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Clinical significance of group B streptococcus testing in late pregnancy

Q. Du[1], X. Liu[1], T. Shi[2], J. Long[1], W. Ma[1], G. Huang[1], X. Zhang[1]

[1] Clinical Laboratory Department, Sichuan Academy of Medical Sciences & Sichuan Province People's Hospital, Chengdu
[2] Luzhou Medical College, Luzhou (China)

Summary

Purpose: This study aimed to detect the maternal group B streptococcus (GBS) by real-time PCR method, aiming to understand its germ-carrying situations and clinical significance. *Materials and Methods:* The secretions of one-third inferior segment of maternal vagina of 1,540 pregnant and postnatal women were collected for GBS detection by real-time PCR method, and the impacts of positive results on the fetus were observed. *Results:* The detection rate of GBS was 5.6% (86/1540); the premature birth rate of GBS-positive pregnant women was 29.1% (25/86), the miscarriage rate was 19.8% (17/86), the premature rupture rate was 26.7% (23/86), and the fetal distress rate was 24.4% (21/86). *Conclusions:* The GBS germ-carriers showed increased rates of premature birth, miscarriage, premature rupture, and fetal distress, thus forming adverse effects towards the maternal and infant outcomes.

Key words: Group B streptococcus (GBS); Real-time fluorescence PCR; Impacts on mother and child.

Introduction

Group B Streptococcus (GBS) can cause a variety of diseases; it is a facultative anaerobic streptococcus, because it can also cause cows to suffer from the mastitis, thus it is also known as Streptococcus agalactiae. GBS infection was considered as the major cause of neonatal pneumonia, sepsis, and death in Western societies since 1970s [1].

In recent years, certain study have found that GBS is a very important perinatal pathogen, which could infect the uterus and fetal membranes mainly through the ascending-spreading along the parturient canal, thus causing an intrauterine infection and fetal asphyctic death, as well as chorioamnionitis, endometritis, and urinary tract infections, etc [2]. Meanwhile, it is also the pathogen in neonatal bacteremia, sepsis, pneumonia, and meningitis; the ankyrins on the GBS surface can adhere to the epithelium and endothelial cells, thus causing infections, and death cases of neonatal meningitis, which is through the key step of BBB permeation [3], often occurring in preterm children [4]. Western countries have given great importance to it for a long time. Edmond *et al*. [5] performed a meta-analysis and revealed that the average incidence rate of live births within three months was 0.053%, with an average mortality rate of 9.600%. USA Centers for Disease Control and Prevention (CDC) had developed the GBS screening and treatment guidelines, which has considerably reduced the incidence and hazards of perinatal GBS. According to the report, about 10% -30% pregnant women carried or were infected by this vaginal bacteria, and about half would transmit it to the newborn during childbirth, leading to the early (within seven days of birth) or late (seven days after birth) invasive infection. Breast milk contamination-caused late-onset infection cases are gradually increased [6]. The early invasive infections are mainly sepsis, meningitis, and pneumonia, with the mortality rate as about 5%. GBS infection could also cause premature rupture of membrane and amniotic infection. A study [7] reported that the PROM incidence of urinary GBS-positive pregnant women was 35%, and the incidence of chorioamnionitis and endometritis was about 21%. In addition, the GBS infections could also cause preterm birth, low-weight birth and very low-weight birth. Schrag *et al*. [8] reported that the common pathogen of early onset sepsis (EOS) of premature infants was still GBS. Scholars in Chinese Taiwan region believed that after giving antibiotics to prevent GBS prenatally, the early-onset GBS sepsis would significantly decrease [9]. In 2008, Barcaite *et al.* analyzed 31 literatures published from 1996-2006, which included 24,093 women from 13 countries, and found that the germ-carrying rate ranged from about 6.5% to 36% [10]. According to the report of USA CDC, there was on average 8,000 newborns that were infected by GBS annually, and the mortality was about 5% [11]. The domestic awareness on the hazards of perinatal GBS infection towards the mothers and children is relatively low. In recent years, there gradually appeared several GBS-cause serious infections in the mothers and children, suggesting that GBS causes serious harm and

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cannot be ignored. Recently, some domestic death cases caused by the GBS infection were reported. Deng *et al.* [12] performed the GBS detection towards 234 cases of paraffin specimens of neonatal lung tissues that died of pneumonia in Beijing Children's Hospital and the detection rate was 65%. The results showed that among the cases of newborn pneumonia death, GBS was first place of pathogens. The GBS carrying rate varies with races, geographies, and ages. According to the statistics, vaginal germ-carrying rate of pregnant women in Beijing was about 13%. The rectal and vaginal germ-carrying rate of Chinese pregnant women was about 5% to 20%, which could be transient, intermittent, and chronic. The pregnant women that carried the germ could cause much more serious complications towards the mothers and children and GBS was the most common neonatal infection pathogen in the developed countries [13]. Based on the GBS infection-related symptoms in pregnant women and newborns, as well as the severities of diseases, prenatal GBS screening was particularly important. The spread during birth was the main way of neonatal GBS infection. GBS-positive patients could be given am antibiotic prophylaxis, which could effectively reduce the neonatal early invasive infections triggered by the vertical transmission of GBS.

Currently, the GBS detection methods include the culture method and the real-time PCR method. The advantages and disadvantages of the former: it is easily performed, while time-consuming, requires more than 48 hours, the culture difficulties are high, it might be affected by many factors, especially by antibiotics, and the sensitivity is low. Real-time PCR technology refers to the addition of fluorophores into the PCR reaction system, then to the accumulation of fluorescent signals that is used to monitor the process of real-time PCR, and finally the template is analyzed by the amplification curve. With the emergence of real-time PCR technology and relevant PCR instrument, the conventional distal-end method used for the gene detection in the past is completely changed. Real-time PCR technology could rapidly and reliably perform GBS screening. This study used this technology to detect the GBS situations in pregnant women at 34-37 gestational weeks, aiming to understand their germ-carrying situations in the third trimester in Chengdu, as well as its impacts on the mothers and children.

Materials and Methods

Specimen collection

The study included 1,540 pregnant women, aged 21-45 years old, at 34-37 gestational weeks, and insisted on regular check-ups in the Obstetric Department of Sichuan Provincial People's Hospital during pregnancy. Vaginal swab: firstly, the excessive secretions were wiped from the genital tract, one sterile polyester swab was then placed in the one-third inferior segment of the genital tract to gently rotate and take the secretions along the genital tract wall, then placed back into a 2.0 pml preservation solution containing sterile swab casing, sealed for detection submission. Anal swab: the swab was carefully inserted into the anus at a depth of at least two to five cm above the anal sphincter, gently rotated along the intestinal wall to obtain the specimens, then placed back into the 2.0-ml preservation solution containing sterile swab casing, and sealed for the detection submission. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Sichuan Provincial People's Hospital. Written informed consent was also obtained from all participants.

Preparation of nucleic acid

The vaginal and rectal secretions were fully shaken, then squeezed the cotton swab, and drew 1.5 ml liquid into the centrifuge tube for centrifugation at 12,000 r/min for five minutes. The supernatant was then discarded, the precipitate was then added to 1.5 ml sterile saline and shaken evenly, followed by centrifugation at 12,000 r/min for five minutes. The supernatant was discarded, the precipitate was then washed three times, and re-suspended with 50 ul saline, then the extract solid content was added and high-speed vortexing was performed for five minutes, followed by instantaneous centrifugation, for ten minutes at 95°C heating, then ten minutes ice-bathed immediately and centrifugation at 12,000 r/min for another two minutes. The supernatant was then restored for the future detection.

Real-time PCR

Five-ul of the above supernatant was added to the prepared PCR reagents for PCR amplification reaction. The amplification parameters were 37°C for two minutes, 94°C for two minutes, 94°C for 20 seconds, and 55°C for 45 seconds, for 40 cycles.

Results analysis the condition settings

The setting principles of the threshold values of the GBS detection fluorescein FAM and the internal reference fluorescein Texas Red were the following: the thresholds were just above the highest point of the amplification curve of normal negative control (random noise threshold), and the Ct values, which were automatically analyzed and calculated by the instrument and shown in the Reporter window, were recorded.

GBS negative (below the detection limit): FAM Ct value = 40, Texas Red (internal reference) Ct value < 40, and there was a good logarithmic growth curve.

GBS positive: FAM Ct value ≤ 33, and there was a good logarithmic growth curve, reference Ct value (Texas Red) ≤ 40.

Invalid reaction: FAM Ct = 40, and Texas Red (internal reference) Ct value = 40, was re-determined.

Experimental gray zone: FAM 33 < Ct value < 40, caused by systematic or human uncertain factors, would normally lead to a repeat of the test twice for confirmation.

Quality control

Negative control GBS (FAM) Ct value = 40, reference (Texas Red) Ct value < 40, and there was a good logarithmic growth curve. Positive control GBS (FAM) Ct value < 33, and there was a good logarithmic growth curve; internal reference (Texas Red) Ct value ≤ 40.

Statistical analysis

The SPSS13.0 software was used for the statistical analysis, the χ^2 test was used, with $p < 0.05$ considered as the statistical significance.

Table 1. — *Comparison of GBS-DNA positive and negative towards the mothers and children [n(%)].*

Kind of distress	Cases	Preterm birth	Miscarriage	PROM	Fetal
GBS-positive	86	25 (29.1%)	17 (19.8%)	23 (26.7%)	21 (24.4%)
GBS-negative	1454	194 (13.3%)	60 (4.1%)	203 (14.0%)	152 (10.5%)
χ^2	8.33	16.58	5.93	9.63	
p		< 0.005	< 0.005	< 0.05	< 0.005

Results

The GBS-carrying rate detected in the perinatal pregnant women by the real-time PCR method was 5.6% (86/1540) (Table 1).

The preterm birth rate of GBS-positive pregnant women was 29.1% (25/86), and that of GBS-negative pregnant women was 13.3% (194/1454); the comparison exhibited a statistical significance of $p < 0.05$.

The miscarriage rate of GBS-positive pregnant women was 19.8% (17/86), and the comparison with that of the GBS-negative pregnant women (4.1%, 60/1454) revealed a statistical significance of $p < 0.05$.

The PROM rate of GBS-positive pregnant women was 26.7% (23/86), and the comparison with that of the GBS-negative pregnant women (14.0%, 203/1454) revealed a statistical significance of $p < 0.05$.

The fetal distress rate of GBS-positive pregnant women was 24.4% (21/86), and the comparison with that of the GBS-negative pregnant women (10.5%, 152/1454) revealed a statistical significance of $p < 0.05$.

Discussion

GBS is the conditioned pathogen that parasitizes in the human inferior digestive tract and urogenital tract. CDC (USA) specifically developed the GBS screening and treatment guidelines [14]. The literature reports a GBS infection rate from 5% to 35% [10, 15]. The clinical data of Thailand showed that between 1996 and 2001, the incidence of GBS infection decreased significantly, while the mortality rate still remained high and that 40% of early-onset children might die from this [16].

Different domestic and international studies have shown that the germ-carrying rates of pregnant women in late pregnancy varied in different regions. In Shanghai, the GBS-carrying rats in the third trimester was 3.7%, while in Beijing, it was 9.2%, and this study investigated 380 cases of pregnant women, and reported a GBS infection rate of 6.8%, close to that of Henan Province (6.3%). Among the 1,540 pregnant women in this study, the GBS-carrying rate was 5.6%, which might be closely related to the irregular application of antibiotics, Shanghai's living level and economic development is better in China, belong to the first-line city, therefore the density of its resident population towards medical intervention acceptance and antibiotics application was higher, and it could be an important factor that led to the lower detection rate. In addition, the populations with better living standards and economic conditions would have better personal hygiene habits that could also be an important factor that could reduce the detection rate. In such developed countries as USA that have much more standard antibiotics application, the GBS detection rate is relatively higher. Of course, the germ-carrying rate varies according to race, geography, and age, and is also affected by many factors such as gynecological inflammation, detection method, frequency, etc., and might also be related to the study's sample size. The sampling in late pregnancy could more accurately assess the situation [17].

In recent years, Western countries have conducted much research in GBS, and most scholars believe that is obviously related to the preterm birth, PROM, miscarriage, fetal distress, puerperal infection, neonatal pneumonia, and the neonatal mortality could be as high as 20% to 50% [18].

Previous study [19] considered that the GBS infection was one of the most important causes of premature birth, through the stimulation of intrauterine infection-released inflammatory mediators, such as the interleukin (IL) IL-1, IL-6, IL-8, IL-12, and other cytokines, as well as the phospholipase A and prostaglandin, which promote uterine contractions and caused the premature birth. PROM might also indirectly lead to the premature birth. In the present study, the premature birth rate of GBS-positive pregnant women was 29.1% (25/86), significantly higher than that of GBS-negative pregnant women (13.3%, 194/1454). However it should be noted that the current conventional treatments all prompted that GBS might easily lead to PROM, intrauterine infection, even neonatal sepsis, thus causing serious consequences towards the mothers and children, so that some medical institutions would perform an over-medical intervention in GBS-positive pregnant women, which might be one reason of high preterm birth rate in GBS-positive pregnant women.

It had been previously confirmed that the PROM rate of the patients with urogenital tract-GBS carrying was higher than those non-carriers, and in the PROM patients, the GBS positive rate was significantly higher than the normal pregnant women [12]. The infection is a major pathogenetic factor of PROM, among various pathogens that could cause infection such as Escherichia coli, mycoplasma urealytium, and GBS, etc. GBS exhibits the strongest adsorption and penetration abilities towards the chorion, therefore its complications are among the worst. Pregnant women that carry GBS would be prone to the occurrence of ascending infection, the direct invasion of proteolytic enzymes produced by the retrograde bacteria, combined with the phagocytosis of inflammatory cells produced by the stimulation of bacterial infections towards the body that would reduce the local tension of fetal membranes, leading to PROM. The

study [20] found that the PROM rate of GBS-positive parturients was higher than the negative ones, while among the parturients with PROM, the GBS-positive rate was higher than the normal ones. Another study reported that among 2,745 cases, the PROM rate of GBS-bacteriuria patients was 35%, while that of the non-bacteriuria patients was only 15%. Among the 60 PROM cases, 15 cases were found as the GBS positive, accounting for 25%, among which the cervical germ-carriers accounted for 53%, the vaginal germ-carriers accounted for 73%, and the anal germ-carriers accounted for 100% [21].

In this study, the PROM incidence of GBS-positive pregnant women was 26.7% (23/86), while that of the negative pregnant women was 14.0% (203/1454), the difference was statistically significant, therefore the PROM rate of perinatal GBS-positive women was significantly higher than the negative ones. In addition, the rates of abortion and fetal distress of GBS-positive pregnant women were also significantly higher than the GBS-negative cases, and the difference was statistically significance. Shi *et al.* [20] detected the rates of cesarean section and fetal distress in the GBS-positive pregnant women that were also significantly higher than the GBS-negative ones, and the difference was statistically significant ($p < 0.05$).

In summary, GBS infections could seriously affect maternal, fetal, and newborn health and positive and effective prevention and treatment measures would be important to reduce the incidence.

While in the clinical practices, the preventive measures are not widely applied, more than 90% GBS-DNA-positive pregnant women in this study were not subjected to prenatal and intrapartum preventive measures, but only administered ampicillin and clindamycin for postpartum infection prevention. Therefore, clinical practices should extend preventive measures to prenatal GBS-positive patients: firstly, the clinics should increase the screening efforts towards the advanced maternal and neonatal GBS infections, while continuously improving the specificity and sensitivity of detection methods, thus the GBS detection rate could be improved. In this study, the real-time PCR method was used as a supplement to the traditional bacterial culture method, and became a rapid, sensitive, and specific method towards prenatal GBS screening; thus it was worthy of the clinical application. Secondly, in order to reduce the serious harm caused by the GBS infections, the women at childbearing age should adopt good personal hygiene habits, maintaining the vulva clean, and timely treating gynecological inflammations, while reducing the chances of infection. In addition, in order to avoid the spread caused by healthcare workers when in contact with the fetus, the they should prepare their own personal hygiene, and carry out health education and psychological care towards the GBS- positive patients before birth, and timely identify the high-risk GBS-positive patients, eliminate their doubts in using antimicrobial drugs during pregnancy, and timely prenatal and postnatal interventions should be well-performed.

In short, GBS is a common pathogen that could seriously threat the health of both mothers and infants. It is very important for the pregnant women to perform vaginal and (or) rectal screening at 34 to 37 gestational weeks; the European and American countries have been already widely carried out this screening, and are achieving good results. The experience of advanced foreign countries provided the present authors with good reference, the comparison of GBS-carrying screening inside the suitable candidates between the present country and foreign countries was an important research topic. The fluorescent quantitative PCR is considered fast, highly accurate, and highly sensitive towards GBS detection [22]. The GBS-carrying rates in the pregnant women varied greatly in different foreign regions, for example it was 6.5%~36.0% in the European countries [23], 2% to 29% in USA, and 13% in Korea [22].

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Address reprint requests to:
Q. DU, M.D.
Department of Clinical Laboratory
Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital
49 Huanhua Binhe Road
Chengdu 610072 (China)
e-mail: qiongducn@126.com