Polymerase Chain Reaction as Screening Method for Group B Streptococcus in Third Trimester of Pregnancy

Gebeliğin Son Trimesterinde Grup B Streptokok Tarama Yöntemi Olarak Polimeraz Zincir Reaksiyonu

Ufuk YEŞİLDAĞER,[a]
Mehmet YILMAZER,[a]
Mine KANAT PEKTAŞ,[a]
Mustafa ALTINDİŞ,[b]
Evren YEŞİLDAĞER,[a]
Dağıstan Tolga ARIÖZ,[b]
Gülengül KÖKEN[b]

[a]Department of Obstetrics and Gynecology, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar
[b]Department of Microbiology, Sakarya University Faculty of Medicine, Sakarya

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Yazışma Adresi/*Correspondence:*
Mine KANAT PEKTAŞ
Afyon Kocatepe University
Faculty of Medicine,
Department of Obstetrics and Gynecology,
Afyonkarahisar,
TÜRKİYE/TURKEY
minekanat@hotmail.com

ABSTRACT Objective: The present study aims to evaluate the efficiency of a rapid real-time polymerase chain reaction (PCR) assay in order to determine maternal group B streptococcus (GBS) colonization in the third trimester of pregnancy. **Material and Methods:** This prospective study reviewed 70 healthy women with term pregnancies who were consecutively admitted to the study center for routine prenatal care from June 2012 to December 2012. **Results:** The prenatal GBS colonization rate was 15.7% by the PCR technique. Perinatal complications including abortus imminens, fetal growth restriction, premature rupture of membranes, preterm delivery, meconium stained amniotic fluid and chorioamnionitis were significantly more frequent in women with GBS positivity (respectively p=0.011, p=0.023, p=0.001, p=0.001, p=0.023 and p=0.001). Puerperal fever was significantly more frequent in women who were GBS positive (p=0.001). The need for neonatal intensive care unit and neonatal pneumonia were significantly more frequent for the neonates that were born to women with GBS positivity (p=0.001 for both). **Conclusion:** The real-time PCR assay can be defined as an accurate test to identify the GBS carriers at the third trimester of pregnancy. This easily applicable tool could enhance the exact identification of candidates for chemoprophylaxis, including women carrying a risk for premature rupture of membranes or preterm labor.

Key Words: Perinatal care; polymerase chain reaction; *streptococcus agalactiae*

ÖZET Amaç: Bu çalışma, gebeliğin son trimesterinde maternal grup B streptokok (GBS) kolonizasyonunu belirlemek amacıyla hızla sonuç veren gerçek zamanlı polimeraz zincir reaksiyonu (PCR) yöntemi etkinliğinin değerlendirilmesini amaçlamaktadır. **Gereç ve Yöntemler:** Haziran 2012 ile Aralık 2012 arasında rutin gebelik takibi için çalışma merkezine başvuran ve gebeliğinin son trimesterinde bulunan ardışık 70 sağlıklı gebe kadın ileriye dönük olarak incelenmiştir. **Bulgular:** Doğum öncesi dönemde grup B streptokok kolonizasyonu sıklığı %15,7 olarak hesaplandı. Grup B streptokok kolonizasyonu olmayan kadınlara göre, grup B streptokok kolonizasyonu olan kadınlarda abortus imminens, fetal büyüme kısıtlılığı, prematür membran rüptürü, preterm doğum, mekonyumlu amnion sıvısı, koryoamnionit ve puerperal ateş gibi perinatal komplikasyonlar anlamlı olarak daha sık bulundu (sırasıyla p=0,011, p=0,023, p=0,001, p=0,001, p=0,023, p=0,001 ve p=0,001). Grup B streptokok ile kolonize kadınların bebeklerinde yoğun bakım gereksinimi ve yenidoğan pnömonisi sıklığı, kolonize olmayanlara göre anlamlı olarak daha yüksekti (her ikisi için p=0,001). **Sonuç:** Gerçek zamanlı PCR, gebeliğin son trimesterinde grup B streptokok taşıyıcısı olan kadınları kesin olarak belirleyebilecek bir yöntemdir. Kolaylıkla uygulanabilen PCR, prematür membran rüptürü veya preterm eylem bakımından risk taşıyan ve grup B streptokok için ilaç profilaksisi gerçekleştirilebilecek olguların atlanmadan tanımlanmasını sağlayabilir.

Anahtar Kelimeler: Perinatal bakım; polimeraz zincir reaksiyonu; *streptococcus agalactiae*

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Group B streptococcus (GBS) is usually a microorganism that asymptomatically colonizes the vaginal and rectal areas of 10-30% of pregnant women. The prevalence of GBS colonization varies significantly with respect to geographic location and socioeconomic status.[1,2]

The GBS colonization in pregnant women can be associated with preterm labor, chorioamnionitis, postpartum endometritis, wound infection and sepsis. As for the infants who are born to colonized mothers, GBS colonization may occur on their mucosal surface and skin as well. Neonatal GBS colonization may lead to the GBS infection of the newborn which is a major cause of morbidity and mortality. Neonatal GBS infection occurs in two distinct clinical forms. One of these is the early-onset GBS disease which is usually acquired from the colonized maternal birth canal during labor or from the ascending spread of the organism into the amniotic fluid after the rupture of membranes. The other routes of transmission include hematogenous dissemination through the placenta, retrograde seeding from the peritoneal cavity and accidental introduction at the time of invasive procedures.[3-5]

In order to reduce the incidence of early-onset GBS infection, all of the pregnant women should be screened for GBS and intrapartum antibiotic prophylaxis should be administered to GBS-colonized mothers.[6] Therefore, since 2002, the Center of Disease Control in the United States has recommended a universal vagina-rectum GBS screening at 35-37 weeks of gestation.[7] Accordingly, since 2001, in France, a universal lower vagina culture screening has been recommended at 35-38 weeks.[8] Recently, polymerase chain reaction (PCR) has become commercially available for rapid, sensitive and specific detection of GBS during pregnancy and labor.[9,10]

The present study aims to evaluate the efficiency of a rapid real-time polymerase chain reaction (PCR) assay in order to determine maternal group B streptococcus (GBS) colonization in the third trimester of pregnancy.

MATERIAL AND METHODS

STUDY DESIGN

This prospective study was approved by the Institutional Review Board and Ethical Committee of Afyon Kocatepe University Hospital where the study was conducted. Written informed consent was obtained from each participant of the study.

The present study reviews 70 healthy women with singleton third trimester pregnancies who were consecutively admitted to the outpatient clinic of the study center for routine prenatal care, during the period from September 2012 through December 2012. The women scheduled for planned cesarean delivery, women with diabetes mellitus, women with immunological disorders, women using corticosteroids and women with a history of any kind of antibiotic use in prior 2 weeks were excluded. At the study center, routine GBS screening was performed by conventional microbiological methods between 35th and 37th weeks of pregnancy.

For every participant, fetal well-being was carefully assessed by transabdominal ultrasonography with 3.5 and 5 mHz convex probes (Voluson 730 Pro, GE Healthcare, Buckinghamshire, UK). Fetal well being is specified if one pocket of amniotic fluid measured at least 2 cm in two perpendicular planes, and one episode of fetal breathing movements continued for at least 30 seconds, at least three discrete limb movements were observed and there was at least one episode of active extension with return to flexion of fetal limbs or trunk in 30 minutes. The data related with the participants and the neonates born to them were obtained from the medical records. Fetal growth restriction is identified when estimated fetal weight is below the 10th percentile for its gestational age and abdominal circumference is below the 2.5th percentile. Preterm birth is the delivery of a baby before 37 completed weeks' gestation. The diagnosis of chorioamnionitis was made retrospectively based on histological findings in women with a high index of suspicion (maternal fever, maternal leukocytosis, maternal tachycardia, uterine tenderness and purulent, foul amniotic fluid).

MICROBIOLOGICAL ASSESSMENT

Culture samples were collected with a sterile cotton swab without using an antiseptic solution and speculum from the distal third of the vagina and rectum of

women. The same swab was first rubbed into the vaginal mucosa and then inserted approximately 2.5 cm beyond the anal sphincter and gently rotated to sample the anal crypts. Afterwards, the swabs were placed in modified Stuart medium and sent to laboratory. Culture samples were incubated at 37 °C with the addition of 5% CO_2 for 24 hours in Todd Hewitt broth media containing nalidixic acid (15 mg/mL) and gentamycin (8 mg/mL) and subcultured to a 5% sheep blood plate at 37°C. Gram-positive and catalase-negative organisms forming β-hemolytic or non-hemolytic colonies were examined for positive CAMP (Christie, Atkins and Munch-Peterson) test, resistance to bacitracin and trimethoprim-sulphamethoxazole, positive sodium hippurate hydrolysis and negative bile esculin reaction for presumptive GBS identification.

POLYMERASE CHAIN REACTION (PCR)

Another swab was used to perform PCR for the detection of GBS colonization. The real-time PCR was performed using the GeneXpert system (Cepheid), which integrates the complete process of DNA extraction, amplification, and detection in a completely automated fashion. The DNA target sequence is the 3'-adjacent region of the GBS cfb gene. A single-use cartridge holds the sample and all necessary reagents and hosts both the extraction and PCR process. Each test cartridge contains a sample processing control and an internal control. The sample processing control validates the sample nucleic acid extraction step, and the internal control monitors the PCR reaction for inhibitors. A computer collects amplification data to detect the presence or absence of the GBS target. The GBS colonization is defined as negative when the cycle threshold is 0 or 142 and the GBS colonization is defined as positive otherwise. Antenatal GBS culture results and molecular test results were read independently of each other. For discrepant results, PCR was repeated twice to confirm the initial findings.

STATISTICAL ANALYSIS

Collected data were analyzed by Statistical Package for Social Sciences version 18.0 (SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as mean±standard deviation (range: minimum-maximum) whereas categorical variables were denoted as numbers or percentages. Independent samples t-test and Pearson χ^2 test were used for comparisons. Two-tailed p values less than 0.05 were accepted to be statistically significant.

A power analysis was carried out to calculate the sample size before our study was commenced. Studies conducted in Turkey yielded GBS prevalence varying between 8% and 32% among pregnant women.[11-14] Thus, the sample size was found to differ between 58 and 170. However, the sample size was limited to 70 because only 70 PCR swabs could be obtained due to the paucity of financial support. A *post hoc* analysis was carried out to determine that a cohort size of 70 women had 53.9% power to detect a difference at the 0.05 significance level.

RESULTS

Table 1 compares the culture and PCR results for GBS screening in the study cohort. Five specimens that were tested to be negative by the conventional microbiological methods were found to be positive for GBS by the molecular technique. In order to assess these five specimens more thoroughly, the amplicon was removed from the reaction tube and run on a 1% agarose gel. A well-characterized GBS clinical isolate was run along with a negative control and a 100-base pair DNA ladder. Therefore, it was confirmed that molecular GBS test correctly identified the specimens as positive for GBS. Each pregnant woman who tested positive for GBS was treated with ampicillin. After a loading dose of 2 grams was given intravenously, maintenance treat-

TABLE 1: Comparison of culture and polymerase chain reaction results.

		Polymerase Chain Reaction		
		Positive	Negative	Total
Culture	Positive	6	0	6
	Negative	5	59	64
Total		11	59	70

TABLE 2: Sociodemographic characteristics of pregnant women.

	GBS positive (n=11)	GBS negative (n=59)	p
Age (years)	24.8±4.8	25.0±5.4	0.908
Gravidity	2.1±0.7	2.1±1.4	0.971
Parity	0.9±0.7	1.1±0.8	0.771
Marital duration (years)	3.5±2.9	4.2±0.2	0.625
Smoking	3 (27.3%)	3 (5.1%)	0.016* (χ^2=5.824)†
Intrauterine device	3 (27.3%)	9 (15.2%)	0.332 (χ^2=0.943)†

*p<0.05 was accepted to be statistically significant.

†Pearson χ^2 test was used for all comparisons.

ment was carried out by administering 1 gram ampisillin in every four hours during an interval of ten days.

Table 2 demonstrates the sociodemographic characteristics of pregnant women with respect to GBS colonization. Smoking habit was significantly more frequent in GBS positive women (p=0.016). Table 3 enlists the obstetric outcomes of the participants according to the GBS colonization. Perinatal complications including abortus imminens, fetal growth restriction, premature rupture of membranes (PROM), preterm delivery, meconium stained amniotic fluid and chorioamnionitis were significantly more frequent in GBS positive women (respectively p=0.011, p=0.023, p=0.001, p=0.001, p=0.023 and p=0.001). Moreover, puerperal fever was significantly more frequent in GBS positive women (p=0.001). Table 4 displays the clinical characteristics of neonates that were born to the women. The need for neonatal intensive care unit and the frequency of neonatal pneumonia were significantly higher for the neonates born to GBS positive mothers (p=0.001 for both). The two cases of neonatal pneumonia diagnosed in the study cohort were attested as nosocomial infections as Klebsiella pneumonia was isolated from the blood cultures of the affected newborns. No maternal, fetal or neonatal deaths occurred during the study period.

TABLE 3: Obstetric outcomes of participants.

	GBS positive (n=11)	GBS negative (n=59)	p
Gestational age at delivery (weeks)	38.2±2.1	38.7±2.2	0.496
Cesarean delivery	4 (36.4%)	14 (23.7%)	0.379 (χ^2=0.775)†
Indications for cesarean delivery			
Malpresentation	2 (18.2%)	6 (10.2%)	0.443 (χ^2=0.588)†
Fetal distress	2 (18.2%)	6 (10.2%)	0.443 (χ^2=0.588)†
Cephalopelvic disproportion	-	2 (3.4%)	0.536 (χ^2=0.384)†
Duration of hospital stay (days)	1.9±0.5	1.5±0.8	0.104
Perinatal complications			
Abortus imminens	4 (36.4%)	5 (8.5%)	0.011*(χ^2=6.436)†
Fetal growth restriction	4 (36.4%)	6 (10.2%)	0.023*(χ^2=5.195)†
Premature rupture of membranes	4 (36.4%)	2 (3.4%)	0.001*(χ^2=12.863)†
Preterm delivery	2 (18.2%)	-	0.001*(χ^2=11.043)†
Meconium stained amniotic fluid	4 (45.5%)	6 (10.2%)	0.023*(χ^2=5.195)†
Chorioamnionitis	2 (18.2%)	-	0.001*(χ^2=11.043)†
Postpartum complications			
Vaginal lacerations	3 (27.3%)	9 (15.2%)	0.332 (χ^2=0.943)†
Uterine atonia	2 (18.2%)	6 (10.2%)	0.588 (χ^2=0.443)†
Puerperal fever	2 (18.2%)	-	0.001*(χ^2=11.043)†
Wound infection	-	2 (3.4%)	0.536 (χ^2=0.384)†

*p<0.05 was accepted to be statistically significant.

†Pearson χ^2 test was used for all comparisons.

TABLE 4: Clinical characteristics of neonates born to participants.

	GBS positive (n=11)	GBS negative (n=59)	p
Newborn sex			
Male	5 (45.5%)	30 (50.8%)	0.743 (χ^2=0.108)†
Female	6 (54.5%)	29 (49.2%)	
Birthweight	2972.7 ± 449.0	3145.7 ± 586.8	0.332
First minute Apgar score	7.5±1.8	8.2±1.6	0.134
Fifth minute Apgar score	8.6±1.5	9.3±1.4	0.107
Need for neonatal intensive care unit	4 (36.4%)	1 (1.7%)	0.001*(χ^2=16.801)†
Neonatal pneumonia	2 (18.2%)	-	0.001*(χ^2=11.043)†

*p<0.05 was accepted to be statistically significant.

†Pearson χ^2 test was used for all comparisons.

DISCUSSION

Early onset GBS disease is the leading infectious cause of morbidity and mortality among infants in developed countries. Currently, intrapartum antibiotic prophylaxis is the only effective method to prevent early onset GBS disease in infancy. The main goal of the prevention strategies against GBS is the accurate identification of pregnant women who are colonized with this microorganism.[3-5] Accordingly, optimal microbiological methods should be adopted to specify the real GBS carriers during pregnancy and delivery. Although the utilization of rectovaginal culture for GBS screening has been recommended universally, routine clinical practice requires a faster and more efficient method than culture. The reason is that the effectiveness of any screening policy depends on the administration of antibiotic prophylaxis at the most convenient time.[15-19]

In order to overcome the need for a sensitive but rapid screening test, PCR-based tests have been introduced. However, all these real-time PCR require well-trained operators, specific laboratory equipment, and consequently, more expense. Besides, contradictory results regarding the sensitivity of these tests have been yielded.[20-22]

Prenatal screening for GBS is routinely performed between 35th and 37th weeks of pregnancy at the study center. Conventional microbiological techniques are normally used and the results are obtained in 48 to 72 hours. Our clinical practice shows that a considerable number of pregnant women do not get their results so that some pregnant women who are GBS positive remain untreated. Therefore, this study has been designed to determine whether PCR can be used as a rapid and efficient method for screening GBS in women with term pregnancies.

The next step towards improving PCR technology for the procurement of optimal GBS screening is to implement a system which can be easily used by any operator so that results can be obtained rapidly during any time period. The utilization of GeneXpert GBS assay within the outpatient clinics has succeeded to identify the GBS positive women accurately. A negative culture result and a positive PCR result may co-exist because PCR detects only bacterial genes, not viable bacteria colonies.[10,15]

The GeneXpert GBS assay usually gives results in less than 2 hours whereas 48 to 72 hours are required to obtain the results of culture-based screening. This may provide an advantage in emergencies (such as preterm labor or PROM) or in pregnant women who are unable to attend the routine prenatal follow up. This technique also helps to avoid losing time either in bringing the sample to the laboratory or in waiting for the interpretation of the results.[10] One disadvantage of PCR is its inability to determine antibiotic susceptibility. However, even with penicillin-resistant or rare GBS colonies,

PCR can be used to confirm the DNA sequence for the identification of GBS and, thus, make a choice of appropriate antibiotics.[9,10]

The rates of GBS isolation from the genital or lower gastrointestinal tract cultures of both pregnant and non pregnant women range from 5 to 40%. Such a variation is associated with the differences in geographic locations, sociodemographic features as well as the distinct sampling and microbiological methods used for the detection of the microorganism.[1-3] The rate of GBS carriage amongst the women with term pregnancies was reported to be 9.2%, 8.0% and 10.6% in three clinical studies that were carried out in western Turkey.[11-13] On the other hand, a rate of 32% was estimated by a study focusing on the prevalence of GBS colonization in eastern Turkey.[14] Our study reveals the prenatal GBS colonization rate as 15.7% which is consistent with the geographic location of the study center. The study center is situated in a city which may be considered as a bridge between western and eastern Turkey.

Moreover, the aforementioned studies declared that advanced age and increased parity are related with decreased rates of GBS carriage in pregnant women.[11-14] Yet, our study was unable to define any significant difference between the GBS negative and GBS positive pregnant women with respect to age, parity and contraceptive method. Such discrepancy may be attributed to the factors that limit the power of our study. These factors include the relatively small cohort size, small number of GBS positive women, and the lack of data about the cost-effectiveness of PCR assay and the colonization of newborns with GBS.

CONCLUSION

Our study defines the real-time PCR assay as an accurate test to identify the GBS carriers at the third trimester of pregnancy. This new tool, which is easy to use, could enhance the exact identification of candidates for chemoprophylaxis, including women carrying a risk for PROM or preterm labor. Therefore, the adoption of this test as a component of prenatal screening could result in a reduction of early-onset GBS disease in neonates. It may be argued whether this PCR-based technique is cost-effective or not. Further research is warranted to clarify the reliability, efficiency and cost-effectiveness of GBS screening by PCR-based assays in women with third trimester pregnancy.

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