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Original Article

High Burden of Group B Streptococcus: An Invasive Bacterium among Pregnant Women Referring to Health Centers of Sanandaj, Iran

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Abstract

Aim of study: Group B Streptococcus (GBS) is known as an invasive bacterium. This bacterium is common in all over the world and reported to be responsible for early-onset newborn disease due to vertical transmission and colonization of the infant during delivery. The present study was carried out to determine the colonization of GBS in anorectum and endocervix among pregnant women referring to Beasat hospital of Sanandaj.

Methods: During Jan 2011 to Sep 2012 a total of 200 pregnant women at 28-38 weeks of gestation were enrolled. Four hundred anorectal and endovaginal swabs were collected from pregnant women by a Gynecologist. Each swab was added to PBS immediately after the sampling and then transported to Cellular and Molecular Research Center, school of medicine, Kurdistan university of Medical Sciences and stored at -70^{0}C. Polymerase chain reaction (PCR) was carried out to detect the *cfb* gene.

Results: The pregnant women were ranged from 18 to 37 years old (mean age, 24± 6 years). Based on PCR results, out of the 400 swabs (200 rectal and 200 vaginal), 160 (rectal) and 123 (endovaginal) were positive for GBS respectively..

Conclusion: Our results showed that GBS can be detected rapidly and reliably by a PCR assay in vaginal secretions and rectal swabs from women at the time of delivery. This study also showed that the rate of incidence of GBS is high in Sanandaj, Iran.

Keywords: Group B *Streptococcus*, Pregnant women, vaginal secretion

Introduction

Some viral and bacterial infections increase the risk of abortion. Infection with group B streptococcus (GBS) is one of the most important infectious causes of neonatal morbidity and mortality in the newborns and their mothers (*Bergseng et al.* 2007; *Berardi et al.* 2004). The recognition of maternal colonization with the organism is a key factor in the occurrence of GBS associated neonatal morbidity and mortality, which has been highlighted in many studies (*Shet and Ferrieri,* 2004; *Katherine et al.* 2011; *Bakhtiari et al.* 2012).

The most likely reservoir of GBS is the gastrointestinal tract and the most frequent site of secondary spread is the genitourinary tract. A newborn can acquire *S. agalactiae* and

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develop neonatal GBS infections by contact with the genital tract of the mother during labor and delivery (*Aleksandra et. al*. 2010). Infants who have such infections may require prolonged hospitalization, and those who survive may have mental retardation or visual loss (CDC 1996). Therefore, identification of GBS colonized women is critical for prevention of neonatal GBS infection. Furthermore, the Center of Disease Control (CDC) recommends the screening of all pregnant women for vaginal and rectal GBS colonization between 35 and 37 weeks of gestation (*Schrag et al.* 2002).

In order to detect GBS, many rapid tests have been developed, such as antigen-based tests, hybridization-based methods and PCR assays. PCR-based assays offer promising tools for sensitive, specific, and rapid detection of GBS. Recently, one of the PCR assays for the identification of GBS had been developed, which targeted the *cfb* gene. Many published studies, using real-time PCR assays, targeting the *cfb* gene or *ptsI* gene in GBS, and different methods for nucleic acid extraction, have demonstrated sensitivities from 0.45 to 1.0 compared to enrichment broth culture.

There are few data from different areas of Iran on the prevalence of GBS and the *cfb* gene among pregnant women (*Bakhtiari et al. 2012*); however, based on our knowledge there is no data on prevalence of GBS among pregnant women in west of Iran. We therefore aimed at finding out the prevalence of GBS among pregnant women in Sanandaj, Iran.

Materials and Methods

This cross-sectional study was conducted during Feb. 2011 to Jan. 2012 in the Gynaecology and Obstetrics ward, Beasat hospital, Sanandaj, and department of Microbiology School of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran. Samples were taken from the mucus of the anal and vaginal of 200 pregnant women at 28-38 weeks of gestation.

For anal specimens, a cotton 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 taken by a Gynecologist and transported to the microbiology department (within maximum one hour). GBS were isolated and identified based on biochemical tests (*Pat et al*. 2007).

Detection of *cfb* gene

DNA was extracted according to the manufacture guidelines (Bioflux, Kit, China) and 3 µl aliquot of the purified DNA solution was used as a template for PCR. For the PCR reactions we used the Sag59: 5-TTTCACC AGCTGTATTA GAATA-3` and Sag190: 5-GTTCCCTGAACATTATCTTTGAT-3 specific primers which producing a 153-bp fragment. The PCR was performed as following: denaturation at 94ºC, annealing at 47^{o}C and extension at 72˚C. After electrophoresis the sizes of DNA fragments were compared to 100 bp ladder (Bio-Rad, Laboratories) as DNA molecular size standards.

The amplified PCR product of clinical samples was sequenced and the *cfb* sequence obtained was compared to Group B *Streptococcus*, which was obtained of National Reference Center laboratory and used as a positive control in all PCR reactions. The negative control consists of all PCR reagents without DNA.

Results

The pregnant women were ranged from 18 to 37 years old (mean age, 24± 6 years). Of the 400 swabs (200 rectal and 200 vaginal) which were taken from the pregnant women, 160 (rectal) and 123 (endovaginal) were positive for GBS respectively based on PCR.

Discussion

Although GBS are pathogens which have been associated with preterm labor, premature rupture of membranes, and neonatal sepsis (*Schrag et al.* 2000; *Sahar et al.* 2009) but testing of asymptomatic people in society may control this communicable disease and therefore prevent morbidity and mortality.

In our experience, despite CDC recommendations to use culture as GBS screening in pregnant women (CDC 2010), this study reports the high prevalence of GBS by use of *cfb* gene as amplification target by PCR screening test. GBS colonization rates in anal and vaginal were 75% and 61.5% respectively by using PCR. Based on our knowledge there is no any report showing GBS detection as high as our study. In another Iranian study, the GBS colonization rate was estimated by 11.2% on the basis of PCR assay (*Bakhtiari et al.* 2012). However, the prevalence of GBS colonization could vary widely with geographic location, age, parity and socio-economic status.

A study in Egypt (*Shabayek et al.* 2010) GBS was detected in 30.6% of women by using the *cfb* PCR assay. Similarly, GBS colonization rate among term pregnant women in Saudi Arabia (*El-Kersh et al*. 2002) is relatively high (27.6%). The results of these studies are quite different from our study because of the carriage rate in our study was quite high. The difference might be because of the difference in the timing of collection of the specimens and the geographical difference. Increasing data suggests that treating GBS infected pregnant women is more expensive than preventing the infection and that properly implemented prevention programs can significantly decrease illness and death resulting from GBS disease. It has been estimated by the CDC that $300 million dollars were spent in a year to treat almost 7,500 cases of GBS (*NomuraI et al.* 2006). Although great progress has been made in preventing GBS, however, quite a few reports from Iran have produced comprehensive data on GBS colonization rate in different regions of Iran (Reference?)

Attention should be focused on prevention of GBS infection in neonates which can only be possible by identification and treatment of carrier mothers, so that potential lethal consequences can be prevented.

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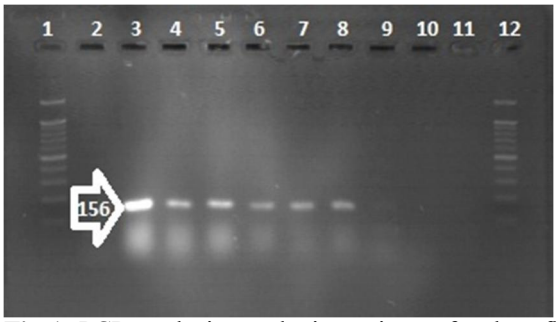

Fig 1. PCR analysis employing primers for the *cfb* gene. Lane 1 and 12: 100 bp ladder; Lane 2: negative control; Lane 3: positive control; Lanes 3-8: positive samples; Lanes9-11: negative samples.

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