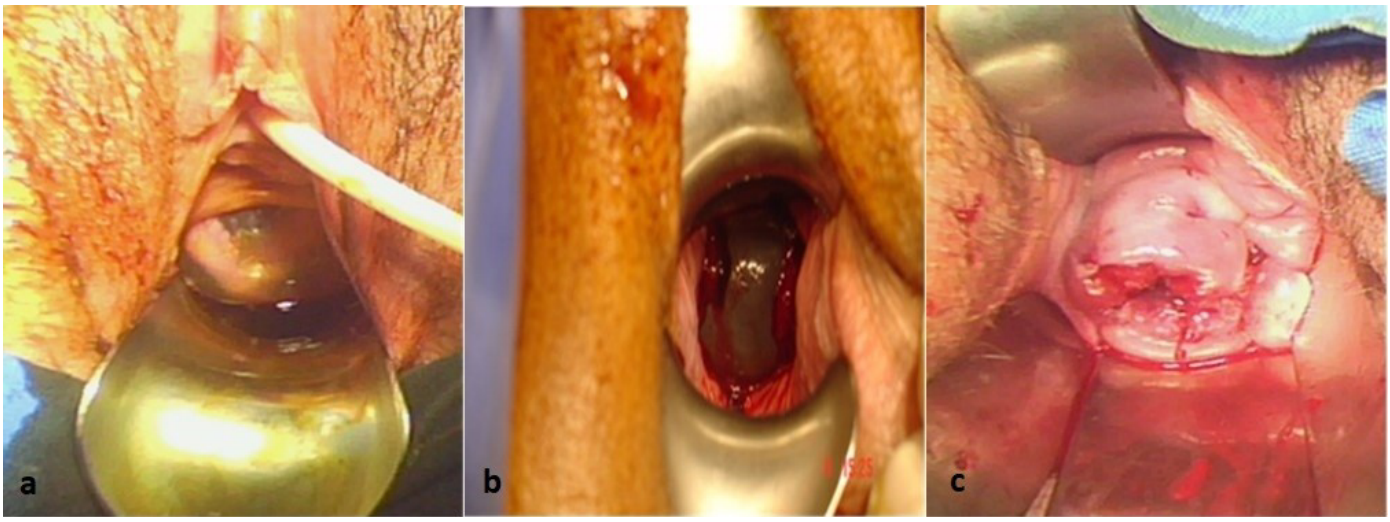

Figure 2. a) During examination with a speculum, the "hourglass-shaped" amniotic membrane is observed to be intact and tense before the amniodrainage process. The left foot of the fetus is observed just behind the highly protruded amniotic membrane, at the level of the hymen.
b) In the pelvic examination of the same patient, it is observed that the tension of the amniotic membrane decreases and shifts proximally after amniodrainage. c) In the same patient, the cervix is observed after emergency cerclage.

Table 2. Perinatal outcome in PCR-positive and PCR-negative patients in the cerclage group.

Cerclage group (n = 19)		**PCR-positive (n= 9)**	**PCR-negative (n= 10)**	**p value***
Positive PCR, n		9	0	< 0.001
Gestation time at cerclage (day), mean ±SD		140 ± 15	143 ± 21	0.78
Mode of Delivery, n	**Vaginal birth**	2	6	N/A
	Cesarean (C/S)	1	4	
	Abortion	6	0	
Chorioamnionitis†, n (%)		5 (26.3%)	0	= 0.001
< 7 1. minute Apgar score, n		8	9	N/A
< 7 5. minute Apgar score, n		8	6	0.303
Birth weight (g) ‡, mean ±SD		383.8 ± 193.6	1245.6 ± 981.5	0.022
NICU§ necessity, n		2	8	0.023
Lenght of NICU§ stay (day), median (IQR)		0 (0 - 1)	7.5 (0.75 - 50.25)	0.010
Newborn with sepsis, n		3	0	= 0.01
Neonatal mortality‖, n		9	4	0.011

†: Patients were diagnosed with chorioamnionitis with the presence of two or more of these fever (> 37.5 ˚C), uterine sensitivity, abdominal pain, malodorous vaginal discharge, maternal and/or fetal tachycardia, and leukocytosis (> 15,000). ‡: grams. §: NICU: Neonatal intensive care unit. ‖: Live-born neonates who died postpartum. *p< 0,05 significant. N/A: not available.

Perinatal outcomes were compared between PCR-positive and PCR-negative patients in the cerclage group (Table 2). The gestational ages of PCR-positive and PCR-negative patients at the time of cerclage were not statistically significant (p>0.05), suggesting that there was no significant difference in the gestational ages between the two groups at the time of cerclage. Five cases of chorioamnionitis were detected in PCR-positive patients but not in PCR-negative patients, and this difference was statistically significant (p<0.001). The mean birth weight of all live or stillborn infants was statistically significant (p=0.022). However, it was not determined whether this difference was between PCR-positive and PCR-negative patients or within each group. In PCR-positive patients, three newborns developed sepsis, and this difference was statistically significant (p=0.01). Neonatal mortality in PCR-positive patients was significantly higher than in PCR-negative patients (p=0.011). Overall, these findings suggested that PCR-positive patients in the cerclage group had a higher incidence rate of chorioamnionitis, neonatal sepsis, and neonatal mortality compared to PCR-negative patients. In addition, there were no significant differences in gestational ages at cerclage placement, fifth-minute APGAR scores, or the necessity and length of neonatal intensive care unit (NICU) stay between the two groups.

The clinical data and prognosis of PCR-positive and PCR-negative patients in the cerclage group were compared in Table 3. The presence of postoperative uterine contractions in PCR-positive patients was found to be significantly higher than in the PCR-negative patients (p<0.01). No significant difference was detected in white blood cell (WBC) count, C-reactive protein (CRP) value, and postoperative fever (p>0.05). Infants of all PCR-positive patients died while six out of 10 patients with negative PCR results (60%) were discharged from the NICU as healthy (p<0.008). Compared

Table 3. Evaluation of clinical data and prognosis of PCR-positive and PCR-negative patients in the cerclage group.

Cerclage group (n = 19)	PCR-positive (n = 9)	PCR-negative (n = 10)	p value*
Fever (> 37.5 °C)‡, n (%)	2 (22%)	0	0.21
The presence of uterine contraction[b], n (%)	8 (88%)	3 (30%)	< 0.01
CRP (> 1) elevation†,‡, n (%)	8 (88%)	8 (80%)	0.81
Leukocytosis (> 15.000)‡, n (%)	3 (33%)	3 (30%)	0.63
Cerclage success (More than a week), n (%)	6 (66%)	7 (70%)	0.63
The take-home baby alive, n (%)	0	6 (60%)	< 0.008
Gestational age at birth (day), mean ±SD	148 ± 16	189 ± 47	< 0.02
The mean extended gestation period (day), mean ±SD (the shortest – longest number of extended days, n)	8 ± 9.1 (1 - 28)	45 ± 53.3 (2 - 142)	= 0.13

*p< 0,05 significant, †: normal CRP level: 0 - 0.05 mg/dl; CRP level in advanced inflammation: > 1 mg/dl; ‡: Presence of fever, CRP elevation, leukocytosis, uterine contractions starting within the first 48 hours postoperatively.

to PCR-negative patients, the gestational age at birth was significantly lower in PCR-positive patients.

The organisms detected by PCR were Enterococcus faecalis (n=2), Streptococcus agalactiae GY102 (n=2), Klebsiella HaNA22 (n=2), Bacterium NLAE-zl-H51 (n=1), Staphylococcus spp clone JPL-53 (n=1), Escherichia fergusonii ATCC 35469 (n=1), and Escherichia sp. ASG34 (n=1). Only one patient was positive for Klebsiella pneumoniae when an AF culture was performed, which was concordant with the bacteria detected by PCR. The AF Gram stain was also positive for this culture. In another patient, whose AF PCR was also positive for Klebsiella, the organism was detected only in urine and cervicovaginal cultures, but not in blood and AF cultures.

DISCUSSION

Due to the heterogeneous nature of this disorder, there are contradictory reports in the literature. Patients with different stages of cervical dilatation, various etiologies, and different management strategies were examined, within the same study (4,5,14-16). To avoid these limitations, only patients with cervical insufficiency (dilated to at least 6 cm) accompanied by amniotic membrane protrusion were included in this study. The amniotic membranes of these patients protruded from the cervix to a position distal to the urethral entrance of the bladder. These patients showed no clinical signs of inflammation, and their vital signs were stable before cerclage. Many studies have emphasized that the success of cerclage is higher when the appropriate patient is selected (5,17). The results of this study demonstrated that PCR amplification and subsequent sequencing of the 16S rRNA species from AF had a strong predictive value for the success of cerclage. In a similar study by Satılmış et al.,16S rRNA PCR was shown to be effective for the diagnosis of sterile body site infections, especially in cases of meningitis and infective endocarditis where routine cultures fail (12).

The primary objective of employing EC for managing amniotic sac protrusion during the second trimester is to extend the duration of the pregnancy as much as possible (14). We assessed the efficacy of EC based on the prolonged duration of pregnancy post-cerclage, perinatal mortality rates, and birth weight as primary outcomes. A systematic review conducted by Ehsanipoor et al. compared the effectiveness of cervical cerclage in second-trimester pregnancies with cervical dilatation and membrane prolapse identified by physical examination. The researchers found a significant improvement in neonatal survival rates (71% compared to 43%; RR 1.65, 95% CI 1.19-2.28) and a prolongation in the gestational period (mean difference 33.98 days, 95% CI 17.88-50.08) (18).

In cases of protruding amniotic sac, the prognosis for pregnancy is often reported to be poor (7,14). During the placement of EC, the goal of amnioreduction is to relieve the pressure on the prolapsed amniotic membranes, thereby facilitating the cerclage procedure and reducing the risk of accidental membrane rupture. This approach also helps decrease the rates of prematurity and related neonatal morbidity (Figure 2b) (19). Depending on the patient, 110-230 cc AF is removed for this purpose. After the amniocentesis performed in this study, during 16-18 weeks of gestation, the AF should be replaced within 14-30 hours, as it typically takes about 3-4 hours to replace 20-25 cc of fluid. Therefore, during follow-up examinations, AF was replaced within 14-30 hours, and no permanent oligohydramnios was observed in our patients. Genetic amniocentesis for karyotyping in pregnant women is widely recognized as reliable. Consequently, the potential adverse effects of amniocentesis on maternal and fetal outcomes are considered negligible.

In a study, pleura, cerebrospinal fluid, peritoneal fluid, and synovial fluid were cultured using the BacT/Alert blood culture system. Reproduction was detected in 18 (95%) of the samples, while only 11 (58%) were detected using the classical culture method (20). This finding led us to use the BacT/Alert blood culture system in our experiments for isolating microorganisms from AF, while routine culture procedures were employed for culturing urine, blood, and cervicovaginal samples.

Despite the existing scientific consensus suggesting challenges in treating intra-amniotic infection or

inflammation in the presence of cervical insufficiency (10), successful treatment of both intra-amniotic infection and inflammation with antimicrobial agents is still possible (10,14,19,21). The use of indomethacin in the treatment of protruded amniotic membranes should be limited to 48 hours to allow time for corticosteroid therapy while minimizing neonatal complications (22). It has been noted that the use of prophylactic tocolytics suppresses uterine contractions and reduces intrauterine pressure, thereby preventing the protrusion of the amniotic membrane. Indomethacin reduces AF production in addition to its anti-inflammatory effects. With cerclage, the inflammatory-like process responsible for the initiation of contraction is also reduced (17). In this study, rectal indomethacin was administered for 48 hours as part of prophylactic tocolysis.

It has been demonstrated that applying double cerclage with two separate sutures to the cervix can improve the success rate, particularly in cases involving amniotic membrane protrusion (1). Among various cerclage techniques with similar success rates (14), the McDonald procedure was chosen due to its ease of implementation, especially in emergencies. To further enhance the success of the cerclage, a specific modification was employed, which involved the use of 1.0 Vicryl suture material placed more distally as the second suture following the initial placement of Mersilen tape. This modification was likely intended to provide additional reinforcement and support to the cervix, thereby improving the effectiveness of the cerclage procedure in preventing premature cervical dilation and its associated complications.

The potential contribution of infection in cervical insufficiency during the second trimester has been suggested, highlighting the importance of screening for infection before cerclage placement as a way to predict prognosis (17). Various preoperative procedures, such as cervicovaginal cultures, urine and blood cultures, Gram staining, and evaluation of inflammatory status in the endocervix, have been proposed to identify patients who would benefit from cerclage, while also recognizing those in whom this intervention might be harmful (3).

However, these laboratory procedures can be time-consuming, and expensive, and may delay surgical intervention. Cerclage placement in emergencies for amniotic sac prolapse is not fundamentally different from prophylactic cerclage The critical distinction between EC and prophylactic cerclage lies in the timing of the procedure. To address this challenge, cervicovaginal, urine, blood, and AF cultures, along with AF Gram staining and PCR analysis, were evaluated. The goal was to identify a diagnostic method that would facilitate rapid patient selection without compromising effectiveness. Despite the uncertain timing of cerclage (23), delaying the procedure could increase the risk of infection, as protruding membranes are more exposed to vaginal bacteria (17).

Emergency cerclage is an effective procedure for reducing the rate of premature birth in patients with cervical insufficiency and amniotic membrane protrusion challenges, favorable outcomes have been reported (15). Despite the challenges, favorable outcomes have been reported. Caruso et al. conducted a study involving 23 patients, finding an average gestational prolongation of 28 days and a survival rate of 46%. The average gestational age at birth was 25 weeks, with a mean birth weight of 700 grams, which was considered a successful outcome (24). Shivani et al. reported that EC, used as a salvage measure for pregnancies at high risk of preterm delivery or mid-trimester miscarriage, extended pregnancy duration by up to 71.2 days following rescue cerclage placement (6). Similarly, Ciancimino et al. reported an average pregnancy prolongation of 89.9 days and a newborn survival rate of 83.3% after EC in 12 patients (25). In another study by Rius et al., EC in 39 patients resulted in a mean gestational prolongation of 49.1 days, with a mean gestational age at birth of 28.6 weeks and a neonatal survival rate of 82.4% (26). A literature review by Cockwell et al., spanning 10-years, suggested that EC significantly prolonged pregnancy. In 25 studies involving 638 patients who underwent EC, the average pregnancy extension was 7 weeks and 1 day, with an average neonatal survival rate exceeding 70%. These findings underscore the efficacy of EC in extending pregnancy and improving neonatal outcomes in cases of cervical insufficiency and protrusion of amniotic membranes (27).

In the study, variables such as age, BMI, gravidity, WBC, CRP values, as well as gestational age at cerclage and birth, and birth weight were not found to be statistically significant ($p>0.05$) (14). However, gestational age at birth was significantly lower in PCR-positive patients. Although most PCR-positive patients experienced miscarriage and had shorter gestational periods, statistical significance was not observed. No significant difference was found in the gestational age at the time of cerclage between PCR-positive and PCR-negative patients. However, significant differences were noted in gestational age at birth, with PCR-positive patients having a mean of 148 days and PCR-negative patients having a mean of 189 days. The mean extended gestation period was 8 days for PCR-positive patients and 45 days for PCR-negative patients. Emergency cerclage extended the gestational period by an average of 45 days in PCR-negative cases. Significant differences were observed in the rates of chorioamnionitis, neonatal sepsis, and neonatal mortality between PCR-positive and PCR-negative patients. Unfortunately, all infants born to PCR-positive patients died, while 60% of infants born to PCR-negative patients survived. A 60% live birth rate should be considered a successful outcome for mid-trimester EC in cases with protruding membranes.

CONCLUSION

Our findings underscore the complexity of identifying patients who would benefit from EC based solely on conventional clinical evaluations such as gynecological examination, genitourinary cultures, WBC count, or CRP levels. This study suggests that women undergoing EC due to amniotic membrane prolapse may harbor subclinical intra-amniotic or extra-amniotic inflammation, reflecting the advanced stages of a process where avoiding cerclage placement could potentially reduce maternal morbidity.

A significant portion of cases in this study (47.3%) were PCR-positive, indicating microbial invasion of the membranes and suggesting a poor prognosis for cerclage in patients with acute cervical insufficiency and subclinical intra-amniotic infection or inflammation (28). We propose that a combination of gynecological examination, history of previous cervical surgeries, prior preterm deliveries, and the absence of intraamniotic inflammation -confirmed by AF PCR- may help identify patients who genuinely require EC placement.

The patients in this study benefited from EC, which potentially improved perinatal outcomes. Despite the anticipated poor prognosis in some cases, proper selection based on these criteria can lead to successful results. We suggest that the success of the cerclage procedure could be predicted based on 16S rRNA analysis PCR results.

Although the number of patients in the cerclage group was limited, preventing definitive conclusions, a larger sample size of the patients who underwent EC would have yielded more statistically reliable results. Additionally, measuring the levels of inflammatory markers, such as interleukins could have provided valuable insights into the changes associated with EC and its impact on inflammation.

This study emphasizes the importance of integrating molecular diagnostic techniques, such as PCR analysis of AF for microbial infection, alongside clinical parameters to more accurately identify candidates for EC placement and ultimately improve perinatal outcomes.

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