Vitamin D supplementation and vitamin D status in children of immigrant background in Norway

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Abbreviations:

DBS	Dried Blood Spot
DEQAS	Vitamin D Quality Assessment Scheme
Hb	Hemoglobin
LC–MS/MS	Liquid Chromatography–tandem Mass Spectrometry
S-25(OH)D	Serum 25-hydroxyvitamin D

Keywords: vitamin D; Hemoglobin; children with immigrant background; Dried Blood SPOT (DBS), breastfeeding.

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Conflict of Interest:

TEG and AMH work at VITAS. TEG is a CEO of the contract laboratory Vitas AS (www.vitas.no), where he also is a stock owner.

Authorship:

AAM and HEM planned the study. AAM carried out the data collection, performed data analysis and prepared the manuscript. AMH performed the analysis of DBS samples. HEM and TEG commented on the draft, contributed to the interpretation of the findings and approved the final version of the manuscript.

Abstract

OBJECTIVE: Sufficient vitamin D status during infancy is important for child health and development. Several initiatives for improving vitamin D status among immigrant children have been implemented in Norway, and the aim of the present study was to evaluate the vitamin D status and its determinants in children of immigrant background in Oslo. DESIGN: Cross-sectional study. SETTING: Child health clinics in Oslo SUBJECTS: Healthy children with immigrant background (n =102) aged 9-16 months were recruited at the routine one-year check-up from two child health clinics with high proportions of immigrant clients. Blood samples were collected using the dried blood spot technique and analysed for serum 25-hydroxyvitamin D (s-25(OH)D) concentration using liquid chromatography-tandem mass spectrometry. RESULTS: The mean s-25(OH)D was 52.3 (SD 16.7) nmol/L, with only three children below 25 nmol/L and none below 12.5 nmol/L. There were no significant gender, ethnic or seasonal variation in s-25(OH)D. However, compared to breastfed children, s-25(OH)D concentration was significantly higher among children who were around the age of 1 year and not breastfed. Around 38% of the children were anemic, but there were no significant correlations between s-25(OH)D and haemoglobin (Pearson correlation, r = 0.1, P=0・33). CONCLUSION: In this study few children had vitamin D deficiency, but around 47 % of the children in the study population were under the recommended s-25(OH)D sufficiency level of ⩾50 nmol/L.

Introduction

Vitamin D is important for calcium absorption. Young children grow fast and have a high demand for calcium to build their skeleton. The classical outcome of severe vitamin D deficiency in children is rickets (1). Vitamin D deficiency is far more prevalent among immigrants in Norway than among ethnic Norwegians (2-4). In 2004-2006, we conducted a cluster randomized intervention study among children of non-Western immigrant background in Norway. The intervention provided free vitamin D drops at the ages of 6 weeks and 3 months, as well as information material in various languages about the importance of vitamin D and instructions for how the drops should be given to the infants. Blood samples were taken from the children and the vitamin D content in the blood was measured. Serum levels of 25-hydroxyvitamin D (s-25 (OH) D) increased substantially more (28 nmol/L) in children who had received free vitamin D drops and whose mothers had received customized information experienced compared to the control group that received standard care at the child health clinics (5). Some of the children were also followed up at 8 months of age, and it turned out that s-25 (OHD) D remained at a high level (not published data). Vitamin D supplementation is particularly important during the first six months, especially because exclusive breastfeeding is recommended during these months and the levels of vitamin D found in breast milk are not sufficient to meet the child's needs.

Based on the positive results of the intervention study, the Norwegian Directorate of Health introduced in 2009 a nationwide package program that provides free distribution of vitamin D drops to all infants of non-Western immigrant background and information material on vitamin D for their parents, available in six different languages. The package is available until the children reach six months of age (one bottle at the 6 week control and one bottle at the 3 month control), after which the parents are encouraged to continue the supplementation on their own. In addition, infant formula and most baby cereals are fortified with vitamin D.

Given these measures for improving vitamin D status among immigrant children, the aim of the present study was to evaluate the vitamin D status of one-year-old children of immigrant background in Oslo

Material and methods

The Norwegian Directorate of Health recommends screening for anemia in all Norwegian children of non-Western immigrant background at the routine one-year checkup. The first stage is to test hemoglobin values for all children. Those with Hb< 11 g/dL are referred to their doctors for further investigation. We wanted to utilize this existing, routine practice for recruiting children to this study. After contacting all potential child health clinics in Oslo, we found out that a few with high proportions of immigrant clients did comply with the screening for anemia. Of the three identified clinics, one declined to participate in this study due to inconvenience. We included, therefore, two clinics with high proportions of immigrant clients who agreed to participate.

The public health nurses at the child health clinics were requested to invite all mothers of immigrant background (Africa, Asia and Middle-East) who brought their children for a routine one-year check-up to participate with their child in the study. Those willing to participate signed a form of consent and were included in the study. For those who declined to participate, the reasons for not participating should be noted but it did not materialized. The study was conducted between February and September 2015 in Oslo, Norway.

Data collection

Background information about the infants, including breastfeeding practices, introduction of complementary feeding and current use of vitamin D and other vitamin/mineral supplements, was collected by public health nurses using a short interview-administered questionnaire. Generally, the public health nurses knew the mothers and were confident completing the questionnaire without using interpreters.

Dried Blood Spot (DBS) and vitamin D analysis

Capillary blood was collected after the fingertip was pierced, using an automated lancet. The first drop of blood was removed with a sterile cotton swab. The blood was collected for hemoglobin measurement and then a few blood drops were applied directly on the sampling filter card within pre-marked circles. The cards were then air dried for 2 hours and sample cards were stored in low-gas permeable zip-lock bags with desiccant packages. Samples were kept refrigerated and delivered to Vitas AS in Oslo (www.vitas.no) for analysis. Serum concentrations of 25-hydroxycholecalciferol (s-25(OH)D3) were quantified using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Punches from the DBS were added to water, shaken and diluted with 2-propanol containing the internal standard 26,27 hexadeuterium-25-OH-Vit D3. After mixing and centrifugation, the supernatant was transferred to an insert and centrifuged again, and an aliquot of 100µl was injected into the HPLC system. HPLC was performed with an Agilent 1260/1290 liquid chromatograph (Agilent Technologies, Palo Alta, CA, USA) interfaced by atmospheric pressure chemical ionization to an Agilent mass spectrometric detector operated in Multiple Reaction Monitoring mode. Vitamin D analogues were separated on a 4·6mm×150mm reversed-phase column with 2·7 µm particles. The column temperature was 20°C. A one-point calibration curve was made from analysis of DBS calibrators with known vitamin D concentrations. The LC-MS/ MS DBS method was internally validated. The intra- and inter-assay CV were 11,1% and 4,0 %, respectively. The detection limit was 5 nmol/L. The analysis refer only to s-25(OH)D3 but in in the text we have used s-25(OH)D. The LC-MS/MS DBS method was chosen as a minimally invasive and convenient technique for pediatric research participants. The laboratory performing the analysis is part of the Vitamin D Quality Assessment Scheme (DEQAS) and is compliant.

The DBS assay for 25OHD has been fully validated according to the Food and Drug Administration (FDA) and The European Medicines Agency (EMA) guidelines for validation of bioanalytical methods(6) and included specificity, precision, accuracy, matrix effects and DBS stability at 25 ^{0}C and 50 ^{0}C (See supplementary material). As a part of the validation, a comparison between DBS and the corresponding plasma from 78 human volunteers was performed (figure 1).

In order to measure hemoglobin concentration, a HemoCue system was used in two child health clinics. The concentration of Hb was recorded on the study questionnaire by the nurse. The HemoCue was calibrated daily using the calibration cuvette provided by the manufacturer. Anaemia was defined as a haemoglobin concentration of < 11 g/dL for children (7).

A universal standard for the normal range for s-25(OH)D does not exist; however, we chose to use the commonly used cut-off points, with respect to which vitamin D status is classified as severely deficient (<12·5 nmol/L), moderately deficient (12·5–25·0 nmol/L), mildly deficient (25–49·9 nmol/L) and sufficient (>50 nmol/L). Serum concentrations below 25 nmol/L are often accompanied by elevated levels of parathyroid hormone and disturbances in calcium homeostasis and bone mineralization and are therefore frequently considered as beyond the cut-off for vitamin D deficiency (8-10).

Statistical methods

Analysis of the data was performed using the IBM SPSS statistical software (V.22 SPSS Inc, Chicago, Illinois, USA). Descriptive statistics are presented as means and standard deviations. To compare the mean s-25(OH)D concentrations according to explanatory variables, we used an independent-sample t test (two-tailed) and one-way ANOVA. The relationships between s-25(OH)D and potentially associated variables were tested primarily using linear regression models.

Ethical clearance

The study was approved by the Regional Committee for Medical and Health Research Ethics (study code: 2014/408). All parents gave written informed consent for participation in the study.

Results

A total of 102 children with a mean age of 12.5 months were included in the study, but samples were not available from two of the children because their samples were spoiled when the blood drops were applied within the pre-marked circles on the sampling filter card. Characteristics of the study population are shown in Table 1.

Feeding practices

Fifty percent of the children were currently breastfed, 56% used infant formula (Table 1). Furthermore, 30% were currently using cow milk/milk products and around 62 % of the children were introduced to solid food at the age of four months while 34 % were introduced between the ages of five to six months. The majority of the children (84%) ate porridge/gruel and over 86 % ate fruits and vegetables every day. The mothers reported that around 20 % of the children ate fatty fish daily.

Supplements

The majority of the children (94%) took vitamin D-containing supplements and only 3 children had never used vitamin D-containing supplements. Among those taking D-containing supplements, 82% took the supplement daily. The majority of the children took either vitamin D-drops (42 %) or cod liver oil (47 %) (Table 2).

Vitamin D status

S-25(OH)D concentrations ranged from 22-109 nmol/L with a mean concentration of 52.3 (SD 16.7) nmol/L. For infants sampled in April–September and February–March, the mean concentrations of s-25(OH)D were 52 (SD 15.2) and 53 (SD 18·3) nmol/L, respectively, and did not vary significantly with the season (P=0·70). As shown in Table 3, only three children had s-25(OH)D concentrations below 25 nmol/L, while 50% of children had s-25(OH)D concentrations above 50 nmol/L. Six percent of the participants had deficiency according to the Institute of Medicine (IOM) definition (25(OH)D < 30 nmol/l. The s-25(OH)D concentrations did not differ significantly with respect to the variables of gender, age and ethnic background (data not shown).

Feeding practices and serum 25-hydroxyvitamin D

S-25(OH)D concentration was significantly higher among children who were currently not breastfed (55·7 (SD 15·6) nmol/L) compared with those currently breastfed (48·6 (SD 16.4) nmol/L) (P<0·001). All three children with s-25(OH)D<25 nmol/L were currently breastfed.

Hemoglobin

The mean haemoglobin concentration was 11.3 (SD 1.1) g/dL. Approximately 38% (40 % for girls and 36 % for boys) of the children were anemic (Haemoglobin< 11g/dL) (Table 4). There were no significant correlations between s-25(OH)D and haemoglobin (Pearson correlation, r=0.1, P=0·33).

Discussion

In this sample of one year old children with non-western immigrant living in Oslo, mean s-25(OH)D concentration was 52.3 nmol/L, which is higher than that which we observed previously among 6 weeks old infants of immigrant background (mean concentration of 41.7 nmol/L) [(2)]. Only three children had a s-25(OH)D concentration less than 25 nmol/L. There are limited data on vitamin D status among children in Norway, but our results are similar to those observed in a study of one-year-old ethnic Norwegian children that was conducted in Oslo from April-June 2000 (n=249) [(10)].

In Norway, exclusive breastfeeding for 6 months and vitamin D supplementation (10 µg/daily) from the age of 4 weeks is recommended. A scheme of free vitamin D drops for all Norwegian infants of non-Western immigrant background until six months of age is in place. Although we have not calculated the vitamin D intake of the children, over 90 % of the children reported taking vitamin D supplements. The majority reported taking supplements daily, indicating that the official recommendations are followed.

There is, at present, no common consensus as to which levels should be regarded as optimal with regard to health(8, 11-13). In accordance with the Nordic Nutrition Recommendations, the Norwegian Directorate of Health recommends that serum 25(OH)D concentrations, in the population in general and including in infants and children, should be maintained at 50 nmol/L [(14)]. This cut-off point is also suggested by the Institute of Medicine and others as well [(11, 12)]. We found that nearly 50% of children included in this study, regardless of the season, had s-25(OH) D concentrations below 50 nmol/L. Low vitamin D status may have negative consequences for the skeletal development of young children. However, clinical signs of vitamin D deficiency, such as rickets, are rarely seen in Norway today. We have recently carried out a nationwide register-based cohort study of nutritional rickets in Norway showing that nutritional rickets is rare in the Norwegian population. Although nearly all cases had non-western immigrant background, the number of children with rickets was also low in these groups[(15)] . Apart from rickets, the other health consequences of low vitamin D status in these age groups are poorly described.

Various factors that contribute to the increased risk for vitamin D deficiency in children of non-Western immigrant background have been documented, such as prolonged breastfeeding, consumption of cow milk, poor diet, low intake of vitamin D-enriched formula milk, high degree of skin pigmentation and low intake of vitamin D supplements or fortified food [(13, 16-20)]. WHO recommends exclusive breastfeeding (EBF) for the first 6 months of life and introduction of complementary food after 6 months along with continued breastfeeding up until 2 years(21). In this study the proportion of partially breastfed children at one year is 50% while around 46% of ethnic Norwegian children are still breastfed at one year (22).
In the WHO European Region the proportion of continued breastfeeding at 1 year varies from 1–78% (23).

In this study three children had s-25(OH)D below 25 nmol/L. All were 12 months old and currently breasted, and two of them were not taking any vitamin D supplements. In this study, only prolonged breastfeeding was associated with vitamin D status. Non-breastfed children had 7.1 nmol/L higher s-25(OH)D concentrations than those currently breastfed. According to the Norwegian regulations infant formula and cereal-based baby foods for infants and young children, should be fortified with vitamin D (1.1µg/100 kcal) and iron (1-3 mg/100 kcal), in this study, over 50 % of the children were reported to consume infant formula 84% cereal based porridges daily. However, we have not measured the amount of cereal-based products consumed by the children daily and therefore could not estimate the contribution of baby food to vitamin D intake, but among 12 months old ethic Norwegian infants who were not breastfed infant formulas contributed with 16% of the intake of Vitamin D(24). Other sources of vitamin D in the Norwegian diet are fatty fishes. In this study around 20% of the children reported consuming fatty fish daily, this is similar to what is reported among ethnic Norwegian children where 22% eat fatty fish daily but the mean intake is only 0.4 g/day. We believe this will not contribute much to the vitamin D intake of the children.

Non-modifiable factors, such as ethnicity and skin pigmentation, did not appear to explain the observed difference. The similar high prevalence of low s-25(OH)D concentration has been observed among breastfed one-year-old ethnic Norwegian children where 34% of the children had levels below 50 nomol/L (10). The similar results has been found among Danish infants were 53% partly breastfed at 9 months(25). In this context, calcium intake is important and although we have not calculated the calcium intake of the children in this study, results from a recent study found that the median daily calcium intake was 777 mg in Norwegian-Somali and 633 mg Norwegian-Iraqi infants, which is in accordance with Norwegian dietary recommendations. The same study revealed that vitamin D supplements and fortified infant formula are frequently used (26).

Strengths and limitations and of the study

Strength of this study is that the data were collected by public health nurses as part of their existing routines. Drawing blood samples from young children is always difficult, so to avoid additional discomfort for children and a high workload for the nurses, we utilized the existing, routine blood collection at the child health clinics. By using the DBS method, we were able to collect the blood sample simultaneously with the collection of blood for hemoglobin concentration determination. DBS is a minimally invasive means of obtaining s-25(OH)D measurements, particularly in blood handling, sample storage and transport. However, DBS requires proper training. DBS has been found to be suitable for the status determination of 25(OH) D and the DBS assay for 25OHD has been fully validated according to international guidelines. Due to assay variability it might be difficult to differentiate the categories of mildly or moderate deficiency. However, the analysis had been done in one batch in liquid chromatography-tandem mass spectrometry which is considered the gold-standard assay and VITAS complies with international standardization efforts.

The present study has several limitations. First, the recruitment took place at only two of the child health clinics. We hence lack information about vitamin D status and the status of routine distribution of vitamin D drops at other child health clinics. However, according to the public health nurses, very few mothers declined to participate and children of diverse ethnic backgrounds, which reflect the district's immigrant population, were included. Secondly, in order to make the study feasible and demand as little extra effort from the nurses as possible, we included only a short questionnaire. Therefore, we could not calculate the intake of vitamin D and calcium. We also did not collect information about sun exposure and time spent outdoors, which are factors that previous studies have identified as effecting the s-25(OH)D concentrations in children. The validation data shows that performing the analysis of s-25(OH)D in whole blood and converting this to serum values return results comparable to values obtained by direct analysis of serum from the same subjects. The conversion does however, relay on a normal hematocrit in the subjects. In the case of a very high or low hematocrit the calculation of the serum values will be somewhat less accurate.

In this study we found that approximately 37% of the children had anaemia, but none of the children had severe anaemia. Our results from this study is similar to that found in previous Norwegian study where the proportion of Norwegian children aged 12 months with anemia (Hb<11 g/dL) was 39% (27). According to the Norwegian regulations infant formula and cereal-based baby foods for infants and young children, should be fortified with iron (1-3 mg/100 kcal). As mentioned above it is reported that the majority of the children consumed infant formula and porridges, but we can't estimate the contribution of these commodities to the iron status of the children, however, improved iron status among Icelandic (6–12-month olds) was explained by consumption of iron fortified foods such as infant porridges and iron-fortified formulas(28). We have used HemoCue system which is the method generally recommended for use in surveys to determine the population prevalence of anaemia. However, results from studies among children have been inconclusive regarding assessments of Hb concentrations in capillary blood and in venous blood. Some studies found that, Hb concentrations in capillary blood are lower than concentrations in venous blood when analyzed with the same method(29), while others showed that Hb measurements of capillary blood with HemoCue, Hb concentrations in capillary blood are higher than assessments of Hb concentrations in venous blood(30). However, hemoglobin determined by the HemoCue method is comparable to that determined by the other methods(31). Although the HemoCue system used by Hb determination was standardized we did not measure other iron parameters and did not corrected for haematocrit content, therefore the results should be used carefully. Young children are particularly vulnerable to the effects of iron deficiency because the first three years of life is a period of rapid growth and development of the brain and nervous system. Therefore, a package of public health measures addressing all aspects of anaemia is needed.

Conclusion

In this study none of the children had severe vitamin D deficiency, but almost half of the children in the study population were under the recommended s-25(OH)D sufficiency level of ⩾50 nmol/L. Vitamin D supplementation and prolonged breastfeeding appeared to be the strongest explanatory factors for the observed difference in s-25(OH)D concentrations, suggesting that targeted interventions to improve vitamin D supplementation among immigrant children beyond the first half year of life may be successful at increasing the vitamin D status of non-Western immigrant children.

Key Messages

- Severe vitamin D and iron deficiencies are rare in the Norwegian children with immigrant background
- Either lack of vitamin D supplementation or prolonged breastfeeding were associated with lower s-25(OH)D concentrations in the immigrant children.
- The use of the DBS method is useful and convenient of obtaining s-25(OH)D measurements, particularly in a community setting.

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Table 1 Characteristics of the study population (n=102)

Age, months, mean(min-max)	12.5(9.3- 16.1)
Sex	
Girls N (%)	55 (55)
Boys N (%)	47 (45)
Ethnic background n (%)	
Africa	31 (30)
Asia	34 (33)
Middle-East	26 (26)
Others†	11 (11)
Vitamin D supplements % (n)	
Yes	94 (92)
No	8 (8)
Proportion of currently breastfeeding infants, % (n)	50 (49)
Proportion of formula feeding, % (n)	56 (57)
Season blood taken % (n)	
April - September	64(64)
February -March	36(36)
S-25(OH)D (nmol/L), mean(SD)*	
Total	52.3(16.3)
Girls	51.5(17.2)
Boys	53.2(17.2)
Hb (g/dL), mean(SD)	
Total	11.3 (1.1)
Girls	11.2(1.1)
Boys	11.3 (1.1)

* range (22-109)

† including Turkey, Kosovo

Table 2 Percentage of children used vitamin D-containing supplements (n=94)†

Frequency	Vitamin D-drops*	Cod liver oil	Other vitamin D containing supplements
Daily	34	40	3
4-6 times/week	4	4	1
1-3 times/week	4	3	1
Total	42	47	5

*The drops are oil based and contain vitamin D3

† Only children that with vitamin D supplementation

Table 3 Vitamin D status on the basis of s-25(OH)D concentration levels in children (n=100)

	S-25(OH)D cut-off (nmol/L)	N (%)
Severe deficiency	>12·5	0
Moderate deficiency	12.5-25	3 (3)*
Mild deficiency	25–49·9	47(47)
Sufficient	>50	50(50)

* s-25(OH) D range (22-24)

Table 4 Hemoglobin levels in children (n= 102)

	Hemoglobin values (g/dL)	N (%)
Severe deficiency	<7	0
Moderate deficiency	7.0-9.9	17 (16.6)
Mild deficiency	10–10·9	22 (21.6)
Non –Anemia	≥11	63 (61.8)

Figure 1 Showing correlation of $25(OHD)_3$ in plasma and DBS in 78 samples