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CLINICAL ARTICLE

Maternal 25-hydroxyvitamin D level and the occurrence of neural tube defects in Tunisia

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Objective: To determine whether low vitamin D levels in pregnant women are associated with the occurrence of neural tube defects (NTDs) in Tunisia. *Methods:* In a prospective study, pregnant women were recruited at a center in Tunis between January 1, 2012, and December 30, 2013. Women carrying a fetus with a severe NTD were recruited before elective termination. Matched, healthy pregnancy women were enrolled into a control group. Plasma levels of 25-hydroxyvitamin D were measured by a competitive chemiluminescence immunoassay. *Results:* Overall, 68 women formed the NTD group and 64 the control group. The mean maternal vitamin D level was significantly lower in the NTD group (20.65 ± 10.25 nmol/L) than in the control group (28.30 ± 13.82 nmol/L; $P < 0.001$). Vitamin D deficiency was recorded for 53 (78%) women in the NTD group and 39 (61%) in the control group. Vitamin D insufficiency was recorded for 15 (22%) women in the NTD group and 20 (31%) in the control group. Vitamin D sufficiency was found only in the control group ($n = 5$ [8%]; $P < 0.001$). *Conclusion:* The findings confirm an association between a decreased vitamin D level in pregnant women and the risk of fetal NTDs.

1. Introduction

Vitamin D is a fat-soluble vitamin that has key roles in calcium–phosphorus homeostasis and bone mineralization. The main source is synthesis in the skin following exposure to ultraviolet light, with dietary intake making only a small contribution to the body's vitamin D supply [1]. The impact of vitamin D on aspects of health has attracted much attention. Not only does vitamin D deficiency lead to rickets and reduced bone mineral density, but it has also been linked with many other health conditions, including diabetes mellitus, hypertension, stroke, autoimmune diseases, epilepsy, multiple sclerosis, and cancer [2].

In pregnancy, an adequate vitamin D status is imperative for fetal skeletal growth, maternal health, and optimal maternal and fetal outcomes. Low vitamin D levels during pregnancy and infancy can lead to adverse outcomes, such as neonatal hypocalcemia, a low birth weight, bone fragility, poor postnatal growth, and autoimmune diseases [3]. A meta-analysis of observational studies [4] confirmed an association between vitamin D insufficiency and small-for-gestational-age (SGA) birth. However, a previous observational study [5] did not find an association between vitamin D deficiency and birth weight or length. Javaid et al. [6] reported that bone mass was decreased among the children of mothers with reduced vitamin D concentrations, but a subsequent prospective and well-designed study [7] did not find any association. Additionally, a systematic review [8] indicated that vitamin D status during pregnancy could affect risk of multiple sclerosis among newborns, particularly in areas with poor sunlight exposure.

In a Spanish cohort [9], a positive correlation was found between maternal vitamin D status and child mental and psychomotor development scores at age 14 months. Maternal vitamin D insufficiency has been linked with impaired language development in school-aged children [10] and with autism spectrum disorder [11], indicating that vitamin D has crucial roles in neuronal function, gene regulation, and brain development. Animal studies have indicated that key enzymes and receptors implicated in the metabolism of vitamin D are expressed in the rat brain [12], and an in utero deficit of vitamin D has been correlated with irregular brain development in experimental animals [13]. In another study in rats [14], it was shown that a lack of vitamin D leads to considerable changes in brain development by affecting cellular proliferation, the gross morphology of the brain, and growth factor signaling.

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Neural tube defects (NTDs) are fairly common congenital malformations that arise during neurulation in early embryonic development. Spina bifida and anencephaly—caused by incomplete closure of the neural tubes—are the most prevalent subtypes, occurring in one in 1000 pregnancies [15]. A previous study [16] highlighted a significant increase in the prevalence of NTDs in Tunisia from 1991 to 2011, with a median prevalence during this period of 2.03 ± 0.87 per 10 000 births. The etiology of NTDs is multifactorial and complex: genetic, lifestyle, and environmental factors all have a role [17]. Published evidence on the association between vitamin D levels in pregnant women and NTDs is limited. A previous study [18] found that vitamin D was not associated with the occurrence of such defects.

As far as we are aware, there has been no study of the association between vitamin D status and the risk of NTDs in Tunisia. Therefore, the present study was conducted to determine whether NTDs are associated with low vitamin D levels in pregnant women.

2. Materials and methods

A prospective study was conducted in the embryo-fetopathology unit of the Maternity and Neonatology Wassila Bourguiba Center in Tunis, Tunisia. This unit receives referrals of pregnant women carrying a fetus with a severe NTD from all regional hospitals and private clinics in Tunisia, in addition to those from the center. Pregnant women were recruited between January 1, 2012, and December 30, 2013. Women carrying a fetus with a severe NTD were enrolled before elective termination. For each women enrolled into the NTD group, a healthy pregnant woman with normal ultrasonography and a normal obstetric history (no previous spontaneous abortion, fetal death, stillbirth, or fetal intrauterine growth restriction) was enrolled into a control group when attending the study unit. Women in the control group were matched to those in the NTD group by date/month of conception and use of folate supplementation. Participants could come from any region across the country. Women with hypertension, cardiac disease, or atherosclerosis were excluded from the study. Ethics approval for the study was obtained from the Ethics Committee of the Maternity and Neonatology Center La Rabta in Tunis. All participants gave written informed consent.

A fasting blood sample was collected from each study participant, and the plasma was separated and stored at –80°C until analyzed. Plasma levels of 25-hydroxyvitamin D were measured by a competitive chemiluminescence immunoassay cospecific for vitamin D3 and vitamin D2 using the Liaison autoanalyzer and specific reagent kit (both from DiaSorin, Stillwater, MN, USA). The participants were grouped into vitamin D status categories on the basis of the 2011 cutoff levels defined by the Institute of Medicine [19], with vitamin D deficiency, insufficiency, and adequacy defined as a 25-hydroxyvitamin D concentration of less than 30 nmol/L, 30–50 nmol/L, and more than 50 nmol/L, respectively.

The length of pregnancy at the time of blood sampling was calculated from the date of the last menstrual period. Face-to-face interviews with participants were also undertaken by K.N. to establish maternal and fetal characteristics. Medical records were reviewed.

A pilot study including 10 women carrying a fetus with an NTD and 10 control mothers showed a prevalence of vitamin D deficiency of 80% among cases and 60% among controls. The sample size for the present study was calculated on the basis of these data using the Power and Sample Size program (Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN, USA). A total of 68 participants in each group were needed to give a power of 80% in an independent two-sided *t* test with an α level of 0.05 to detect a difference of 22% in the frequencies of vitamin D deficiency between the two groups.

The statistical analysis was performed using SPSS version 18 (SPSS Inc, Chicago, IL, USA). Continuous variables were examined for normality using the Kolmogorov–Smirnov test. The χ [2] test was used to compare categorical data such as gravidity, and parity. Differences in vitamin D and maternal age means between NTD and control groups were examined by the *t* test. $P < 0.05$ was considered statistically significant. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated to investigate the possible association between feto-maternal characteristics and vitamin D deficiency.

The reporting of the present study conforms to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for observational studies.

3. Results

Overall, 68 women aged 22–43 years were included in the NTD group and 64 women aged 21–41 years were included in the control group. Data for four mothers selected for the control group were missing because the mothers refused to participate in the study. In the NTD group, 2 (3%) women had had a previous NTD pregnancy. There were no differences in maternal age, consanguinity, and pregnancy duration between the two groups, but gravidity and parity differed significantly between the groups (Table 1).

The mean 25-hydroxyvitamin D concentration was significantly lower among women in the NTD group (20.65 ± 10.25 nmol/L, range 5.40–43.75) than among women in the control group (28.30 ± 13.82 nmol/L, range 7.57–76.00; $P < 0.001$). Vitamin D deficiency and insufficiency were more common in the NTD group than in the control group (Fig. 1). In the NTD group, 53 (78%) women had vitamin D deficiency and 15 (22%) had vitamin D insufficiency. In the control group, 39 (61%) women had vitamin D deficiency and 20 (31%) had vitamin D insufficiency. Vitamin D sufficiency was only found in the control group (n = 5 [8%]). Vitamin D status varied significantly between groups ($P < 0.001$).

In the NTD group, vitamin D status and vitamin D deficiency were not associated with maternal age or parity (Table 2). The mean 25-hydroxyvitamin D level was higher among mothers with two or more pregnancies than among those with one pregnancy, but the difference was not significant ($P = 0.075$) (Table 2). Additionally, vitamin D deficiency was more common in mothers with only one pregnancy, but the difference was not significant ($P = 0.05$) (Table 2).

Women in the NTD group with a consanguineous marriage had a higher 25-hydroxyvitamin D concentration than did those without such a marriage, although the difference was not significant ($P = 0.071$) (Table 2). Vitamin D deficiency was not associated with consanguinity either.

The season of blood sampling was significantly associated with the vitamin D status in the NTD group, with the highest 25-hydroxyvitamin D concentrations found in mothers with blood drawn in summer

Table 1
Maternal characteristics.[a]

Characteristic	NTD group (n = 68)	Control group (n = 64)	*P* value
Age, y			0.65
≤30	27 (40)	23 (36)	
>30	41 (60)	41 (64)	
Length of pregnancy, wk			0.95
≤20	29 (43)	27 (42)	
>20	39 (57)	37 (58)	
Gravidity			0.016
1	23 (34)	10 (16)	
>1	45 (66)	54 (84)	
Parity			<0.001
0	31 (46)	10 (16)	
≥1	37 (54)	54 (84)	
Consanguinity			0.11
Consanguineous marriage	10 (15)	4 (6)	
Non-consanguineous marriage	58 (85)	60 (94)	

Abbreviation: NTD, neural tube defect.
[a] Values are given as number (percentage) unless indicated otherwise.

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