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Prevalence of Hypovitaminosis D in Full Term Appropriate for Gestational Age Exclusively Breastfed Neonates in Central India

KEYWORDS: Exclusively breastfed , Full Term, Neonates, 25 hydroxy vitamin D3,ALP.

***Dr.Manoj Bhatnagar**
Assistant Professor, Pediatrics, Indira Gandhi Government Medical College, Nagpur, Maharashtra, India. *Corresponding Author

Dr. Shamama Subuhi
Assistant Professor, Pediatrics, Indira Gandhi Government Medical College, Nagpur, Maharashtra, India.

ABSTRACT *Objective: To study prevalence of Hypovitaminosis D in full term appropriate for gestational age, in exclusively breastfed neonates.*

Methods: This observational study was conducted in neonates, in tertiary care hospital in central India. Period of study was from December 2012 to July 2014. After clinical assessment, gestational age was calculated as per New Ballard Score. Sample size was 86 enrolled from Neonates visiting Immunization center and Outpatient Department for routine checkup. Parameters measured were Serum 25 hydroxy vitamin D (25 OHD) and Alkaline phosphates (ALP) levels.

Results: Out of 86 neonates, 66 (76.7%) were found to be deficient, 8(9.3%) were found to be insufficient, and 12(14%) were found to be normal. but for statistical purpose deficient and insufficient neonates 74(86%) were considered as deficient. Significant correlation was found in our study. Correlation coefficient (r) was found to be -0.91602, and p = 0.0000 (<0.05). Significant correlation between 25-OH Vit D3 (1.30-26.92) and Alkpo4 (36-378) means low value of Sr.25-OH Vit D3 is associated with high Value of Sr.Alkpo4 or vice a versa

Conclusions: Significant prevalence of 25 hydroxy vitamin D Deficiency is observed in exclusively breast fed neonates.

Introduction
Exclusive breast-feeding is recommended up to 6 months of age with all its beneficial effects on child survival. Globally as many as 1.45 million lives are lost due to suboptimal breast-feeding in developing countries. The increase in the practice of breast-feeding, associated with the belief that "breast is best" and that breast milk does not require supplementation because it is a baby's "perfect food," may lead to decreased 25-hydroxy vitamin D(25-OHD) intake from other sources and thereby causing rickets[1].

Vitamin D is the essential precursor of 1, 25-hydroxyvitamin D, the steroid hormone required for calcium absorption, bone development and growth in children. During the first 6-8 weeks of life, the vitamin D status of infants is determined by the vitamin D levels at birth, which depend on the vitamin D status of the mother. Breast milk concentration of vitamin D is low (<20 IU/l) and is inadequate for the needs of the growing infant. Vitamin D in breast milk relates to mothers' vitamin D intake, skin pigmentation and sunlight exposure. This implies that babies born to mothers with vitamin D deficiency are very likely to develop vitamin D deficiency unless supplemented from outside or adequately exposed to sunlight [2].

Against this background we planned a study with following objectives

Primary Objective:
To study prevalence of Hypovitaminosis D in full term appropriate for gestational age exclusively breastfed neonates.

Secondary Objectives:
1) To asses Vitamin D deficiency in different community.
2) To study Hypovitaminosis D in different socio economic group.
3) To asses Vitamin D deficiency according to dark pigmentation (Color of the skin) and sun exposure.

Materials & Methods
The present study is observational study which is carried out in neonates visiting Immunization center and Out Patient Department (OPD) for routine checkup. Total number of neonates was 86. Sample size was calculated by expected prevalence of the event in the study group ,p= 0.667, expected absolute allowable error in the d = 0.10 value of normal deviate at Z1-ά /2 = 1.96, 95% level of confidence, n = { Z21-ά /2 P(1-P) } / d2 = { (1.96) 2 × (0.667)(1-0.667) }/ (0.10)2 = 85.86 = 86, Sample size is minimum 86 of either sex, fulfilling the inclusion criteria, from December 2012 to July 2014.Institutionl ethical committee approval was obtained.

Exclusively full term breastfed neonates without vitamin D supplementation were included in this study. Preterm infants (<37 weeks) who are not exclusively breastfed, neonates those received vitamin D supplementation and presence of chronic diseases in mother , any gross congenital anomaly in neonates leading to feeding problem or illness requiring hospitalization in neonatal period were excluded. Use of glucocorticoids and vitamin D deficiency secondary to illness of vitamin D metabolism in mother during pregnancy were also excluded.

An informed consent was obtained from the parents of patients who were enrolled in the study. Eligible patients underwent detailed history and clinical examination and finding were recorded in case report form. After taking informed consent and proper counseling of parents blood sample of patients taken in plane bulb to estimate Sr.25H Vit D3 levels and ALP levels.Vitamin D deficiency was defined as 25OHD < 15 ng/ml, severe vitamin D deficiency as 25OHD <5 ng/ml and insufficiency as 25OHD 15-20 ng/

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ml. [3].

Alkaline phosphatase in IU/L at 37°C (98.6°F) using p-nitrophenol phosphate buffered with 2-Amino-2 Methyl-1- Propanol (AMP) kinetic in Newborn(1-30 days) is 95-368 IU/L. [4].

1) Serum.25 Hydroxy vitamin D3 (25OH Vit D3)
Serum.25 Hydroxy vitamin D3 (.25OH Vit D3) estimation done by CLIA (Chemiluminescent immunossay) method. Machine which was used ROCHE MODULAR ANALYTICS E170 and material–VIT- D TOTAL CALSET. The Roche Vitamin D3 (25-OH) assay is a direct competitive electrochemiluminescence immunoassay for human serum or plasma intended for use on Roche automated immunoassay analyzers.the Modular Analytics E170 analyzer was used. The assay employs microparticles coated with streptavidin and a polyclonal sheep antibody against 25 (OH) D, which is labeled with ruthenium. In the first incubation, 25(OH) D3 in the sample competes with biotin labelled 25(OH) D for binding with the anti-25(OH) D antibody. In the second incubation, the biotin-25(OH) D/anti- 25(OH) D antibody immunocomplex becomes bound to the microparticles via interaction of biotin and streptavidin. The microparticles are then magnetically captured onto the surface of an electrode. A voltage is applied to the electrode to produce a chemiluminescent emission, which is measured by a photomultiplier and is inversely proportional to 25 (OH) D concentrations. [5].

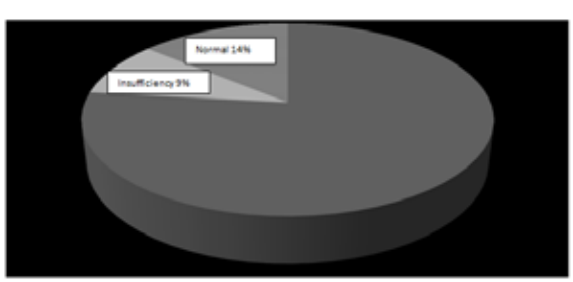
2) Serum. Alkaline Phosphatase (ALP)
Serum Alkaline Phosphatase (ALP) was done in the department of biochemistry, tertiary care hospital from central India by using Auto analyzer. Serum sample collected in plain bulb.

Principle: "Alkaline phosphatase catalyzes the hydrolysis of p- Nitro phenyl phosphate at pH 10 and liberates p– Nitro phenyl phosphate. The liberation of p- nitro phenol results in increase in absorbance at 405 mm which is proportional to the concentration of alkaline phosphates in the sample". This is the kinetic type of reaction. [6–9].

Different statistical test which are used Yates Corrected Chi Square, Fisher Exact test, Pearson's Chi Squar. Statistical software package which was used Open Epi . p value of < 0.05 was taken as significant.

Results
Prevalence of Hypovitaminosis D in full term Appropriate for Gestational Age exclusively breastfed neonates. (Fig .1).

Fig.1- Prevalence of Hypovitaminosis D in full term appropriate for gestational age exclusively breastfed neonates.



In Fig.1; Out of 86 neonates, 66 (76.7%) were found to be deficient, 8(9.3%) were found to be insufficient, and 12 (14%) were found to be normal. but for statistical purpose deficient and insufficient neonates 74(86%) were considered as deficient.

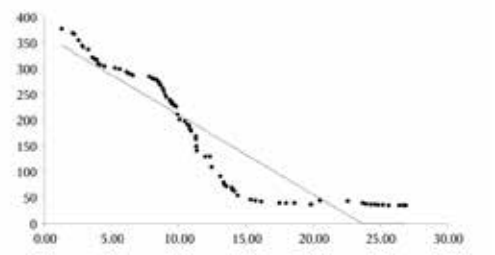


Fig. 2 Diagram showing correlation between 25-OH Vit D3 (ng/mL) and Alkpo4 (IU/L)

Significant correlation was found in our study. Correlation coefficient (r) was found to be - 0.91602, and p = 0.0000 (<0.05). Significant correlation between 25-OH Vit D3 (1.30-26.92) and Alkpo4 (36-378) means low value of Sr.25-OH Vit D3 is associated with high Value of Sr.Alkpo4 or vice a versa (fig 2). Standard deviation of 25OHD = 6.68, ALP= 112.32 and mean of 25OHD = 11.71, ALP = 184.63. Confidence intervals of 25OHD (10.29, 13.12) and ALP (160.90, 208.37). Deficiency 77%, Insufficiency 9% & Normal 14%

Parameters were taken in the study, like sex, age group, sun exposure, dark pigmentation, religion, education, occupation, socioeconomic status, wearing burkha, dietary habit, and seasonal variation. Out of 86 neonates there was 47 Male and 39 Female. Following parameters which were studied here found to be statistical non significant. Deficiency of 25OHD found to more in neonates of age more than 21 days as compare to neonates with age less than 21 days .Yates's Corrected Chi Square = 0.1651,(p=0.6845). Neonates whose mothers consume

flesh food and drinks milk were found to be less deficient in 25 OH Vit D3 as compare to neonates whose mothers did not consume flesh food and not drink milk. Fisher Exact test.(p = 0.1675).Also no significant difference found in parents education. Fisher Exact test. (p=0.1392), Parents occupation. Yates's corrected chi square = 0.02454. (p=0.8755) and seasonal variation. Fisher Exact test. (p =0.9251) (Fig.3)

Fig .3 Diagram showing deficiency and normal value of 25 OH Vit D3 (ng/mL) in statistical non significant factors.

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Statistical significant difference found in following parameters.

Neonates whose mothers dark in color were found to be more deficient in 25 OH Vit D3 as compare to neonateswhose mothers had fair color. Yates's Corrected Chi Square = 4.567 (p =0.006). Also neonates in Muslim community were more deficient in 25OHD as compare to neonates of Hindu community. Fisher Exact test. (p= 0.00573). Deficiency of 25OHD in neonates of mother those were exposed to sun more frequently was found to be less as compared to those expose to sun occasionally. Yates's Corrected Chi Square = 12.04. (p = 0.0005208). Deficiency of 25OHD in neonates of mother those wearing burkha is found to be more as compare to those were not wearing burkha (Fisher Exact test) (p=0.007304).Neonates whose parents from lower socioeconomic class or from Below Poverty Line (BPL) found to be more deficient in 25OHD as compare to those from upper socioeconomic class or from Above Poverty Line (APL) . Pearson's Chi Square = 3.89. (p =0.048). (Fig.4)

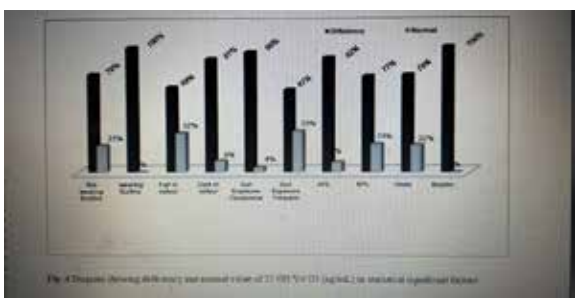

Fig .4 Diagram showing deficiency and normal value of 25 OH Vit D3 (ng/mL) in statistical significant factors.

Discussion

We found a high prevalence of vitamin D deficiency in full term, appropriate for gestational age and exclusively breastfed neonates.

In our study, out of 86 neonates, 74 (86%) have deficiency of 25 OH Vit D3. Study done in Pakistan by Atiq M,Suria A et al (1998) shows high prevalence of Vitamin D deficiency in 62 healthy breastfed infants which was 55% [10].

Indian study by Agrawal M et al (2003), shows that out of 78 infants 82% had Hypovitaminosis D and had elevated-serum alkaline phosphates [11]. Study done by Vandana Jain et al from New Delhi, India (2011) showed out of 98 infants 66.7% was found to be having deficiency of 25 OH Vit D3 [2].This incidence is much higher than the studies done by other European and American investigators like S H Dijkstral et al (2007), Netherland suggest out of 87 newborns of healthy mothers the prevalence of vitamin D deficiency was 42.5% [12]. In a Study done by frank R Greer (2008) American Society for Clinical Nutrition out of 40 breastfeeding infants 30% had low level of 25 (OH) D concentrations [13].

In our study, the physiological relevance of Hypovitaminosis D is corroborated by an almost ubiquitously elevated ALP concentration; Significant correlation found in our study. Correlation coefficient (r) was found to be - 0.91602. Significant correlation between 25OHD and ALP indicates that low value of Sr.25OHD is associated with value of Sr.ALP or vice versa. We found that deficiency of 25OHD in neonates of mother those were exposed to sun more frequently (67%) is found to be less as compared to those mother who expose to sun occasionally (96%).

Study done by Ozkan B et al (2005) in Turkey suggests out of 42, 29 infants (69%) had Hypovitaminosis D.

Most infants (83%) were exclusively breast-fed without supplemental vitamin D. All mothers had limited sunlight exposure and 33 of 42 mothers (78.6%) were concealing clothing [14]. We found that neonates whose mothers were dark in color found to be more deficient (92%) in 25 OH Vit D3 as compared to neonates of fair colored mother (65%). We found that deficiency of 25OHD in neonates of mother wearing burkha (100%) is found to be more as compared to those not wearing burkha.

In a Dutch study carried out by Wielders JP et al (2006), severe vitamin D deficiency was found in 54% of newborn infants of non-European origin compared with 6% of Dutch / West European newborn infants. This study did not report data on pigmentation or clothing habits and their possible associations with vitamin D deficiency [15].

Study done by S H Dijkstral et al (2007), suggest out of 87 newborns of healthy mothers with either dark skin and/ or concealing clothing (risk group) had higher prevalence of vitamin D deficiency (63.3%) as compared with light skin (control group) (15.8%) . [12].

Study done by S H Dijkstral et al (2007), Rotterdam, Netherlands, found a comparable prevalence (63.3%) of vitamin D-deficient newborn infants from the risk group defined by intermediate/dark skin or veiling. In addition, the newborn infants of the veiled mothers showed an extremely high prevalence of vitamin D deficiency (90.9%). [12].

We found that neonates those born in winter season (100%) had more deficiency of 25OHD as compare to those born in summer season (85%). Study done by S H Dijkstral et al (2007), Rotterdam, Netherlands, Vandana Jain et al (2011) from New Delhi, India. suggest there is no seasonal variation in deficiency of 25 OH Vit D3 in newborns [12 & 2]. Parents from lower socioeconomic class (BPL card holder) had more deficiency (92%) of 25OHD in there neonates as compare to upper socioeconomic class (APL card holder) (77%).

Study done in Pakistan by Atiq M,Suria A, et al (1998) shows that Significantly higher levels of vitamin D were found in infants of lower socioeconomic class and in those living in mud houses. A high prevalence of vitamin deficiency was found in breastfed infants and nursing mothers, predominantly among those belonging to the upper socioeconomic class. Infants of the lower socioeconomic class had comparatively higher levels of serum 25OHD[10].

Conclusion

From the results of this study, we have concluded that though breast feeding is undoubtly best feeding, but as 86% neonates on exclusive breast feeding are deficient in serum Vitamin D3 levels, so we strongly advocate supplementing Vitamin D3 to every neonate irrespective of gestational age, Weight and feeding practices since birth.

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sr.25OHD.

Contribution- Mr.S.Agrawal statistician dept of Community Medicine conceptualized the study and supervised the statistical analysis.

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