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Original article

Comparative Molecular and Microbiologic Diagnosis of Vaginal Colonization by Group B Streptococcus in Pregnant Women during Labor

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Abstract

Objective(s)
Rapid tests for detection of *Streptococcus agalactiae* or Group B Streptococci (GBS) at the onset of labor are needed to permit early intrapartum antibiotic prophylaxis. This study aimed to evaluate the PCR assays targeting the 16S ribosomal RNA gene (16S rDNA) for detection of the GBS in comparison with a specific culture method.
Materials and Methods
Two swabs were used to obtain vaginal specimens from the 330 pregnant women attended delivery room at Hedayat hospital, Tehran, Iran. One swab was analyzed by direct plating onto selective GBS agar medium (ISLAM) and the other swab was used for a PCR assay, which amplified the 16S rDNA of *S. agalactiae*. Comparative study between the selective culture and the PCR assay was done among the 330 tested women.
Results
The GBS colonization rate based on the culture results was 20.6% (68/330). Both culture and PCR methods were positive for 56 and negative for 253 women. The culture method was positive and PCR was negative in 12 women. The culture was negative and the PCR positive for 9 women. Sensitivity of the PCR assay was 82.3% and specificity was 96.5%. The positive predictive value was 86.15% and negative predictive value was 95.4%.
Conclusion
ISLAM diagnostic procedure and PCR are rapid and reliable analyzing methods, which might be useful for accurate diagnosis of GBS colonization in pregnant women at the time of delivery.

Keywords: Early diagnosis, PCR , rRNA gene, *Streptococcus agalactiae*, Streptococcal infection

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Introduction

The *Streptococcus agalactiae* known as the Group B Streptococci (GBS) is a species of the normal flora of female urogenital tract (1). GBS normally colonizes the vagina in many women asymptomatically. In pregnancy, GBS can infect the amniotic fluid, and during labor, vertical transmission may infect the newborn leading to neonatal sepsis and meningitis, which are associated with a high mortality. Approximately 10-30% of women are colonized with GBS in vagina during pregnancy and 50-75% of their infants acquire this organism through birth canal (2). Thus, identification of GBS-colonized women is critical for prevention of neonatal GBS infection.

In 1996, Center for Disease Control and Prevention (CDC) published guidelines for the prevention of prenatal group B streptococcal disease and recommended intrapartum antibiotic prophylaxis during labor in women with recognized risk factors (fever in labor, preterm delivery at < 37 weeks gestation and prolonged rupture of membranes > 18 hr) and GBS colonized women based on prenatal culture screening (3). Whereas, up to 50% of infants who developed GBS disease were born from carrier mothers without risk factors (4), in 2002 CDC guidelines were revised and offered bacteriological screening for all pregnant women at 35 to 37 weeks of gestation (5).

GBS colonization status may be transient, chronic or intermittent and the duration of colonization varies, therefore diagnosis of GBS in vagina at the certain points in pregnancy can not assure the existence of this organism at the time of delivery (6). Rapid screening tests for detection of GBS which can identify carrier mothers at the onset of labor would obviate the need for prenatal screening at 35-37 weeks gestation (5) and unnecessary administration of prophylactic intrapartum antibiotics in uninfected women. Moreover, rapid methods may be advantageous for women with insufficient prenatal care and in the cases of preterm labor.

Specific media and molecular methods are very efficient tools to develop simple, fast, sensitive and cost-effective diagnostic tests. Therefore, this study aimed to evaluate the accuracy of PCR assay and improve the diagnosis of GBS. In this report, we used both a specific culture method and PCR assay for targeting the *S. agalactiae* 16S ribosomal RNA gene sequence. This provides a rapid and precise analytical technique for GBS diagnosis during labor.

Materials and Methods

Study subjects

Three hundred and thirty pregnant women attended delivery room for labor at Hedayat Hospital in Tehran (Iran) from April through July 2008. They were enrolled in this study after signing a written informed consent, which evaluated and proved by Avicenna Ethical Committee. The age of women ranged between 16 and 40 years (mean age 25.83±4.6). Specimen of vaginal fluid was obtained by brushing the lower vagina (vaginal introitus) with two sterile cotton swabs before membrane rupture. A swab was immediately placed into Amies transport medium with charcoal (Hi-Media, India) and another swab was soaked in tube contained 1 ml of phosphate-buffered saline (PBS) with pH 7.2. The specimen samples were transported at room temperature, cultured and analyzed by PCR assay at the Avicenna Research Laboratory within 24 hr after collection.

Microbiologic detection techniques

Vaginal specimens were cultured on selective GBS Agar Base medium (ISLAM) with 5% horse serum for isolation and detection of GBS (Oxoid, UK). The swab was rotated over one-third of the surface of a GBS agar plate and the inoculum was then spread over the plate using an inoculating loop. *S. agalactiae* develops orange-red pigmented colonies in GBS agar plates. The plates were incubated at 35-37 °C under anaerobic conditions in an anaerobic jar (Hi-Media, India) with gas pack for 18–24 hr. Negative plates were reincubated for an additional 24–48 hr before being discarded.

Extraction of DNA from bacteria on vaginal swabs

DNA was extracted using 6% Chelex®-100 (Bio-Rad Laboratories, USA) according to the

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following procedure. Swabs were soaked in 1 ml of phosphate-buffered saline (PBS), (pH 7.2) agitated vigorously to dislodge bacteria and epithelial cells. The sediments were spun down at 10000×g for 5 min (Eppendorf, Digital Centrifuge 5415R), and then washed by resuspending the pellet in PBS and spinning down at 13000×g for 3 min. The pellet was then resuspended in 200 µl 6% Chelex®-100 (Bio-Rad Laboratories, USA), incubated for 20–30 min in a water bath at 55°C. The sample was vortexed for 10 s and boiled at 100 °C for 8 min. The sample was vortexed for 10 s and centrifuged at 13000×g for 3 min. The supernatant containing the DNA was removed gently and stored at –20 °C (7-9).

PCR primers

The selected PCR oligonucleotide primers were designed from highly divergent and species specific regions of the DNA coding for 16S rRNA (10). These hypervariable DNA regions facilitate the design of highly specific primers (11) moreover, the presence of rDNA in many copies, permits signal enhancement (12). The sequences, specificities, position, G+C contents and annealing temperatures are summarized in Table 1. The length of the amplified product is 405 bp. These primers were synthesized by Operon® (Germany).

PCR amplification

The amplification reactions of the DNA template/sample were carried out in 0.2 ml PCR single tube-RNase/DNase/pyrogen free (BOECO, Germany) with hinged flat cap using a Thermocycler (AB Applied biosystem, USA). Each PCR consisted of 2.5 µl of 10×Buffer (without $MgCl_2$) (Roche, Germany), 2 mM of $MgCl_2$ (Roche, Germany), 0.4 mM dNTPs, 0.2 µM of each primer, 1 U of Taq DNA polymerase (Roche, Germany), 5.0 µl of DNA template/sample, and distilled H_2O to a total volume of 25 µl. The PCR amplification was performed as described in Riffon *et al* (10).

Detection of PCR products

To confirm amplicon production, the mixture (10 µl PCR product and 2 µl of loading buffer) was analyzed by electrophoresis in 1.5% agarose gel (Roche, Germany) stained with ethidium bromide. Electrophoresis was carried out in 1×TAE (40 mM Tris-acetate, 1 mM EDTA; pH 8.0) at 100 V for 45 min. The DNA molecular weight marker VIII (19-1114 bp), (Roche, Germany), was run concurrently. Gels were visualized under UV illumination and the results were Photographed (UPP-110 S (Type I), SONY, Japan).

Statistical analysis

The GBS colonization rate was calculated based on the both culture and PCR assay results for the total number of samples. The sensitivity, specificity, and positive and negative predictive values of PCR assay were estimated for PCR assay. The 95% confidence intervals for sensitivity, specificity, and positive and negative predictive values were calculated. The McNemar test was performed to determine the statistical significance of the differences observed between two tests. Statistical analysis was done using the SPSS 13.

Results

Among the 330 pregnant women, the results of culture on GBS agar as a gold standard method, were positive in 68 individuals who have been identified as carriers of GBS (20.6%) (95% CI 16.7-24.5%). The results of PCR assay were also positive for 56 of 68 as carrier identified women based on culture results. Both the culture and PCR assay were negative for 253 women. In 9 pregnant women the PCR assay results were positive whereas the culture results remained negative (Table 2). For specimens that were culture positive and PCR negative, the PCR was repeated two additional times to confirm the results. Sensitivity of the PCR assay was 82.3% (95% CI 78.4 - 86.2%) and specificity was 96.5% (95% CI 94.5 - 98.4%). The positive predictive value was 86.15% (95% CI 82.6 - 89.6%) and negative predictive value was 95.4% (95% CI 93.4 - 97.3%).

The kappa statistic was calculated for comparison of culture and PCR assays. The kappa index of agreement was 0.8 and generally, a Kappa value greater than 0.7 indicates satisfactory reliability.

The McNemar test indicated that there was no statistically significant difference between two applied methods.

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Table 1. Oligonucleotide primer sequences used for PCR.

Name	Specificity	Sequences (5'-3')	Position	G+C content (%)	Annealing temperature (°C)	Reference
Sag40	*S. agalactiae*	CGCTGAGGTTTGTGTTTACA	40-61	48	60	10
Sag445	*S. agalactiae*	CACTCCTACCAACGTTCTTC	445-465	50	60	10

Table 2. Detection rates of colonization with GBS based on applied methods.

Method of detection	Culture positive (%)	Culture negative (%)	Total (%)
PCR assay positive (%)	56 (82.4)	9 (3.4)	65 (19.7)
PCR assay negative (%)	12 (17.6)	253 (96.6)	265 (80.3)
Total (%)	68 (100)	262 (100)	330 (100)

Discussion

Group B Streptococcus (GBS) is an important cause of infection in pregnant women and their newborns. The maternal carriage of GBS varies worldwide because of the socioeconomic, geographical and racial differences (13, 14) and depends on the accuracy of laboratory techniques (15-17). Stoll *et al* in 1998 reported the prevalence of GBS in various geographic regions of the world which follows: Middle East/North Africa, 22%; Asia/Pacific, 19%; Sub-Saharan Africa, 19%; India/Pakistan, 12%; and America, 14% (18). Epidemiological studies among Persian women showed that GBS colonization rates in Iran range from 9.1 to 26.7% (19-22). The wide discrepancies in the percentage of GBS carriers in Iran may be caused by different sample collection sites, bacterial load in swab samples, gestational age at the time of sampling and different diagnostic methods.

Pregnant women who are not screened for GBS are at risk of transmitting the infection to their babies (3). The incidence of GBS infection among infants ranges from 0.5 to >2 per 1000 live births (5, 23, 24). GBS neonatal disease differs with age at presentation and is classified as early onset (within the first week of life), and late onset disease (7-90 days). A decrease in the prevalence of GBS disease has been noted over recent years simultaneously with the release of revised CDC guideline in 2002 (25).

Rapid, accurate and sensitive method for GBS detection at the time of delivery increases the chance of carrier women treatment and decreases the rate of vertical transmission of GBS to newborn (16). Currently, the CDC gold standard method for diagnosis of vaginal GBS colonization is being applied at 35-37 weeks of gestation by culturing the vaginal swabs in a selective broth and subculturing on agar plates. This type of GBS diagnosis is based on bacteriologic criteria, which is sensitive enough to detect both light and heavy colonization. However, this requires at least 36 hr (5) which is not quick enough for GBS identification at the time of labor. Although, due to the importance of prevention of neonatal sepsis and meningitis, many simple and fast tests have been developed which help to identify GBS carrier women rapidly (26), all of them are time consuming even more than a day.

Several special media have been introduced which can identify GBS colonization based on unique ability of GBS in pigment production (27, 28). Votava *et al* estimated the sensitivity of GBS agar medium for detecting GBS carriage in comparison with standard culture method. The sensitivity of the standard method using selective Todd-Hewitt broth was 97% and the corresponding value for GBS agar was 93.9% (17). Accordingly, GBS agar medium would be an accurate and rapid method which can identify GBS colonized women within 18-24 hr after sample collection.

Common molecular methods such as PCR have widely been used for identification of bacteria. PCR assay accuracy depends on the design of species-specific primer pairs. Thus, we selected highly specific oligonucleotide primers designed for the hypervariable region of 16S rRNA gene (10).

In this study, 9 women revealed positive GBS by PCR and not by culture so false positive rate was 13.8% (9/65). These

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disparities can be resulted from the existence of nonviable bacteria in the vaginal swabs or unequal sample collection between the two swabs obtained from pregnant women. Vaginal specimens contain nonviable bacteria which can not grow on media but bacterial DNA would be present for PCR amplification (16). Furthermore, the potency of pigment production in some GBS isolates becomes weak and can not be identified by GBS agar medium. Non-hemolytic group B streptococci are rarely (1-2%) isolated from clinical specimens; although these strains grow perfectly in GBS agar, they can not produce the orange pigment (29, 30).

Twelve cases in this study were culture positive and PCR negative and false-negative rate was 4.5% (12/265). Although PCR assays are rapid and sensitive methods for detection of GBS in pregnant women (15, 31), the bacterial load in the vaginal swabs may have been very low and the PCR amplification cycles may not have met the threshold set to assign a positive PCR result. If the PCR assay had been used alone, these women would not have been considered as GBS carrier and would not have been treated with antibiotic during labor.

Conclusion

In conclusion, we emphasized that the high maternal GBS colonization rate in pregnant women necessitates an appropriate screening management for diagnosis, and therefore prophylactic treatment of the infected women. The impacts of studies on the prevalence of GBS neonatal disease, the preventative treatment and the affects of infected infants are strongly requested in our country in order to find a strategy in fighting GBS. Our study confirmed that direct detection of human beta-hemolytic group B streptococcus using ISLAM and PCR is at least as reliable as other detecting standard cultures. In spite of the fact that PCR techniques are sensitive and specific methods, the process of PCR assay increases the cost and the technology of PCR is not usually available in all laboratories in our country. On the other hand, a clear benefit of using ISLAM culture method is the cost effectiveness in less amount of time. Therefore, the specificity, accuracy and rapidity of GBS agar medium suggest that ISLAM diagnostic procedure would be reasonable method for identification of GBS carriage at the time of delivery. Moreover, 16S rRNA gene based PCR can be considered as a useful approach for detection of maternal GBS colonization in instant situation.

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