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VITAMIN D STATUS AND BONE MINERAL DENSITY (BMD) IN MOTHERS OF RACHITIC INFANTS

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ABSTRACT

Vitamin D deficiency is endemic in the Middle East, including United Arab Emirates. It remains a conundrum why rickets selectively affects certain infants, and not others with similar vitamin D status. During the lactation period, calcium supply to the infant is provided through the breast milk by mobilization of mineral stores from the bone. Our primary aim was to examine the vitamin D status and the maternal bone mineral density (BMD) and the secondary aim to provide a descriptive analysis of the biochemical status of mothers whose infants were diagnosed with nutritional rickets. The study was conducted at Sheikh Khalifa Medical City (SKMC) and analyzed at VPS Healthcare, Abu Dhabi, UAE. Data was retrospectively extracted from the files and computerized database for the period from June 2005 till December 2007. Biochemical testing was performed by methods previously described. BMD was studied by dual energy X-Ray absorptiometry (DXA) using Hologic, Inc. –Discovery QDR Series (Bedford, MA, USA). 57% of mothers had normal BMD, 40% had osteopenia and 2.5% had osteoporosis. The T score of the spine was significantly lower than that of the hip (mean difference 0.44, 95% CI 0.55-0.83, P = 0.02). By multiple regression, only the child's 25(OH) D concentration acted as a predictor of maternal BMD (P= 0.03). The finding of decreased bone density in women of child bearing age raises the possibility of a bidirectional influence between the mother-child dyad. The lactational calcium supply may be limited in children destined to develop rickets.

Keywords: Vitamin D, Rickets, Bone Mineral Density (BMD), Calcium.

INTRODUCTION

Rickets and vitamin D deficiency are surprisingly still prevalent in the Middle Eastern community. In this sunshine rich area, a number of hypothesis have been postulated including cultural dress code, perennially hot weather forcing indoor living, urbanization and flat dwelling, impacting upon rickets etiopathogenesis. In addition prolonged breast feeding has often been suggested as a risk factor for vitamin D deficiency rickets. The basis of this is the low concentration of vitamin D in the breast milk. Raised circulating levels of the calcium regulating hormones, calcitonin, 1,25-dihydroxyvitamin D and parathyroid hormone, have been observed in some studies (Retallack *et al.*, 1977; Specker *et al.*, 1987; Stevenson *et al.*, 1979; Greer *et al.*, 1982) whereas no differences have been seen in others (particularly in early lactation) (Lund

and Seles, 1979; Pitkin *et al.*, 1979; Hillman *et al.*, 1981; Cole *et al.*, 1987; Wilson *et al.*, 1990; Kent *et al.*, 1990; King *et al.*, 1992). However, this presents a conundrum of why vitamin D deficiency rickets manifests only in selected cohort of breast fed infants. In areas where vitamin D deficiency is rare, one could ascribe possible causality to a select cohort of mothers and infants suffering from deficiency. However, it is well established, that vitamin D deficiency is endemic in certain regions of the Middle East. Therefore it is possible that other factors may be implicated in rickets in this region.

It is only recently recognized that primary investigation should be performed in mothers of rachitic infants. These women are at risk of severe vitamin D deficiency and their child's status red flags this subset for the possibility of calcium or vitamin D derangement.

However, most children born to women with a similar vitamin D status (endemic deficiency), are not afflicted. Therefore, certain factors may tip the scale in the favor of rickets development. If these women emerge from a community with endemic vitamin D deficiency, then it is possible that some other factors may be implicated in the pathogenesis of rickets as well.

Studies in the USA and elsewhere, of mothers who are likely to be habituated to relatively high calcium intakes have been unable to demonstrate an effect of dietary intake or the ingestion of calcium-containing supplements on breast-milk calcium concentrations (Kirksey *et al.*, 1979; Vaughan *et al.*, 1979; Picciano *et al.*, 1981; Feeley *et al.*, 1983; Karra *et al.*, 1988). However, low breast-milk calcium concentrations have been reported from regions of the world where maternal calcium intakes are likely to be low (Bassir, 1958; Jansen *et al.*, 1960; Bailey, 1965; Carniero and Dutra de Oliveira, 1973; Laskey *et al.*, 1990; Prentice and Barclay, 1991) and early studies have suggested that calcium supplementation of lactating mothers may increase breast-milk calcium outputs (Morrison, 1952). Previous studies have not examined the status of mothers of rachitic infants in a comprehensive fashion. It is well described that neonates have a correlation with their mother's vitamin D status in the first 2-3 months of life. However, this correlation ceases after this point, the age after which most infants manifest with rickets.

During the lactation period, calcium supply to the infant is provided through the breast milk by mobilization of mineral stores from the bone. A possible hypothesis is that in children destined to develop rickets; this lactational calcium supply is diminished. The aim of our retrospective study, was to provide a cross sectional analysis of the status of the mothers at the time of diagnosis of rickets. Our primary aim was to examine the vitamin D status and the maternal bone mineral density (BMD) and the secondary aim to provide a descriptive analysis of their biochemical status.

METHODS

The study was conducted at Sheikh Khalifa Medical City (SKMC) and analyzed at VPS Healthcare, Abu Dhabi, UAE. Data was retrospectively extracted from the files and computerized database for the period from June 2005 till December 2007. All patients were seen by a single physician (JR) at the pediatric clinic or as inpatients (tetany and seizures for hypocalcemia). Our hospital is mandated to see patients from within the capital city, Abu Dhabi, but patients often present from other Emirates (provinces) as well. The inclusion criteria were all pediatric patients fulfilling the diagnosis of rickets where BMD and biochemical testing was performed on the mothers as well. The inclusion criteria for rickets were the following: elevated alkaline phosphatase, hypocalcemia or

hypophosphatemia, and X-Ray findings consistent with rickets in older infants (young infants who presented with seizures or tetany were also included even if no X-ray changes were present as this finding is not expected at this age). Patients were excluded if their mothers were not routinely investigated. The matching data from the mothers must have included a BMD as well as at least a vitamin D concentration, both attained subsequent to the diagnosis of rickets. In addition, wherever available in both mother and child dyads, recordings were made of serum Ca, PO₄, CBC, PTH, 25 (OH)D and 1,25 (OH)2D. Biochemical testing was performed by methods previously described. BMD was studied by dual energy X-Ray absorptiometry (DXA) using Hologic, Inc. –Discovery QDR Series (Bedford, MA, USA). Status of 25(OH)D was measured with a Waters HPLC 2695 separation module with UV detection using Chromsystems kits (Chromsystems Instruments & Chemicals GmbH, Heimbürgstrasse, Munich, Germany), by using a modified high performance liquid chromatography (HPLC) method. This method was used in 2006 for vitamin D₂ testing and successfully modified and applied to measure both the forms of vitamin D (D₂ and D₃) i.e. total vitamin D metabolites in the same run (Haq *et al.* 2007; Rajah *et al.* 2010; Al Anouti *et al.* 2011; Haq 2013). HPLC method is simple, has high throughput, and is sensitive, accurate and precise for the analysis of serum 25(OH)D₂ and 25(OH)D₃. The study was approved by the Institutional Review Board at SKMC.

STATISTICS

A P value of < 0.05 was regarded as statistically significant and wherever applicable, confidence intervals were calculated. All hypothesis testing was 2 tailed. Descriptive statistics are reported as means and standard deviations. Medians were reported for non-normal distributions. Correlations and associations were determined using Pearson's correlation coefficient and linear regression analysis. To determine predictors of BMD in mothers, multiple linear regressions were performed using physiologically relevant inputs as well as those documented to be significant by bivariate analysis.

RESULTS

There was a significant decrease in the T scores in both the hip and spine in mothers of rachitic infants compared to the reference population. However the T score of the spine was significantly lower than that of the hip (mean difference 0.44, 95% CI 0.55-0.83, P = 0.02). The demographic and biochemical, hematological and densitometry data are summarized in Table 1. The corresponding details of their rachitic infants are summarized in Table 2.

Table 1 – Maternal biochemical, hematological and densitometry data

Variables	Number of subjects	Mean	SD
Age (yrs)	42	29.09	5.11
25 (OH) D (nmol/l)	38	25.78	13.77
1,25 (OH) ₂ D (nmol/l)	40	113.28*	-
Alkaline Phosphatase(U/l)	42	75.89*	-
g/cm ² (SPINE)	39	0.94	0.091
g/cm ² (HIP)	39	0.87	0.12
PTH (pmol/l)	41	8.24*	-
Calcium(mmol/l)	42	2.29	0.13
Phosphate(mmol/l)	41	10.08	0.21
T HIP	40	-0.46	0.93
T SPINE	40	-0.91	0.79
Hemoglobin (g/l)	40	121.95	13.17

*Back transformed after logarithmic transformation

Table 2 – Variables of rachitic infants with vitamin D levels and their mineral product

Variables	Number of subjects	Mean	SD
Age (months)	41	9.84*	-
Calcium (mmo/l)	39	2.11	0.41
Phosphate (mmol/l)	38	1.14*	-
PTH (pmol/l)	35	26.90*	-
25 (OH)D (nmol/l)	35	18.77*	-
1,25 (OH) ₂ D(pmol/l)	30	176.30*	-
Ca*PO ₄ product (mmol/l)	36	2.36*	-

*Back transformed after logarithmic transformation

Table 3 – Regression equation for predictors of bone mineral density (BMD) (T Spine)

Independent variables	Coefficient	Standard error	t	p
(Constant)	-0.940			
25(OH)D mother (nmol/l)	-0.003	0.010	-0.27	0.78
25(OH)D child (nmol/l)	0.010	0.004	2.25	0.031
Age mother	0.004	0.0250	0.15	0.88
PTH	-0.007	0.015	-0.48	0.68
ALP	-0.002	0.005	-0.48	0.63

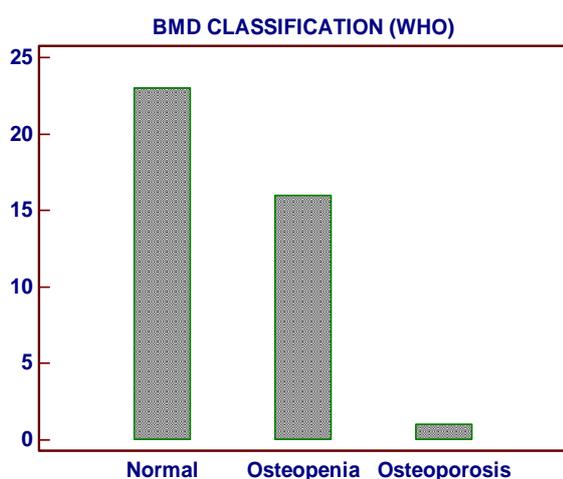


Figure 1: WHO classification of bone mineral density (BMD)

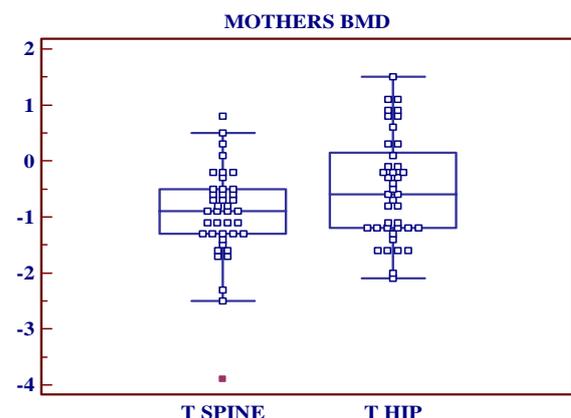


Figure 2: T scores in both the hip and the spine in mothers of rachitic infants

However, as an entire group, the mean T score of 0.9 was within the normal range (Figure 2).

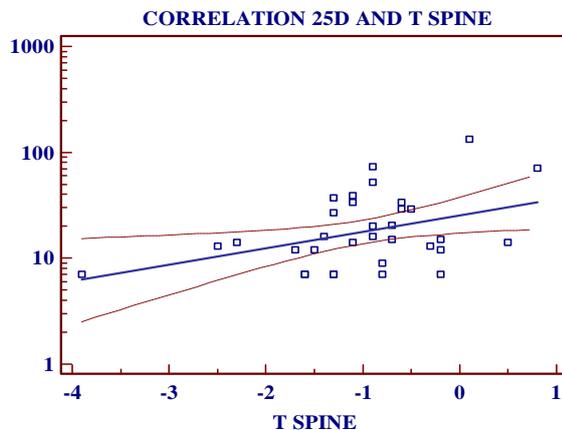


Figure 3: Correlation of 25(OH) D with T spine of children

By bivariate analysis, the correlations tested against BMD (T spine) were maternal 25 (OH)D, child's 25(OH)D, maternal PTH, maternal ALP and maternal age. Subsequently, by multiple regression, only the child's 25(OH) D concentration still acted as a predictor of maternal BMD ($P=0.03$, see Table 3). Maternal PTH was mildly elevated and significantly correlated with both ALP (Pearson's $r = 0.88$, $P < 0.0001$) and T spine (Pearson's $r = 0.572$, $P = 0.0001$). The median parity of the mother was 2.5 children. There was no correlation between maternal and child 25(OH) D concentration ($P=0.96$). Seventy percent of the women were Emiratis and the rest were of mixed origin including India, Pakistan and Arabs of other Gulf regions.

DISCUSSION

Osteoporosis remains an endemic problem worldwide especially in women greater than 50 years. The finding of decreased bone density in this population of much younger women raises the possibility of a bidirectional influence between the mother-child dyad. These mothers may have a lower bone mineral status compared to other child bearing women, which decreases their calcium transport into the baby during lactation. The corollary may also be a possibility, that prolonged breast feeding in these mothers decreases their bone density. This explanation is less likely as breast feeding usually results in an 8% reversible decrease in the BMD.

Even though the mean BMD falls within the normal range, compared to data attained from Middle Eastern women, the BMD of our population is similar to that of women in the above 50 years category. The BMD of the spine was clinically and statistically different from that at the hip. This finding would fit with the pathophysiologic process of osteoporosis whereby bone

mass is initially mobilized from the trabecular bone of the spine compared to the cancellous bone at the hip.

The correlation of the rachitic infants 25 (OH) vitamin D concentration with maternal BMD at first glance may seem intriguing. However, children with the worst vitamin D status may have the most calcium depletion both in the fetal period as well as in early infancy. Hence mobilization of calcium from the maternal source (i.e. bone) during lactation may be enhanced in these patients, accounting for the worst BMD as the child's vitamin D status worsened.

The maternal vitamin D status did not differ from that of Emirati women recently described. This factor alone cannot account for their lower BMD. Other postulates besides prolonged breast feeding remain a possibility. It has recently been recognized in Emirati women that certain haplotypes predisposing to altered calcium regulation are prevalent in this community. Earlier research on BMD between-community difference was still apparent but progressively diminishing in young women during the attainment of peak bone mass at 35-40 years of age (Prentice et al., 1991, Prentice 1993). However, since bone mass is accrued until 30 years, it is likely that the lowered T scores in this population represent women who failed to attain their peak bone mass because of both suboptimal dietary intake of minerals and moderate to severe vitamin D deficiency. This is compounded by multiple gestations starting from an early maternal age. It is possible that these women had suboptimal calcium intake as well as vitamin D deficiency which started in their childhood.

This study is a retrospective study without a control group. It is possible that other confounding variables may impact upon the development of lowered BMD. Also, other factors implicated in the pathogenesis like prolonged breast feeding and multiple gestations may be no different to the population of other child rearing aged Emirati women. A detailed log of the risk factors for osteoporosis was also not within the scope of this study. Furthermore, the dietary intake of calcium was not quantified in any systematic fashion. The calcium required for breast-milk production and infant growth can be a substantial proportion of dietary intakes especially in regions of the world where calcium consumption is low. Insufficient calcium supply might lead to maternal bone loss, reduced breast-milk calcium secretion and impaired infant bone growth. However, changes in calcium absorption and excretion may be sufficient to allow these requirements to be met without affecting maternal or infant health (Prentice 1993).

Previous studies have not examined the status of mothers of rachitic infants. This has only recently been described as a standard of care in rachitic infants. Proper screening of mothers with appropriate counseling and treatment for osteopenia or osteoporosis, may not only impact upon the long term osseous and non-osseous

benefits of vitamin D in the mother, but preclude a similar marginal vitamin D and calcium status in future weaning children. The mean T score at the spine did not fall in the osteopenic range. Nevertheless, this sub-clinical depletion of bone mass may alter the scale in the pathogenesis of rickets in their children and later development of more severe bone loss in mothers. Most data in elderly women suggest conflicting data regarding vitamin D deficiency as a risk for osteoporosis. Recent studies could not bear out the anti-fracture benefit of vitamin D supplementation in women at risk.

Our study reveals that while the mother's BMD is lower than their age matched historical controls, the vitamin D status is similar to that of age matched females. Therefore, further studies especially documenting nutritional practice is warranted. Prentice and Paul, 1990 showed that calcium density of breast -milk was shown to be similar to that of the weaning diet and calculations demonstrated that the cessation of breast-feeding would be unlikely to alter the calcium intakes of these children substantially. The capability of dietary surveys to accurately assess calcium intakes in traditional societies, however, has been challenged (Harris, 1945; Baker and Mazess, 1963) based on the fact that a number of unusual food items have been identified which could make significant contributions to calcium intakes in such communities. Examples include wild leaves, fruits and roots eaten by certain African, American and Australian tribes (Wehmeyer, 1966; Wehmeyer et al, 1969; Brand et al., 1982; Norton et al., 1984; Eaton and Nelson, 1991) and pica and ash eaten by South American Indians (Baker and Mazess, 1963).

The maternal PTH was mildly elevated and positively correlated with maternal alkaline phosphatase but negatively correlated with the T spine. This is consistent with the hypothesis that this cohort of mothers suffer from total body mineral depletion despite normal serum calcium. PTH elevation results in increased mobilization of calcium from bone stores (elevated ALP) and hence osteopenia (decreased T spine). This may happen primarily because these women are a high risk group to start with, or secondary to lactation losses. Prolonged breast feeding may act as a confounding variable in the development of rickets. However it is common Arab cultural practice to continue breast feeding into the second year of life, especially in males. Our study raises the possibility that prolonged breast feeding alone cannot account for the evolution of vitamin D deficiency rickets. We raise the possibility that most mothers, whose infants are destined to develop rickets, have a depleted BMD (and hence impaired calcium transfer) to start with. This study should be viewed as hypothesis generating and does not answer the chicken and the egg question: Do osteopenic/osteoporotic bones affect lactational calcium supply or is the diminished bone density merely a result of the prolonged lactation practice? Therefore, in future

studies it will be worth measuring calcium content in both blood and milk of lactating mothers (both controls as well as in mothers of rachitic infants).

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