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Investigation of *Streptococcus* agalactiae Colonization inThe Last Trimester Pregnants by Using Standard Culture and Molecular Methods

Son Trimester Gebelerde Streptococcus agalactiae Kolonizasyonunun Standart Kültür ve Moleküler Yöntemler ile Araştırılması

Özgün Araştırma
Research Article

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ABSTRACT

Amaç: *Üçüncü trimesterdeki gebelerde grup B streptokok (GBS) kolonizasyonunu gerçek zamanlı polimeraz zincir reaksiyonu (real-time PCR) ve kültür yöntemleri kullanarak tespit etmeyi ve bu yöntemleri karşılaştırmayı amaçladık.*
Yöntem: *Mayıs 2014-Eylül 2014 tarihleri arasında Cerrahpaşa Tıp Fakültesi Kadın Doğum Anabilim Dalı Polikliniğine 35-37. gebelik haftaları arasında başvuran 100 kadından GBS taraması için vajinal swab örnekleri alındı.*
Bulgular: *GBS kolonizasyon oranları; kültür ve real-time PCR yöntemleriyle sırasıyla % 5 ve % 7 idi. Kültür altın standart kabul edildiğinde gerçek zamanlı PCR için duyarlılık ve özgüllük sırasıyla % 100 ve % 97,9 idi. GBS kolonizasyonu ile yaş grupları, eğitim düzeyleri, önceki gebelik sayıları, sigara içme alışkanlıkları, antibiyotik kullanım öyküsü ve kontraseptif yöntemleri arasında anlamlı bir fark yoktu.*
Sonuç: *Gerçek zamanlı PCR tekniğinin kültür yöntemi kadar duyarlı olduğu kanıtlanmıştır. Ayrıca, gerçek zamanlı PCR, GBS tespitinde hızlı bir tanı yöntemi olarak daha etkili bir intrapartum antibiyotik profilaksisi sağlar. Bu sayede infantlarda morbidite ve mortalitenin düşük olmasına yardımcı olur. Fakat, PCR testinin her laboratuarda kullanılamaması ve yüksek maliyeti handikap oluşturmaktadır.*

Anahtar kelimeler: *S.agalactiae, gebelik, kolonizasyon*

ÖZ

Objective: *We aimed to detect and compare group B streptococcus (GBS) colonization in pregnant women at third trimester using real-time polymerase chain reaction (real-time PCR) and culture methods.*
Methods: *Vaginal swab specimens were taken for screening of GBS from 100 women between 35-37 weeks of gestation who were attending to antenatal outpatient unit of Obstetrics and Gynecology Department of Cerrahpasa Medical Faculty from May 2014 to September 2014.*
Results: *Rates of GBS colonization was %5 and %7 by culture and real-time PCR methods, respectively. Using culture as the gold standard; sensitivity and specificity for real-time PCR were 100% and 97.9%, respectively. Any significant difference was not detected between GBS colonization with age groups, education levels, number of previous pregnancies, smoking habits, history of antibiotic use, and contraceptive method.*
Conclusion: *Real-time PCR technique has proven to be as sensitive as the culture method. Also, real-time PCR may provide a rapid diagnostic tool for GBS detection potentially allowing a more effective intrapartum antibiotic prophylaxis and lower infant morbidity and mortality. However, the inability to use PCR test in every laboratory and its high cost creates a handicap.*

Keywords: *S.agalactiae, pregnancy, colonization*

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INTRODUCTION

Group B streptococcus (GBS) is a leading cause of sepsis, menengitis, and death among newborns [1,2]. GBS colonization in pregnant women is the most important risk factor. As many as 40% of all pregnant women have rectal and/or vaginal GBS colonization. The incidence of neonatal GBS infection is 0.5 per 100 live births. Vertical transmission from mother to the neonate accounts for up to 75% of the cases with neonatal GBS colonization, and 1% to 2% of these infants will develop early-onset GBS infection [2,3].

Since 2002, the Center for Disease Control and Prevention (CDC) recommends GBS screening for pregnant women using culture-based method. This approach is preferable, resulting in more effective prevention than clinically risk-based chemoprophylaxis [4]. The standard method for the diagnosis of GBS colonization consists of simultaneous culturing of both vaginal and anal swabs in a selective broth medium that inhibits the growth of non-GBS microorganisms. Unfortunately, although the standard culture is a highly sensitive and specific method, it requires 48 hours for complete identification of GBS. Moreover, negative culture results are observed in some women whose infants subsequently develop GBS infection [5-10].

A rapid test that could accurately detect GBS carriage at the time of labour may be an ideal component of a screening programme. Many techniques have been tested in order to validate a fast and efficient method of GBS screening to replace the culture. Nowadays, molecular biology- based assays, such as polymerase chain reaction (PCR), have become the focus of investigation of GBS colonization in pregnant women. A good target for GBS amplification is the *cfb* gene because it is well studied in this species [11,12]. In 2004, FDA approved a real-time PCR method for the identification of GBS in vaginal, and rectal samples [13].

Our prospective study was designed to detect and to compare GBS colonization in pregnant women at third trimester using real-time PCR and culture methods.

MATERIAL and METHOD

Samples and Microbiological Tests

After the ethics committee approval was obtained for the study, vaginal swab specimens from 100 women between 35-37 weeks of gestation who were attending to antenatal outpatient unit of Obstetrics and Gynecology Department of Cerrahpasa Medical Faculty were obtained for screening of GBS from May 2014 to September 2014. Two vaginal swabs for each woman were collected from distal 1/3 of the vagina by a trained gynecologist and were transferred to the microbiology laboratory of Cerrahpaşa Faculty of Medicine. Culture swabs were placed in selective enrichment culture media (Todd-Hewitt Broth) and incubated for 24-48 hours at 37°C, and then subcultured onto Columbia CNA agar plates which were again incubated at 37°C for 24-48 hours. After incubation, the plates were inspected for the presence of β-hemolytic colonies. When β-hemolytic colonies were observed after 24 hours, gram staining was performed. The β-hemolytic colonies whose morphology was consistent with group B streptococci were subjected to the catalase test. For the catalase-negative suspected colonies, additional tests as bacitracin (Oxoid) and trimethoprim/sulfamethoxazole (Oxoid) resistance tests, CAMP test (standard strain *Staphylococcus aureus* ATCC 25923), bile-esculine test, PYR (L-pyrrolidonyl-beta-naphtylamide, Oxoid) test were performed to identify GBS. Latex agglutination test (Avipath-Strep, Omega Diagnostics, United Kingdom) was also done to confirm the GBS origin.

Polymerase Chain Reaction

DNA test was based on real-time PCR assay targeting *cfb* gene region. DNA samples were extracted using Instagen Matrix (Bio-Rad, USA), according to a proto-

col explained below:
1-Samples (Cophan Regular Swap and Medium) were transferred from 1000ul medium to 1.5 ml Eppendorf tube. Then, tubes were centrifugated at 13 000 rpm for 10 minutes, and supernatant was removed.
2-A 100 µl of InstaGen matrix (Bio-Rad, USA) was added to the pellet and vortexed for 10 s. Each sample was incubated at 99°C for 20 min in mixer heat block unit.
3-The samples were centrifuged at 13 000 rpm for 5 min. The DNA containing supernatant was used in the PCR assay.

CFX-96 real-time PCR system (Bio-Rad, Hercules, CA, USA) was used for the thermocycling and fluorescence detection. The real-time PCR amplification was performed in a total volume of 20 µl that contained 10 µl of Seegene Universal PCR Master Mix, 2 µl of primer, 3 µl of template DNA and 5 µl of ddH2O. The primers used for PCR were as follows, with forward primer 5'TTTCACCAGCTGTATTAGAAGTA3' and reverse primer 5' GTTCCCTGAACATTATCTTTGAT3'. The cycling program was as follows: one cycle at 95°C for 15 minutes, 40 cycles at 95°C for 15 seconds, 60°C for 30 seconds and 72°C for 30 seconds. One positive control (*Streptococcus agalactiae;* Kuen 1362, 81/50, KÜKENS) and one negative control were included in each run.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 22; SPSS Inc., Chicago, IL, USA). In this study descriptive analyses were used mostly but categorical variables were evaluated using χ^2 test or Fisher's Exact test. A p-value <0.05 was considered as statistically significant.

RESULTS

The mean age of the pregnant women in our study was 29.7.± 5.56 (range: 19 to 44) years. Ninety-one (91%) pregnant women had no smoking history while 9 (9%) of them were smokers. None of the pregnants had a history of alcohol use. When the previous contraception methods were questioned; 57 pregnant women (57%) had not used any method while the remaining pregnants preferred condoms (n:27), pills (n:11), and intrauterine devices (IUD: n:5). Of the pregnant women, 18 (18%) had a history of antibiotic use within 2 weeks before delivery.

In our study, GBS colonization was detected in 7 (7%) pregnant women. GBS growth was detected in the culture of 5 (5%) vaginal swab samples taken from pregnant women. In the real-time PCR test, GBS growth was detected in 7 (7%) specimens, and also in 2 swab samples where GBS growth was not revealed in the culture. Finally, using culture as the gold standard, sensitivity and specificity for real-time PCR were 100% and 97.9%, respectively (Table 1).

Table 1. Comparison between culture and real time PCR for detection of GBS in vaginal swabs of 100 pregnant women at their 35-37 gestational weeks.

		Culture		
		Negative	**Positive**	**Total**
Real - time PCR	Negative	93 (97.9)	0 (0)	93
	Positive	2 (2.1)	5 (100)	7
Total		95	5	100

When the age groups were compared in terms of GBS carriage, GBS positivity in the 21-25 age group (11%, 2/18) was found to be two times higher than the total positivity in the other age groups (5/82, 6%) but without any statistically significant integroup difference (χ^2:1.954, p=0,744). Although GBS colonization was found to be less common in women who had first pregnancy, no statistically significant difference was found between the number of pregnancies and colonization (χ^2:2.192, p=0.533). Although GBS colonization rate was higher in smokers (11% vs 6%), but without any statistically significant difference when compared with nonsmokers (Fisher's test; p:0.447). Although GBS growth was not detected in IUD users, no statistically significant difference was

found when compared with other groups (χ^2:0.457, p=0.938). Also, when the relationship between antibiotic use and GBS colonization was examined, any statistically significant difference was not found between smokers, and nonsmokers (χ^2:0.724, p=0.855). In addition, there was no statistically significant difference between education status of pregnant women and GBS colonization (χ^2:4.667 p=0.332).

When newborn results were evaluated; mean gestational age was 38.75±1.5 weeks and the mean gestational weight was 3332±483 gr. There was no statistically significant difference between GBS and birth weight/gestational week (p>0.05). On the postnatal 7th day and third month follow-up of newborns; none of the infants had any complication(s).

DISCUSSION

In our study, we studied real-time PCR method which was an alternative to classical culture method in the antenatal diagnosis of GBS. As a result of the study, GBS carriage was found to be 5% and 7% by culture and real-time PCR methods, respectively. Our GBS colonization rate was similar to the previous publications in our country, although it was below the rates cited in the current literature (15-20%) [14-16]. In a study from Turkey, 500 pregnant women were examined at a Training and Research Hospital, and GBS colonization was found to be 8% in rectovaginal specimens. Infants of 41 mothers colonized with GBS were followed up twice at the first week and third month after delivery and no complication was found in any of the infants [16]. Similarly, in our study, there was no complication in infants of GBS colonized mothers. In both clinics, undetected GBS neonatal infection could be explained by antibiotic prophylaxis due to intrapartum clinical risk factors, planned cesarean deliveries and low GBS colonization rate throughout the country.

Different reports about GBS carriage rate are linked to various factors such as race, geographic region, age, smoking, number of pregnancies, contraception method, sexual intercourse pattern, diagnostic method (culture, molecular method, etc) and gestational week, area of the sampling (vaginal and/or rectal) [17,18]. Colonization rate increases with parity and decreases with age [19]. In our data, no significant relationship was found between the number of pregnancies, age groups and GBS colonization. In a study which examined smoking and GBS colonization it was shown that colonization rate was two times higher in smokers. The reason for this difference may be the role of the suppression of the immune system with the similar mechanism of increased colonization seen in immune compromised patients [20]. In our study, GBS colonization was found to be - though not statistically significant- higher in smokers. The use of IUD which is one of the controversial factors of GBS colonization, was investigated by Farrag et al. [21]. According to this, colonization rate was found to be significantly higher in patients with IUD users (31%) compared to non-users (14.5%). In our study, although GBS growth was not detected in IUD users, it was not statistically significant since GBS colonization rate was low.

Although CDC recommends taking a vaginorectal specimen, rectal sampling may be a problem for pregnant women. In a study from Egypt, none of the pregnants gave permission for rectal sampling [22]. In our study, we only took vaginal samples.

One of the factors affecting the colonization of GBS is the media used which increase the chance of isolation by up to 50% [23]. In the same Egyptian study, the diagnostic sensitivity of GBS, which was 50% in direct culture, increased up to 90% after subculturing with Lim broth [22]. The most important reason for the decrease of sensitivity by direct incubation on the selective plaque is the effect of other bacteria that are overgrown because of weak colonization of GBS [19].

Although PCR method is a highly sensitive method in GBS screening, a wide range of sensitivity has been reported according to the device and sampling methodology used. Bergeron et al. [23] showed an increase of 62% PCR-positivity with the Light-Cycler PCR method compared to the culture. A GBS PCR method with the GeneXpert assay, which was also approved by FDA, was found to be 95% sensitive in the detection of intrapartum GBS carriage [24]. In the study of Rallu et al. [11], 605 vaginorectal specimens were examined; 2 different PCR tests analyzing 2 different regions (*cfb* and *scpB*) and GBS antigen tests were compared with the standard culture. According to this study, the most accurate results were obtained by *scpB* PCR test (sensitivity 99.6%; specificity 100%), followed by *cfb* PCR test (sensitivity 75.3%, specificity 100%) compared to GBS antigen identification test (sensitivity 57.3%; specificity 99.5%) and the standard culture method (sensitivity 42.3%; specifity 100%). In the literature, rates of sensitivity were reported as 92-99% for PCR and 62-98% for non-enriched samples compared to culture [11]. In our data, the rates of sensitivity,and specificity of PCR were 100% and 97.9%, respectively. An issue that should be discussed is to accept the culture method as the gold standard. Factors such as antibiotic repression, weak GBS colonization, enterococcal growth, contamination, female hygiene products cause PCR positivity and culture negativity. To accept the clinical infection as the gold standard may be a solution. But, unfortunately no newborn infection was observed in our study.

Another important issue is the cost of the tests used. In the study of Picchiassi et al. [25], it was showed that intrapartum PCR test was more expensive than antepartum culture. However its cost may be reduced if the test is widely practiced in the next years. In our study, cost analysis was not performed.

CONCLUSION

Our results have indicated that PCR technique proved to be as sensitive as the culture method. Also, real-time PCR may provide a rapid diagnostic tool for GBS detection potentially allowing a more effective intrapartum antibiotic prophylaxis and lower infant morbidity and mortality. On the other hand, the inability to use PCR test in every laboratory and its high cost creates a handicap.

Declaration of interest:
None.
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None.

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