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ORIGINAL ARTICLE

Study on the Correlation between Genital Tract Microenvironment and GBS Carrier Rate of Late-Stage Pregnant Women in Dongguan

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SUMMARY

Background: **The carrier rate of group B *Streptococcus* (GBS) in the genital tract of women in the late stage of pregnancy and its correlation with the genital tract microenvironment were investigated in a group of pregnant women in Dongguan, China, to provide a basis for the clinical prevention and treatment of GBS.**
Methods: **A retrospective analysis was done of the results of routine testing for GBS, leucorrhea and bacterial vaginosis (BV) in 6,166 women in the late stage of pregnancy (35 - 37 weeks of gestation) who underwent a prenatal examination at Dongguan Southeast Central Hospital from January 2018 to December 2020. GBS positivity was detected by RT-PCR. Normal saline floating microscopy was used to detect routine indicators of leucorrhea, including white blood cells (WBCs), *Lactobacillus* (Lab), vulvovaginal *Candida* (VVC), and trichomoniasis (TV). BV was detected based on an enzymatic reaction. The correlation between GBS infection and age and the vaginal microenvironment was determined statistically.**
Results: **The rate of GBS positivity was 10.53% (649/6,166) and was statistically significant for women 20 years of age ($p < 0.05$). Logistic regression showed that abnormal VVC, TV, BV, WBCs, and *Lactobacillus* were associated with GBS infection. The results of a rank sum test of the WBC group showed that the infection risk in groups with < 15 WBCs/hpf increased as the WBC count increased, but there was no statistical difference between groups with > 15 WBCs/hpf. The rank sum test results for *Lactobacillus* showed a significant difference between the abnormal and normal and other groups, but no significant difference between the other groups.**
Conclusions: **The overall carrier rate of GBS in the genital tract of late-stage pregnant women in Dongguan was 10.53%. GBS infection is related to the genital tract microenvironment. Our results provide a basis for the prevention and treatment of clinically confirmed GBS.**
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KEYWORDS

Dongguan, group B streptococcus, carrier rate, vaginal microenvironment

INTRODUCTION

Group B *Streptococcus* (GBS), or *Streptococcus agalactiae*, is a β-hemolytic Gram-positive coccus and common opportunistic pathogen in the lower digestive

and urogenital tracts of healthy people [1]. However, GBS in the genital tract or rectum of pregnant women can cause a variety of adverse pregnancy outcomes, including premature birth, premature rupture of membranes, stillbirth, and intrauterine infection [2]. Newborns of GBS-positive mothers are at risk for neonatal pneumonia, sepsis, meningitis, and other diseases, and the adverse impact on the neonatal nervous system is irreversible [3]. The infection rate of GBS is between 10% and 30%, with regional, ethnic and socioeconomic-level differences but also depending on the detection method [4]. The GBS infection rate in Dongguan, China, is unclear and there have been relatively few studies on the genital tract microenvironment. Therefore, we investigated the GBS infection rate of late-stage (35 - 37 weeks) pregnant women in Dongguan, China, as well as the relationship between GBS infection and the vaginal microenvironment. Our results provide a basis for the prevention and treatment of clinically confirmed GBS.

MATERIALS AND METHODS

General information

The study population consisted of 6,166 women at the late stage of pregnancy (35 - 37 weeks) who underwent a prenatal examination at Dongguan Southeast Central Hospital from January 1, 2018, to December 31, 2020. The average age of the participants was 28.2 ± 4.8 years (range: 15 - 47 years), including 358 in the ≤ 20 years group (group A), 3,649 in the 21 - 30 years group (group B), 2,104 in the 31 - 40 years group (group C) and 55 in the ≥ 41 years group (group D).

Methods

Specimen collection

GBS test samples were collected according to perinatal GBS prevention guidelines [5] formulated by the Centers for Disease Prevention and Control (CDC) of the United States. In this method, vaginal and rectal samples are collected at the same time as follows. The vulva is cleaned and then a test paper is inserted at a depth of 1/3 below the vagina. The test paper is rotated several times and then inserted into the rectum at a depth of 2 - 5 cm. The paper is again rotated several times before it is removed and placed into the collection tube. The samples are then sent to the laboratory for testing. Leucorrhea samples are obtained after the GBS samples, using sterile cotton swabs to collect vaginal secretions under endoscopic guidance. Two samples are collected: one for routine leucorrhea detection and the other for bacterial vaginosis (BV) detection. The two samples are placed in test tubes and sent to the laboratory for detection within 30 minutes.

Specimen detection

Quantitative GBS detection was achieved using real-time quantitative fluorescent PCR (RT-qPCR) and a kit from the Borcheng Co. (Beijing, China). The quality control sample, the negative and positive control samples, and the standards were also from Borcheng Co. RT-qPCR was conducted on an ABI 7500 real-time fluorescent quantitative PCR instrument (Thermo Fisher Scientific, Waltham, MA, USA) maintained and calibrated according to the manufacturer's instructions. Leucorrhea was determined by normal saline floating microscopy and BV using an enzyme reagent kit (Zhuhai Yinke Co., Guangdong, China). The detection method and interpretation of the results were as described by the manufacturer.

Observation indicators

Information collected from the participants included gestational weeks and age. In the analysis of the GBS test results, the cutoff for positivity was ≥ 1.0e3; values below the cutoff were considered negative. Leucorrhea detection indicators included *Lactobacillus* abnormalities, white blood cells (WBCs), vulvovaginal candidiasis (VVC), trichomoniasis (TV), and BV. Lactobacilli were divided into four grades: high, medium, low, and none. The WBC count was divided into six groups based on the number of cells per high-power field (hpf): 0, 1 - 5, 5 - 15, 15 - 30, and > 30.

Statistical methods

All data were analyzed using Microsoft Excel 2013 (Redmond, WA, USA) and SPSS 23.0 (IBM Corp., Armonk, NY, USA) software. The counting data and rate were compared in a χ^2 test and measurement data between two groups in a *t*-test. A logistic regression analysis was used to identify correlations, and the Wilcoxon test to compare rank data. The test standard was a = 0.05. A p-value < 0.05 was considered to indicate a statistically significant difference.

RESULTS

GBS carrying rate

Of the 6,166 specimens collected in this study, 649 (10.53%) were GBS-positive.

Relationship between GBS infection and age

Group A accounted for 5.81% (358/6,166) of the samples, and the GBS positivity rate was 5.31% (19/358). Group B contributed 59.18% (3,649/6,166) of the samples and had a GBS positivity rate of 10.74% (392/3,649). The corresponding values in group C were 34.12% (2,104/6,166) and 11.12% (234/2,104), and in group D 0.89% (55/6166) and 7.27% (4/55). The difference in the positivity rate between group A and all other groups was significant (p < 0.001), but the differences in the positivity rates of the other groups were not (p > 0.05) (Table 1).

Table 1. Relationship between GBS infection rate and age.

Age group (years)	Number of women (n)	Number of positive cases (n)	Positivity rate (%)
15 - 20	358	19	5.31
21 - 30	3,649	392	10.74
31 - 40	2,104	234	11.12
≥ 41	55	4	7.27
Total	6,166	649	10.53

Table 2. Logistic regression analysis of VVC, TV, BV, WBC, and *Lactobacillus.*

Group	Number of samples (n)	GBS positivity (n)	Positivity rate (%)	p	OR	95% CI
WBC	5,230	617	11.80	< 0.001	3.779	2.630 - 5.429
VVC	975	143	14.67	> 0.001	2.264	2.151 - 3.247
TV	23	5	21.74	0.033	3.052	1.096 - 8.500
BV	537	92	17.13	< 0.001	1.873	1.478 - 2.373
Bacillus	4,157	470	11.31	< 0.001	1.492	1.218 - 2.652

Relationship between GBS infection and the vaginal microenvironment

The WBC results were divided into negative and positive groups according to the presence and absence of bacilli. The VVC, TV, and BV results were divided into negative and positive groups according to normal and abnormal (none and rare) groups. The results of a binary logistic regression analysis showed significant ($p < 0.05$) differences in the GBS positivity rates in each group. The odds ratios (ORs) and 95% confidence intervals (CIs) for groups A, B, C, and D were 3.779 (2.630 - 5.429), 2.264 (2.151 - 3.247), 3.052 (1.096 - 8.500), and 1.873 (1.478 - 2.373), respectively.

Relationship between GBS infection and WBC and bacilli classification

The results of the rank sum test showed that the GBS infection rate in the WBC and *Lactobacillus* normal and abnormal groups was statistically significant ($p < 0.05$). There was a significant difference between the normal *Lactobacillus* group and both the rare group and none groups ($p < 0.05$), but not between the latter two groups ($p > 0.05$). Compared with other groups, the p-values of the WBC 0/hpf, 1 - 5/hpf and 5 - 15/hpf groups were all < 0.05, indicating statistically significant differences. The p-values of the 15 - 30/hpf and > 30/hpf groups and of the full field group were > 0.05, indicating no statistically significant differences.

DISCUSSION

GBS is a conditional pathogen existing in the lower digestive tract and genital tract of healthy people. However, in pregnant women and the immunocompromised it can cause various diseases. Infections in newborns may lead to asphyxia, hypoxia, and other pathologies. The impact on the neonatal nervous system is irreversible. Therefore, screening for GBS in late pregnancy is particularly important. The infection rate of GBS differs depending on the geographic region, population, nationality, age, number of pregnancies, and the sampling and detection methods. There are also large differences between studies. In their meta-analysis of the global infection rate of GSB, Russell et al. [5] determined a positivity rate of 11 - 35%, with significant differences in different regions and populations. For example, in Southeast Asia the positivity rate was 11.1%, and the rate in pregnant women at 35 - 37 weeks of gestation was 10.53%. Similar results were reported in European and American countries. Given the importance of GBS in pregnancy screening, research on GBS infections rates has increased. However, as China is a very large country, regional differences in GBS positivity rates have been reported: 3.1 - 7.0% in northern China [6,7], 4.5 - 11.35% in southern China [8], 3.7 - 9.29% in eastern China [9,10], 4.05 - 14.29% in western China [11,12], and 7.22 - 9.95% in central China. The rates in several cities have been reported as well: Guangming, District of Shenzhen (11.35%), Longhua, District of Shenzhen (4.5%), Huizhou (18%) [13], Zhongshan (6.7%), Fo-

shan (7.09%), Hong Kong (21.8%), and Macao (17.1%).

In this study, vaginal-rectal joint sampling was conducted and the samples were analyzed by PCR. The positivity rate from vaginal sampling alone is lower than that from rectal sampling alone, and both are lower than the rate from combined sampling. American CDC guidelines and some experts in China recommend mixed sampling to improve the detection rate. The culture method is the gold standard for the detection of GBS, but PCR is convenient, fast and allows quality control. While the PCR method has a slightly higher positivity rate, it shows good consistency with the culture method. In this study, the difference in GBS infection rate compared to the published literature persisted after correction for the sampling and detection methods. However, Dongguan is a city with a large and mobile population, such that study participants may have come from all over the country or from other countries. There may also be differences arising from a long vs. a short residency time in Dongguan. Other factors that may affect GBS infection include age and the microenvironment of the genital tract.

The study participants were between 15 and 47 years of age, with most between 21 and 40 years of age. The GBS positivity rate in our study population as a whole was 10.53%, whereas the rate in the 21 - 40 age group was 10.93%. However, due to the small number of participants > 40 years of age, the results should be interpreted with caution. The positivity rate of the group younger than 21 years was 5.3%, which was significantly different from the overall rate; however, there was no statistically significant difference between the other groups. In China, research on the relationship between age and GBS infection has yielded inconsistent results, which may be related to hormone levels, pregnancy times, the vaginal microenvironment and other factors. In participants with VVC, TV, or BV, GBS positivity was significantly increased, by 2.264-, 3.052- and 1.873-fold, respectively. The incidence rate of VVC in pregnant women is higher than that in non-pregnant women, which may be related to factors such as fungal adhesion, extracellular enzymes and morphological changes, as well as the hormone level and immune state of the body. *In vitro* experiments have shown that estrogen and progesterone inhibit the response of *Candida*-specific human peripheral blood lymphocytes and that the response correlates positively with the hormone level. A *Candida* infection inhibits the action of lactobacilli in the vaginal microenvironment, which further increases the risk of GBS infection. The optimum pH for the survival of *Trichomonas* is 5.2 - 6.6, and growth is inhibited at a pH < 5.0. TV is a common sexually transmitted disease; however, pregnant women have increased estrogen levels and are resistant to *Trichomonas* infections. A *Trichomonas* infection causes the breakdown of a large amount of glycogen, which in turn reduces *Lactobacillus* and increases the vaginal pH, often resulting in mixed infections (e.g., BV or VVC) and disruption of the self-cleaning effect of the vaginal microenvironment, facilitating GBS infection. Only 23 of the 6,166 samples were *Trichomonas*-positive. The positivity rate of 0.37% was lower than the 1.7 - 7.0% reported in the literature, but the highest rate reported is 21.74%. BV is usually caused by a decrease in hydrogen-peroxide (H_2O_2)-producing *Lactobacillus* in the vaginal microenvironment, an increase in harmful flora such as *Gardnerella* and an increase in vaginal pH, which further increases the risk of GBS infection.

In this study, lactobacilli were initially divided into four groups according to the standard, but in routine microscopic detection, medium and small amounts were not easy to distinguish, and there was great subjectivity. Therefore, the two groups were combined, resulting in comparisons of normal, rare, and none groups. The rank sum test showed significant differences in the GBS infection rate between the normal group and the other two groups, but not between the rare group and the none group. Rosen [14] reported similar results. Marziali [15] and others have shown that only specific lactobacilli inhibit the growth of GBS and that the H^+ produced by *Lactobacillus* plays an important role in preventing a GBS infection, as do the non-sugar substances on the surface of these bacteria. Therefore, the reduction in lactobacilli, especially H_2O_2-producing strains, increases the risk of a GBS infection. In addition, according to the rank sum test results of leucocytes, for a WBC count < 15/hpf, the GBS infection risk increased with the increasing number of WBCs, whereas there was no significant difference between groups with a WBC count > 15/hpf. Taken together, the WBC and *Lactobacillus* results suggest that changes in the vaginal microenvironment increase the risk of GBS infection. This risk may be related to the fact that the risk increases with the decrease in *Lactobacillus* until a certain level is reached and then does not change after that level is exceeded. However, the roles of different *Lactobacillus* species and virulence of strains of GBS as well as of other factors should be explored in further research. The GBS infection rate of late-stage pregnant women in Dongguan has gained the attention of clinicians and researchers in China. The results of this study suggest that the infection rate is closely related to the vaginal microenvironment. Thus, in the clinical prevention and treatment of GBS infections, monitoring of the vaginal microenvironment is critical.

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Declaration of Interest:

Authors have no conflict of interest to declare.

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