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Original Article

Evaluation of Culture and PCR Methods for Diagnosis of Group B *Streptococcus* Carriage in Iranian Pregnant Women

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Abstract

Background: Group B *streptococcus* (GBS) is one of the most important cause of morbidity and mortality among newborns especially in developing countries. It has been shown that the screening approach rather than the identification of maternal clinical risk factors for early-onset neonatal GBS disease is more effective in preventing early-onset GBS neonatal disease. The objective of this study was to detect GBS among clinical samples of women using PCR and standard microbiological culture.

Methods: Samples were taken from 375 women at 28-38 weeks of gestation during six month from January 15 till June 15, 2011 from a hospital in Tehran, Iran. Samples were tested by standard culture using Todd–Hewitt broth, blood agar and by PCR targeting the *cfb* gene.

Results: Among the 375 women, 35 (9.3%) were identified as carriers of group B *streptococci* on the basis of the results of the cultures of specimens, compared to 42 (11.2 %) on the basis of PCR assay.

Conclusion: We found that GBS can be detected rapidly and reliably by a PCR assay in vaginal secretions from women at the time of delivery. This study also showed that the rate of incidence of GBS is high in Iranian women.

Keywords: Group B *Streptococcus*, Pregnant women, Polymerase Chain Reaction

Introduction

Group B *streptococcus* (GBS) is an important cause of morbidity and mortality in newborns. The incidence of neonatal GBS infection ranges from 0.5 to >2 per 1000 live births in different geographical areas. GBS neonatal disease is classified as either early-onset disease (<7 days) or late-onset disease (>7–90 days) (1). Early onset disease accounts for 70–80% of cases. GBS colonization in pregnant women is the single most important risk factor for early-onset newborn disease due to vertical transmission and colonization of the infant during delivery. Intrapartum antibiotic treatment lowers the incidence of early onset disease (2). Infants who have such infections may require prolonged hospitalization, and those who survive may experience mental retardation or visual loss. Among pregnant women, the prevalence of colonization with group B *streptococci* ranges from 15 to 40%. Women who are carriers are also at risk for severe infections (3). It has been shown that the screening approach rather than the identification of maternal clinical risk factors for early-onset neonatal GBS disease is more effective in preventing early-onset GBS neonatal disease (4). The culture method has a slow turna-

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round time, requiring 36 to 72 h before results can be issued. In addition to being time consuming, this method requires an experienced technician to identify the suspected colonies, which are not always beta-hemolytic (5). The recent commercial availability of a rapid, highly sensitive, and polymerase chain reaction (PCR) assay suitable for the detection of GBS colonization in pregnant women during delivery provides improvements over culture-based and immunological methods. A number of PCR assays targeting different genes for the specific detection of GBS have been developed (6).

The objective of this study was to detect GBS carriers among pregnant women using PCR and standard microbiological cultures.

Materials and Methods

Samples were taken from the mucus of the anal and vaginal tissues of 375 pregnant women at 28-38 weeks of gestation during six month from January 15 till June 15, 2011 from Mirzakuchak khan hospital in Tehran, Iran. For anal specimens, a swab was carefully inserted approximately 2.5 cm beyond the anal sphincter and then gently rotated to touch anal crypts. For vaginal specimens, excessive secretions or discharge were wiped away, and secretions from the mucosa of the lower third of the vagina were obtained with a swab. Each swab was transported in Stuart's bacterial transport medium immediately after the sample was obtained. Samples were tested by standard culture using Todd–Hewitt broth and blood agar (7) and by PCR (8) targeting the *cfb* gene.

Culture

In the laboratory, the same specimen was used for optimized GBS culture and for PCR. Each swab was twisted and twirled in 0.55 ml of sterile saline; 50 µl was seeded on selective blood agar (Columbia CNA agar with 10 mg nalidixic acid l21 and 10 mg colistin l21) with 5% human blood, (9) and 50 µl was used inoculate selective GBS broth (Todd–Hewitt broth with 15 mg nalidixic acid l21 and 8 mg gentamicin). The selective blood agar plates were incubated for 48 h, and the broth was subcultured onto non-selective blood agar plates after 24 h of incubation. Blood agar plates were examined after 24 and 48 h, and *beta*-hemolytic and non-hemolytic, pyrrolidonyl arylamidase-negative colonies were identified as GBS using a commercial latex agglutination test. Growth was semi-quantified using the categories of abundant/moderate (10 GBS colonies per plate), sparse (1–10 GBS colonies per plate) and growth only after enrichment in selective GBS broth (7) . The GBS strain ATTC 12386 was used as positive control and reference to set – up culture media.

PCR

The PCR product amplified by using forward primer TTTCACCAGCTGTATTAGAAGTA and reverse primer GTTCCCTGAACATTATCTTTGAT as previously described (10). For nucleic acid extraction, 300 µl of suspension was added to an equal volume of lysis solution [containing 15 µl lysozyme (Sigma; 20 mg/ml), 6 µl proteinase K (Sigma; 20 mg/ml), 6 µl mutanolysin (Sigma, 10000 U/ml), and 273 µl TE buffer] and incubated for 15 min at 37 °C and 15 min at 65 °C. DNA was purified using a DNeasy Tissue kit (Qiagen) and eluted in a volume of 100 µl. Extracted DNA was stored at -20 °C till utilize. A 2 µl aliquot of the purified DNA solution was used as a template for PCR. The negative control consist of all PCR reagent without DNA (11). Moreover, for confirming of PCR product, an amplicon was eluted from the agarose gel after electrophoresis and was sequenced. Then, the exact sequence was aligned with a

reference *cfb* gene (accession no. **EF694027.1**) by ClustalW software (12).

Results

Among the 375 pregnant women at 28-38 weeks of gestation , 35 (9.3 %) were identified as carriers of group B *streptococci* on the basis of the results of the cultures of combined vaginal and anal specimens, compared to 42 (11.2 %) on the basis of PCR of vaginal and anal specimens. Seven women had a positive result using the PCR method but were identified as negative by culturing, but was not detected any samples vice versa. Sixty percent of women who had a positive PCR result used an IUD, and 33% had genital tract disease (27%/3% for the culture assay). In addition 16.7% of women who had a positive result in the PCR assay received antibiotics (20% for the culture assay). Overall, group B *streptococci* were detected slightly more often by the PCR assay than by culturing. The sensitivity of the PCR assay was 100%, and the negative predictive value was 100%, compared with the culture results. The specificity and positive predictive value of the PCR assay were 98% and 100%, respectively. The amount of time required to obtain the results was 3 hours for the conventional PCR assay and at least 36 hours for culturing.

Discussion

To prevent group B streptococcal disease in neonates, the current recommendation is to screen pregnant women by culturing combined vaginal and anal secretions at 35 to 37 weeks' gestation and to treat those with positive cultures or to treat women with risk factors for disease transmission empirically (13). Exhaustively described in the literature, nucleic acid testing assays based on nucleic acid amplification technologies, such as PCR, offer a great potential for rapid, highly sensitive, and specific detection of various infectious agents directly from clinical samples (14,15). We have used PCR for the detection of group B *streptococci* that have been shown to be specific and sensitive in tests with purified DNA. In addition, we have used the culture assay for comparison (16). Overall, group B streptococcal colonization was detected more often by the PCR assay than by culturing. Although PCR assays are extremely sensitive, the bacterial load in that sample may have been very low, or the sample could have been contaminated during the culturing process (3). So, in spit of culture method which introduced as a gold standard, but it seems that PCR is more sensitive. Antibiotics could theoretically have decreased the specificity of the PCR assays, but for at least the two women who received antibiotics (penicillin and cephalosporins) before the specimens were obtained, the results of the PCR assays and the results of culturing were consistent. The GBS carriage rate of almost 10% in the present study is similar to the rates reported in the majority of published studies, ranging between 10 and 29% (4,17-21). However, higher prevalences of GBS colonization were described in a Danish study from 2004 (22) and a Canadian study from 2006 (23). Also, Shabayek (10) reported GBS was detected in 25.3% of isolates by Islam medium, in 30.6% by using the cfb PCR assay and in 30% by using the scpB PCR assay. The different prevalence rates may be explained by gestational age at culturing, differences in culture sites and culture techniques, a change in the prevalence over time, or real differences in the prevalence in different populations or ethnic groups (20). There may be a higher bacterial load in separate specimens compared to combined specimens, and collecting, processing and analyzing vaginal and rectal specimens separately may lead to the

detection of more GBS-colonized women (24).

Although the importance of infection as a cause of preterm delivery is gaining recognition, little is known about the role of GBS infection in miscarriages. McDonald and Chambers (25) stated that GBS was a key pathogen in unsuspected intrauterine infections underlying spontaneous midgestation abortions. The study of Daugaard et al. (26) demonstrated an association between the presence of group B *streptococci* in the urine and on the cervix and late spontaneous abortions, but El Kersh et al. (27) found no correlation between the presence of group B streptococci and a history of repeated spontaneous miscarriages. In the US, the screening-based strategy has been recommended since 2002 (2). This recommendation was based on data found in a recent study comparing the screening- and risk factor-based strategies. The conclusion of that study was that the screening-based strategy was over 50 % more effective than the risk factor-based strategy (28). The management strategy depends on local factors, such as the percentage of GBS carriers and the percentage of pregnant women with prenatal risk factors within the population, the organization of prenatal care and the local availability of laboratory facilities. The choice of the preventive strategy should be based on rationality, cost-effectiveness and current knowledge and possibilities (29).

We found that GBS can be detected rapidly and reliably by a PCR assay using combined vaginal and anal secretions from pregnant women at the time of delivery. This study also showed that the rate of incidence of GBS is high in Iranian pregnant women. We, therefore, emphatically recommend a screening-based strategy to detect GBS in Iranian pregnant women.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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