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Original Article

Oral *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 to reduce Group B *Streptococcus* colonization in pregnant women: A randomized controlled trial

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ABSTRACT

Objective: This study is to examine the effect of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 taken orally before bedtime on Group B *Streptococcus* (GBS)-positive pregnant women with respect to becoming GBS negative.
Materials and Methods: In total, 110 pregnant women at 35–37 weeks of gestation who were diagnosed by GBS culture as being GBS positive for both vaginal and rectal GBS colonization were randomly assigned to be orally treated with two placebo capsules or two probiotic capsules (containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14) before bedtime until delivery. All women were tested for vaginal and rectal GBS colonization again by GBS culture on admission for delivery.
Results: Of the 110 participants, 99 completed the study (49 in the probiotic group and 50 in the placebo group). The GBS colonization results changed from positive to negative in 21 women in the probiotic group (42.9%) and in nine women in the placebo group (18.0%) during this period (Chi-square $p = 0.007$).
Conclusion: Oral probiotic containing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 could reduce the vaginal and rectal GBS colonization rate in pregnant women.

Introduction

Group B *Streptococcus* (GBS) is an encapsulated Gram-positive coccus that colonizes the gastrointestinal and genital tracts of 15–40% of pregnant women [1]. Although GBS colonization usually remains asymptomatic in these women, vertical transmission may occur when GBS ascends from the vagina to the amniotic fluid after the onset of labor or rupture of the membranes. In some cases, transmission can take place with intact membranes [2,3]. During the past 2 decades, GBS has been considered to be a leading cause of primary neonatal sepsis, pneumonia, and meningitis in the 1st week of life, which is known as early-onset GBS infection [4]. *Intrapartum* parenteral antibiotic prophylaxis for women with a positive GBS culture at 35–37 weeks of gestation is recommended by the Centers for Disease Control and Prevention (CDC). According to the 2010 CDC guidelines, a minimum of 4 hours of *intrapartum* antibiotic prophylaxis prior to delivery is recommended to prevent early-onset GBS infection because therapeutic drug level may not be achieved with < 4 hours of treatment [5]. Preliminary studies and data from a large health maintenance organization showed that 40–50% of GBS-colonized multiparous women are not able to receive antibiotics at least 4 hours before delivery due to the rapidity of their labor [6]. Newborns from GBS positive women with inadequate *intrapartum* antibiotic prophylaxis are deemed at risk. Low-risk infants are recommended to undergo 48 hours of observation. High-risk infants should additionally undergo blood cultures and a complete blood count [5]. GBS disease is not easily resolved by antibiotic treatment of the pathogen. Thus, such traditional approaches need to be re-evaluated.

Lactobacilli are the dominant bacteria of the vaginal flora. They possess antimicrobial properties that regulate other urogenital

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microbiota. The possible mechanisms of the probiotics acting in the vagina include modulation of host immunity, alteration of the microenvironment to be less receptive to pathogens (i.e. the production of lactic acid, bacteriocin, biosurfactants, hydrogen peroxide, and signaling compounds), and dislodging pathogen biofilms [7]. Using probiotic during pregnancy has an excellent safety record [8]. Taking oral probiotics containing lactobacilli daily has been shown to maintain normal lactobacilli vaginal flora [9]. Reid et al [10] showed that *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 are antagonistic to the growth and adhesion of various intestinal and urogenital pathogens, including GBS, *Gardnerella vaginalis*, and uropathogenic *Escherichia coli*. A study by Altoparlak et al [11] showed a negative correlation between the colonization rate of vaginal lactobacilli and GBS in pregnant women. The lactobacilli colonization rate was 21.3% in the GBS positive group, and 47.6% in the GBS negative group [11]. These results suggest that lactobacilli play a role in preventing vaginal colonization by GBS. The study of Velraeds et al [12] has also shown that certain lactobacilli can inhibit the growth and adhesion of streptococci *in vitro*. However, their ability to do so *in vivo* needs further testing.

Most research to date has focused on the potential of probiotics to prevent bacterial vaginosis and preterm labor. The effectiveness of probiotics as a surrogate or adjunctive therapy for *intrapartum* antibiotic prophylaxis in GBS colonized pregnant women has not been evaluated. To our knowledge, the present study is the first to investigate the role of probiotics in preventing and treating vaginal colonization by GBS in pregnant women. The purpose of this study was to examine whether oral *Lactobacillus*-containing probiotics can reduce the vaginal and rectal GBS colonization rate in GBS positive pregnant. Through the results of our study, we try to investigate the role of probiotics in preventing unnecessary tests, admission, and antibiotic treatments in newborns from GBS-positive mothers. We hope our study will have some impact on GBS sepsis protocols.

Materials and methods

This study was a prospective, double-blind randomized clinical trial that was performed in the Obstetric Department of the China Medical University Hospital, Taichung, Taiwan from March 1, 2011 to December 30, 2011. This research study was approved by the China Medical University Hospital Institutional Review Board (DMR99-IRB-309) and registered in the Clinical Trials.gov Protocol Registration and Results System (registration number NCT01577108). Written informed consent was obtained from each participant.

The inclusion criteria of our study were pregnant women, singleton pregnancy, with a positive GBS screening culture at 35–37 weeks of gestation. They agreed, throughout the trial period, to abstain from the use of any systemic or intravaginal antibiotic, antifungal agents, or any other intravaginal product (e.g., contraceptive creams, lubricants, and douches). The exclusion criteria included multiple gestations, pregnant women with impaired immunity, diabetes, or any other kind of significant disease or acute illness that could complicate the evaluation of the results. Pregnant women who received vaginal or systemic antibiotics and antifungal therapy within 2 weeks of the screening visit were also excluded.

Vaginal and rectal GBS screening cultures by swabbing both the lower vagina and rectum (through the anal sphincter) were performed for all pregnant women at 35–37 weeks of gestation in our outpatient department. The specimens were transported to laboratory as soon as possible and inoculated into the Lim broth. Women with vaginal and rectal GBS colonization were invited to participate in our study. Informed consent was obtained from each participant. The trial patients were doubled-blind computerized randomized by the hospital pharmacy. Each woman was assigned a number. Identical looking probiotic and placebo capsules were prepared and distributed in numbered containers by the pharmacy. The patients were provided two capsules of probiotics or placebos to be taken once daily at bedtime until delivery. The probiotic capsules contained dried viable *L. rhamnosus* GR-1 and *L. reuteri* RC-14, and each capsule contained 1×10^9 viable cells of both strains. The *L. rhamnosus* GR-1 and *L. reuteri* RC-14 strains were encapsulated in gelatin capsules, which were produced by Chr. Hansen (Horsholm, Denmark) using good manufacturing practices (U-relax). The placebo capsules contained the same composition (dextrose anhydrate, potato starch, microcrystalline cellulose, and magnesium stearate, gelatin, and titanium dioxide) except for the *L. rhamnosus* GR-1 and *L. reuteri* RC-14 strains. Vaginal and rectal GBS cultures were repeated for all participants at the time of admission for delivery. All the participants were treated according to the CDC's 2010 guidelines on GBS (GBS-positive mothers as assessed by culture at 35–37 weeks of gestation should receive at least 4 hours of *intrapartum* antibiotic prophylaxis) on admission for labor.

The primary outcome was the absence of vaginal and rectal GBS colonization in pregnant women who presented as GBS positive at 35–37 weeks of gestation after probiotics or placebo treatment. The secondary measures were the relationship between parity and newborn transfer units, and the cause of admittance to the neonatal unit. The Wilcoxon rank-sum test and Chi-square test were used to test for significant differences between the two groups in terms of maternal age, maternal weight, education level, parity, gestational week of delivery, duration of drug taking, neonatal birth weight, newborn transfer units, and Apgar scores. The Chi-square test was also used to evaluate the difference in the primary outcome between probiotics and placebo groups, the cause of admission to the neonatal unit, and the relationship between parity and newborn transfer units. Assuming GBS culture to become negative in $> 40\%$ of the participants in the study group and not $> 15\%$ in the placebo group, a sample size of 50 women per group was considered sufficient to reach 80% statistical power. All statistical analyses were calculated using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered to be statistically significant.

Results

The study was conducted in our hospital from March 2011 to December 2011. During the trial period, 1210 women, at 35–37 weeks of gestation, underwent vaginal and rectal GBS screening cultures in our outpatient department; 219 women had positive culture results. The GBS colonization rate was 18.1%. There were 110 pregnant women enrolled in the study. Overall, 99 participants completed the study. Three participants, two in the study group and one in the control group, dropped out of the study because of failure to undergo the GBS culture before delivery. Eight participants, four in the study group and four in the control group, withdrew from the study due to personal reason. A CONSORT flow diagram depicting information about the number of participants at the different stages of the trial is shown in Figure 1.

The demographic and clinical characteristics of the two groups are shown in Table 1. There were no significant differences between the two groups in terms of the following factors: maternal age, maternal weight, education level, parity, gestational week of delivery, duration of drug taking, neonatal birth weight, newborn transfer units, and Apgar score. There were no adverse treatment effects in terms of nausea, vomiting, diarrhea, abdominal pain, skin rash, or systemic infections after taking the capsules in any of the participants during the trial. In total, 24 neonates were transferred