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Original article

Relationship between cord blood vitamin D level and group B *Streptococcus* vaginal carriage rate in pregnant women

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SUMMARY

Background & aims: Group B streptococci (GBS) are a major cause of neonatal infection. The existence of a link between levels of vitamin D and vaginal carriage of GBS was investigated.
Methods: Vitamin D level, assayed from umbilical venous blood at birth, and the status of GBS vaginal carriage during the last term of pregnancy were available from 2246 mother–child couples.
Results: Levels of vitamin D in cord blood at birth was deficient (<10 ng/ml), insufficient (10 to 30 ng/ml), or normal (>30 ng/ml) in 32.1%, 53.0% and 14.9% of tested subjects, respectively. Levels differed significantly depending on the season of delivery, the duration of exposure to sunlight, the skin phototype, and on vitamin D supplementation. The percentage of GBS vaginal carriage was 15.4. A negative association was observed between GBS vaginal carriage and level of vitamin D ($P < 0.01$ by chi-square test). No relationship was established between GBS colonisation (27 cases identified) or infection (7 cases identified) and vitamin D status.
Conclusions: A correct vitamin D level was associated with a lower rate of GBS vaginal carriage during pregnancy. This calls for the monitoring of vitamin D status in pregnant women, a screen which could greatly reduce the need for intrapartum antibiotic prophylaxis.
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1. Introduction

Group B streptococci (GBS) are gram positive bacteria originating from the intestinal flora that can colonize the vagina, especially during pregnancy. They can be transmitted to the newborn during his/her passage through the birth canal, or by upward migration in the event of ruptured membranes, and then colonize skin, nasopharynx or bowel and, in some cases, be responsible for early perinatally-acquired infection, called early-onset disease (EOD), by contrast to late-onset disease (LOD), which is characterized by meningitis occurring after the first week of life and mainly linked to GBS ST-17, a hypervirulent clone of GBS.[1,2] To prevent the potential problems listed above, current guidelines recommend a systematic screening of all the pregnant women through a vaginal or rectal sample taken between the 34th and 38th week of gestation (WG) in France, or, the 35th and 37th WG in North America. This sampling is followed by the use of intrapartum antibiotic prophylaxis for women presenting with a GBS positive result[3,4] (http://www.has-sante.fr/portail/upload/docs/application/pdf/Antenatal_prevention.pdf). The application of these recommendations has resulted in a significant drop in the prevalence of GBS diseases in concerned countries, but may have contributed to the increased resistance of this bacterium to antibiotics.[5–8] Consequently, complementary approaches to antibiotic therapy for the prevention of GBS diseases are welcomed.

During the past years, vitamin D has been increasingly recognized as an important modulator of bacterial infection (for reviews, see[9,10]), notably during the course of pregnancy.[11,12] Vitamin D deficiency in

Abbreviations: GBS, group B streptococcus; NS, not significant; WG, weeks of gestation.

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the mother or the newborn has been associated with increased risk of immune-mediated diseases in the offspring, such as respiratory syncytial virus bronchiolitis, wheezing, and asthma,[13,14] but also bacterial vaginosis in the mother.[15,16] In accordance with these observations, we hypothesized that vitamin D may play a key role in the control of GBS vaginal proliferation in pregnant women.

The main objective of the present study was to explore the relationship between neonatal vitamin D status, which is a good proxy for maternal vitamin D status, and pre-delivery GBS vaginal carriage in the mother. An additional objective was to identify whether factors that influence the level of vitamin D[17] could modify the incidence of GBS vaginal carriage and the subsequent occurrence of neonatal infection.

2. Materials and methods

2.1. Study design

A prospective single centre study was carried out in the maternity ward of the University Hospital of Saint-Etienne, France, from the 1st of December 2006 to the 31st of December 2007 on a total of 3263 mother–child couples. Mother–child couples were eligible for participation in our study if a live birth occurred, whether preterm (defined as born before 37 weeks of gestation) or at term. Prior to obtaining consent, a clear description of the study was provided to all interested and eligible mothers. The study was approved by the local Ethics Committee.

Date of birth, sex and birth-term status were obtained from the medical files. In case of prematurity, the gestational age was recorded in WG.

2.2. Vitamin D measurement and categorization

For each newborn included in the study, an umbilical cord blood specimen was collected in the delivery room, which prevented the use of more invasive venous sampling methods. A 25[OH] D assay was carried out on this sample using a radio-immunological method until July 28th, 2007 (DiaSorin 25[OH] RIA assay®, Antony, France). After this date, a competitive chemiluminescence immunoassay (DiaSorin 25[OH] D Liaison automated immunoassay CLIA®) was used to quantify Vitamin D levels. Both tests were used according to the manufacturer's instructions. According to DiaSorin, the technical sensitivity of the two tests is 5 and 4 ng/ml, respectively, whereas specificity is 100% for 25-OH-D_3 for both tests. Specificity is also at 100% for 25-OH-D_2 for the first test and 104% for the second test. To our knowledge, there are no unanimously recognized reference values for the level of vitamin D in umbilical cord blood.[17–19] Recently, authors proposed a classification based on three categories termed: (i) deficient if under 15 mg/ml, (ii) insufficient if comprised between 15 and 30 mg/ml, and, (iii) sufficient if above 30 mg/ml.[20] In the current study, the value of 10 mg/ml, cited in previous studies,[21,22] was used as detection threshold (and not 15 mg/ml). Except for this difference, the same categories as those proposed above[20] were used.

2.3. Factors contributing to variations in vitamin D level

A questionnaire pertaining to the factors that might affect vitamin D levels was completed by all mothers participating in the study. The following items were recorded:[17] (i) skin phototype as light or dark (olive and black skins were included within the same category because the number of women with black skin was low), (ii) exposure of face and forearms to sunlight during the last trimester of pregnancy (<1 h, 1–2 h or >2 h per day), (iii) consumption of food or dietary supplements known to improve the vitamin D levels (e.g., fatty fish or cod liver oil at least once a week; mushrooms, eggs, milk, cheese or butter at least once a week), and (iv) vitamin D_2/D_3 supplementation.

2.4. Screening for vaginal carriage in the mother

The screening for SGB vaginal carriage was performed according to French recommendations (http://www.has-sante.fr/portail/upload/docs/application/pdf/Antenatal_prevention.pdf). A vaginal sample was obtained between the 34th and 38th WG. The screening test was carried out in different laboratories on a single vaginal swab and consisted of a culture on blood medium without a selective enrichment step. In case of preterm birth, an intrapartum vaginal sample was tested.

For the purpose of the study, results from vaginal testing were collected retrospectively from the patient's obstetric history file. The collected data gave rise to three possible outcomes: absence of GBS by culture, presence of GBS by culture, and no bacterial culture performed.

2.5. Diagnosis of infection in the newborn

When an infectious risk was suspected at birth – notably in case of GBS vaginal carriage, sepsis or fever in the mother, and clinical abnormality, respiratory distress, fever or unexplained prematurity in the neonate-, gastric fluid and possibly swabs from ear, throat and anus were collected within the first twelve hours of life for microbiological cultures, together with the search for central markers of infection including blood count, C-reactive protein and sometimes procalcitonin dosages. When appropriate, placenta, blood or cerebrospinal fluid was cultured.

A GBS disease was defined as the detection of GBS at least in peripheral samples associated to clinical or biological stigma of infection (i.e. C-reactive protein >20 mg/l or procalcitonin >18 ng/l before 12 h of life). Only cases where GBS diseases occurred during the first seven days of life were taken into consideration.

2.6. Statistical analysis

Depending on the nature of the variables that were investigated, the results were expressed in percentage or mean, together with standard deviation (SD). Categorical variables were compared using the chi-square test or the Fisher exact test. Means were compared using variance analysis. The correlation between vaginal carriage and various factors connected with vitamin D was studied by univariate and multivariate logistic regression analyses. Results were considered significant when P values were under 0.05.

3. Results

3.1. Characteristics of the study population

From the 1st of December in 2006 to the 31st of December in 2007 (13 months), a vitamin D measure was available for 2393 newborns. The characteristics of this population were compared to those of the 3263 births having occurred during the same period in our hospital. The two populations exhibited a similar profile with the exception of two items: (i) less assays were performed in premature babies, probably in relation with the suddenness of birth and the lower amount of available blood, and (ii) more questionnaires were filled out by those mothers whose baby was tested for vitamin D (Table 1).

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