Vitamin D Supplementation in SGA Babies

Dr. Jyothi Naik
Prof. Dr. Syed Manazir Ali
Dr. Uzma Firdaus
Prof. Dr. Jamal Ahmed

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Author

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Dedicated to

My Father

Late. Mr. C. B. Naik

ABOUT AUTHOR & CO - AUTHORS

Dr. Jyothi Naik did her MBBS in 2003 from JSSMC, Mysore and Diploma Anaesthesia from MMC Mysore in 2008. She has worked for few years as Anaesthesiologist in BMC, JIPMER, HCG and other hospitals. She took MD Pediatrics in JNMC, AMU Aligarh through All India quot and finished her MD Pediatrics in 2018. She presented papers and posters at various conferences and published papers in reputed journals.

Prof. Dr. Syed Manazir Ali has passed MBBS in 1982, DCH in 1985, MD (Paed.) in 1987 from Aligarh Muslim University, DNB in 1994 from National Board, New Delhi, DCH in 1997 from Royal College of Phys & Surgeon, Glasgow and Fellow in HIV Medicine in 2007 from Regional Training Centre Maulana Azad Medical College Delhi and joined Department of Pediatrics, JNMC AMU in 1987 as a Senior Resident and continuing as Professor since more than 12 years. His areas of interest are Neonatology, intensive care and HIV Medicine.

He has published more than 40 papers in reputed National and International Journals. He has delivered lectures on important pediatrics topics at state and national pediatrics conference/ seminars.

Dr Uzma Firdaus did MBBS in 2000 and MD in Paediatrics in 2005 from J.N.Medical College, A.M.U, Aligarh. Later i joined as Senior Resident for around 2yr and am presently working as Assistant Professor in the department of Paediatrics for the last 7 years..Her thrust area is Neonatal intensive care. She has presented several papers and posters at various National conferences and reputed journals have published her articles.

Prof. Dr. Jamal Ahmad did his MBBS in 1997 and MD Medicine in 1982 from JNMC, AMU, Aligarh. Later he did his DM Endocrinology in 1986 from, PGIMER, Chandigarh, FCCP (USA) in 1987, Ph D Medicine in 1996 and then FRCP in 2008 from UK. He hold various teaching posts from Senior resident to Professor in JNMC, AMU in Medicine and Endocrinology. He was Director of Rajiv Gandhi center for Diabetes and Endocrinolgy and also Dean of JNMC, AMU Aligarh.

He has more than 150 publications in journals and any presentations at various conferences and courses.

PREFACE

Vitamin D is a fat soluble steroid hormone required for calcium absorption and bone growth in children. The deficiency affects all the system of the body i.e. Brain, CVS, Respiratory system, Immune system, Hematological, Renal and also affects the foetus and newborn of the mothers' deficient with vitamin D. Its earliest manifestation in the patients will be in the form of aching muscle weakness, depression, head swelling, tiredness and other health problems. Keeping in view of the above facts the role of vitamin D supplementation during pregnancy plays an important role in the outcome of foetus. So vitamin D supplementation improves the outcome of term small for gestational age (SGA) babies.

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I begin this acknowledgement with deepest gratitude and obligation towards the Almighty for granting me the opportunity to join this noble profession and to pursue further studies in my dream branch, pediatrics. Thanks to GOD for granting me the strength to stay steadfast in the path towards my mission despite of the numerous obstacles I had faced.

This work is the idea of an esteemed, much revered teacher my supervisor ***Prof. SYED MANAZIR ALI, Professor, Department of Pediatrics, JN Medical College, AMU, Aligarh****. An extraordinarily intelligent, talented, patient and kind supervisor is the best of what I could have ever dreamed of. I thank you sir for all the help and support in finishing this work. Your guidance will stay as a part of my journey in this profession. I am also obliged to my co supervisor* ***DR Uzma Firdaus*** *, for her never ending support timely suggestions and constant inspiration in fulfilling the needs of the study.*

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Without doubt, most heartfelt gratitude towards my family my husband ***Dr Nataraj A R*** *,my daughter* ***Hamsini.N,*** *to whom I dedicate this piece of work. They have always been pillars of strength for me and my share of worries has been borne by them. I take this as an opportunity to bow to the people I owe my entire existence to, my father* ***Mr. NAIK*** *who envisioned me to join this profession and whose unconditional love and support had always been my guiding light, my mother,* ***Mrs. Girija Naik*** *to whom I am indebted to eternity for all the prayers, sleepless nights and tiring days that she has spent for me. I also thank my brother Vijay naik who had patiently supported my journey.*

Last but not the least, I extend my acknowledgments to my patients for being a part of the study, and giving me the opportunity to work with them and learn from them in spite of all their pain and suffering.

Dr. Jyothi Naik
Prof. Dr. Syed Manazir Ali
Dr. Uzma Firdaus
Prof. Dr. Jamal Ahmed

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INTRODUCTION

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 wk 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[1]. Breast milk concentration of vitamin D is low (<20 IU/l) and is inadequate for the needs of the growing infant[2]. Vitamin D in breast milk relates to mothers vitamin D intake, skin pigmentation and sunlight exposure[3].

Vitamin D deficiency with a resurgence of rickets is increasingly being reported in infants and toddlers from various parts of the world, especially from temperate regions and among African American babies [4-6] Rickets and hypocalcemic seizures due to vitamin D deficiency in exclusively breastfed young infants have been reported from southern India[7,8 i ii] There are a few reports of vitamin D deficiency among pregnant women and cord blood of their small for date babies and breastfed young infants from India.[9-12]

Daily vitamin D supplementation is considered to be the most appropriate way to prevent vitamin D deficiency and its clinical manifestations such as rickets, growth failure, lethargy, or irritability.[13, 14] Guidelines for vitamin D supplementation in the first year of life differ from country to country and have been modified several times during the past decade.[15] The American Academy of Pediatrics recommends a daily intake of 400 IU vitamin D for all infants, children, and adults.[16] On the other hand, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition recommend daily supplementation of 800 to 1000 IU/ day for preterm infants in the first months of life.[17] The World Health Organization recommends 400 IU to 1000 IU/ day of vitamin D supplementation in low birth weight infants.[18]

Even though many studies in India suggest deficiency of Vitamin D in Newborns we do not have any standard guidelines for Vitamin D supplementation in these patients. Many studies suggest daily oral supplementation of 400 IU for term babies and 800 IU for pre term babies. The recommended dose of Vitamin D of 400 IU seems to be inadequate to normalize the serum Vitamin D level. [19] The effective dose of Vitamin D to prevent its deficiency is yet to be defined for Indian population. Another important issue in our study population is that majority of the healthy children in northern India (70-80%) are deficient in Vitamin D as suggested by myriad of studies conducted on this topic.[20] Recent meta-analysis suggests that maternal vitamin D deficiency during pregnancy is associated with an increased risk of small for gestational age (SGA).[21] But we have very limited data on the status of Vitamin D in SGA babies and its daily supplementation dose required.

We did a randomized trial comparing two different regimes of oral Vitamin D supplementation (i.e.400 IU vs. 800IU) on serum vitamin D levels in exclusively breast fed term Small for date infants at 3 months. To the best of our knowledge no similar study has been done in this part of the country. The main objective of the study is to evaluate the level of Vitamin D in small for date newborns and compare the effect of 800 IU & 400 IU of Vitamin D supplementation on Vitamin D levels.

AIMS AND OBJECTIVES

Hypothesis: Daily supplementation of 800IU of Vitamin D should increase Vitamin D levels more than 400 IU in term SGA babies who are exclusively breast fed

Research Question: Is there a difference in vitamin D levels at 3month of age with vitamin D supplementation -at a dose of 400IU v/s 800IU

AIM AND OBJECTIVES

Aim: To compare the effect of vitamin D supplementation in a two different dose regimens i.e. 400IU v/s 800IU on vitamin D status in healthy term small for gestation age babies upto 3 months

Objectives

1. To study the level of vitamin D at birth in term small for date newborns

2. To study the effect of vitamin D supplementation by 2 different doses at 3 months of age

REVIEW OF LITERATURE

VITAMIN D

The term "vitamin D" refers to compounds vitamin D3 (cholecalciferol) or vitamin D2 (ergocalciferol). Vitamin D3 is derived from 7-dehydrocholesterol by ultraviolet irradiation of the skin. Vitamin D3 is also found in animal food sources e.g., fatty fish (e.g., salmon, mackerel and tuna) cod liver oil, milk, etc, [22] Vitamin D2 is found in vegetable sources like sun-exposed yeast and mushrooms. Notably, most dietary sources are not sufficiently rich in their vitamin D content. Vitamin D is a fat soluble vitamin and its synthesis in the body is dependent on multiple factors like latitude, [23] atmospheric pollution, clothing, [24, 25]skin pigmentation and duration and time of exposure to sunlight.

PHYSIOLOGY OF VITAMIN D METABOLISM

Vitamin D3 (cholecalciferol) is taken in the diet (from fortified dairy products and fish oils) or is synthesized in the skin from 7-dehydrocholesterol by ultraviolet irradiation.[26] In order to be biologically active and affect mineral metabolism and to have effects on numerous other diverse physiological functions including inhibition of growth of cancer cells and protection against certain immune mediated disorders,[27] vitamin D must be converted to its active form.

Vitamin D is transported in the blood by the vitamin D binding protein (DBP, a specific binding protein for vitamin D and its metabolites in serum) to the liver. In the liver vitamin D is hydroxylated by vitamin D 25 hydroxylases resulting in the formation of 25-hydroxyvitamin D3 (25(OH) D).[28] 25(OH) D, the major circulating form of vitamin D, is transported by the DBP to the kidney. In the proximal renal tubule 25(OH)D is hydroxylated resulting in the hormonally active from of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)2D) which is responsible for most, if not all of the biological actions of vitamin D. The cytochrome P450 monooxygenase 25(OH)D 1α hydroxylase (CYP27B1; 1α(OH)ase) which metabolizes 25(OH)D to 1,25(OH)2D is present predominantly in kidney. This enzyme is also found in extra renal sites including placenta, monocytes and macrophages. [29]

Studies using mice deficient in DBP have resulted in new insight into the role of DBP in vitamin D metabolism and action. Although DBP null mice have markedly lower total serum levels of 25(OH) D and 1,25(OH)2D than wild type (WT) mice, the levels of serum calcium and PTH are normal in the DBP mice.[30] In patients with reduced levels of circulating DBP, serum calcium levels have also been reported to be normal.[31] More recent studies using DBP null mice have shown that DBP is important for total circulating 1, 25(OH) 2D but DBP does not influence the pool of 1, 25(OH) 2D that enters cells and affects the synthesis of vitamin D target proteins. [32]

Thus direct measurement of 1, 25(OH) 2D may not, in all cases, reflect the biologically active 1, 25(OH) 2D. This may be, in part, why 25(OH) D, which is also more stable than 1, 25(OH) 2D, is used to assess clinical vitamin D status. It has been suggested that the maintenance of normal serum calcium levels in the DBP null mice may be due to the ability of the vitamin D receptor to concentrate 1,25(OH)2D in tissues due to its high affinity for 1,25(OH)2D, resulting in transcriptional regulation of genes involved in maintenance of calcium homeostasis.[32]

In addition to 1, 25(OH) 2D, the kidney can also produce 24, 25 dihydroxy vitamin D3 (24, 25(OH) 2D), a relatively inactive metabolite when compared to 1, 25(OH) 2D. 25-Hydroxyvitamin D 24 hydroxylase (CYP24), also a mitochondrial P450 enzyme, can hydroxylate both 25(OH) D and 1,25(OH)2D[29]. It has been suggested that the preferred substrate for 24(OH)ase is 1,25(OH)2D.[33] Thus, 24(OH)ase limits the amount of 1,25(OH)2D in target tissues both by accelerating the catabolism of 1,25(OH)2D to 1,24,25(OH)3D resulting in calcitroic acid or by producing 24,25(OH)2D thus decreasing the pool of 25(OH)D available for 1 hydroxylation (Fig 1).

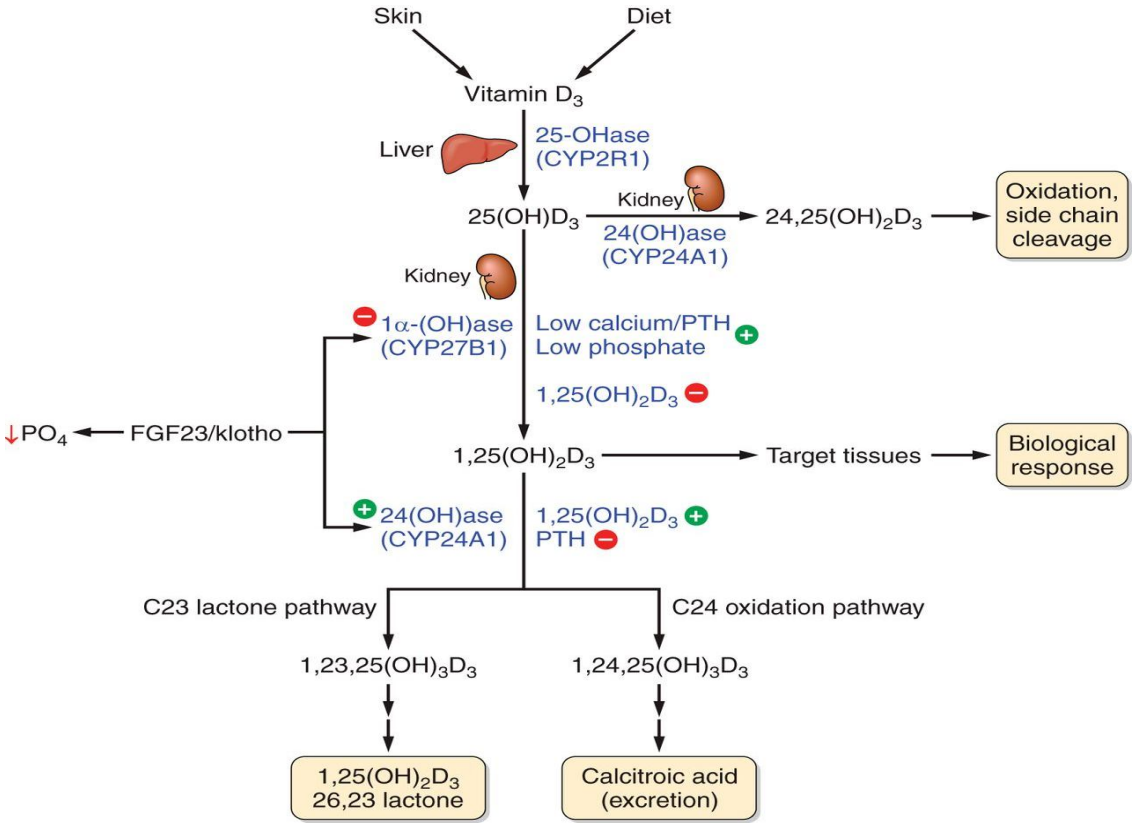


Figure-1: Vitamin D metabolism

FUNCTIONS OF VITAMIN D

The most well recognized function of 1,25(OH)2D involves regulation of calcium and phosphorus balance for bone mineralization and remodeling. Without adequate levels of 1,25(OH)2D in the bloodstream, dietary calcium cannot be absorbed. Low calcium levels lead to an increase in serum PTH concentration, which leads to increased tubular reclamation of calcium in kidneys and resorption from the skeleton at the cost of lowering bone density. In the long term this leads to weakened and brittle bones that break easily.[22]Approximately 40%–60% of total skeletal mass at maturity is accumulated during childhood and adolescence. Rickets will results from inadequate mineralization of growing bone. Thus it is a childhood disease and it is manifested as bone deformities, bone pain and weakness. Biochemical abnormalities consistently include hypophosphatemia, elevated alkaline phosphatase levels and serum 25(OH)D levels are usually below 5 ng/mL.[22] Biochemical studies have implicated vitamin D deficiency in many chronic diseases including, but not limited to, infectious diseases, autoimmune diseases, cardiovascular diseases, diabetes and cancer.[34-36]

SERUM 25(OH) D LEVELS AS INDICATIVE OF VITAMIN D DEFICIENCY, INSUFFICIENCY OR SUFFICIENCY

Maintenance of adequate levels of serum 25(OH)D is essential to sustain the claimed pleiotropic effects, whether skeletal (classical) or extra-skeletal (non classical). The threshold levels of serum 25(OH)D required to optimize its effects may not be the same in the various target organs. Based on classical skeletal effects, vitamin D deficiency is defined as serum levels of 25(OH)D < 20 ng/mL (50 nmol/L) with consequent and consistent elevation of PTH and reduction in intestinal calcium absorption. Vitamin D insufficiency is defined as serum 25(OH)D levels in the range of 20–29 ng/mL.[37-39] At serum 25(OH)D levels of 30 ng/mL intestinal calcium absorption reaches its peak, and PTH levels continue to fall until this level of 25(OH)D is attained. Thus, vitamin D sufficiency is defined as serum levels of 25(OH)D 30–32 ng/mL. A desirable and safe range of serum 25(OH)D levels would be 30–100 ng/mL.[38] This range would be sufficient for most known effects of vitamin D and also significantly lower to obviate concerns pertaining vitamin D toxicity.[40]

Serum concentration of 25(OH)D is the best indicator of vitamin D status. It reflects vitamin D produced cutaneously and that obtained from food and supplements and has a fairly long circulating half-life of 15 days. 25(OH)D functions as a biomarker of exposure, but it is not clear to what extent 25(OH)D levels also serve as a biomarker of effect (i.e., relating to health status or outcomes). Serum 25(OH)D levels do not indicate the amount of vitamin D stored in body tissues. In contrast to 25(OH)D, circulating 1,25(OH) 2 D is generally not a good indicator of vitamin D status because it has a short half-life of 15 hours and serum concentrations are closely regulated by parathyroid hormone, calcium, and phosphate.[22] Levels of 1,25(OH) 2 D do not typically decrease until vitamin D deficiency is severe.

Immunoassays such as radioimmunoassay (RIA), enzyme linked immunosorbant assay (ELISA), chemiluminescence immunoassay and protein binding assays are used in routine testing of 25(OH)D in clinical laboratories. LCTMS (liquid chromatography tandem mass spectrometry) is the widely accepted reference method for 25(OH)D measurement. However, LCTMS is tedious, expensive and time consuming and therefore seldom used commercially.[22]

DEFINITION OF VITAMIN D STATUS

Different Classifications that have been used so far to classify Vitamin D deficiency on basis of 25(OH)D status are as shown in Table I.[41] Recent evidence suggests maintaining levels above 20ng/mL for maximizing health benefits like good immune function and overall growth.[42] Various studies discussed here have used different cut-off levels to define Vitamin D deficiency, insufficiency and sufficiency levels on basis of 25(OH)D levels either as nM (nanomoles per liter) or ng/mL. For uniformity and ease of comparison, in this review all the data on 25(OH)D levels are presented in a single concentration unit for serum 25(OH)D levels-ng/mL.

Table-I: Different classifications of Vitamin D deficiency on basis of 25(OH) D status by various Associations and organizations

Serum 25(OH)D levels (ng/ml)	US Endocrinology[40]	US Institute Of Medicine [43]	Pediatric Endocrinology Society [44]	Lips [45]	Indian Academy of Pediatrics (IAP) [46]
Deficiency	<20 ng/mL	<15 ng/mL	<15 ng/mL	<20 ng/mL	>20 ng/mL
Sufficiency	21-29 ng/mL	>20 ng/mL	>30 ng/mL	>30 ng/mL	12-20 ng/mL
Insufficiency	>30 ng/mL	-	-	-	<12 ng/mL
Risk of Toxicity	>150 ng/mL	>50 ng/mL	-	-	>100 ng/mL

RDA OF VITAMIN D

Infants (0–1 yr.) require at least 400 IU/d (IU = 25 ng) of vitamin D and children 1 yr and older require at least 600 IU/d to maximize bone health.[40] Whether 400 and 600 IU/d for children aged 0–1 yr and 1–18 yr, respectively, are enough to provide all the potential nonskeletal health benefits associated with vitamin D to maximize bone health and muscle function is not known at this time. However, to raise the blood level of 25(OH) D consistently above 30 ng/ml (75 nmol/liter) may require at least 1000 IU/d of vitamin D.[40]

The endocrine society clinical practice guidelines suggest that obese children and adults and children and adults on anticonvulsant medications, glucocorticoids, antifungal such as ketoconazole, and medications for AIDS be given at least two to three times more vitamin D for their age group to satisfy their body's vitamin D requirement.[40]

VITAMIN D SCENARIO IN INDIAN CHILDREN

It is a vast tropical country extending from 8.4on latitude to 37.6° n latitude. Aligarh lies at a latitude of 27.88°N and longitude of 78.08°E and 178m above sea level. Majority of its population lives in areas receiving ample sunlight throughout the year and hence there was disbelief that Vitamin D deficiency is uncommon in India. [47]

However from the data available in the published literature, Vitamin D deficiency is very common in India in all the age groups and both sexes across the country.[48-51] The mean vitamin D levels in the study on children from northern India was 11.8±7.2ng/mL.[43] Vitamin D deficiency is a common problem in India due to several factors:

1. Changing food fads and food habits contribute to low dietary calcium and Vitamin D intake.
2. High fibre diet containing phosphates and phytates which can deplete Vitamin D stores and increase calcium requirement.[45]
3. Genetic factors like having increased 25(OH)D-24hydroxylase which degrade 25(OH)D to inactive metabolites.[52]
4. It has been shown that increment in serum 25(OH)D in response to treatment depends on the heritability of Vit D binding protein.[53]
5. With modernization, the number of hours spent inside has reduced thereby preventing adequate sun exposure. This is particularly true in urban Indians.
6. Increased pollution can hamper the ultraviolet rays to adequately synthesize vitamin D in the skin.[54]
7. Cultural and traditional habits prevalent in certain religions like Burqa and the Pardah system in Muslims have been well known.
8. Repeated and unplanned, unspaced pregnancies in dietary deficient patients can aggravate Vitamin D deficiency in the mother and the fetus.

PREVALENCE OF VITAMIN D DEFICIENCY IN INDIAN CHILDREN AGED 0-6 MONTHS (NEW BORNS AND BREAST FEEDING GROUP)

Studies as summarized in the Table II were conducted in the age group of newborns up to 6 months and showed prevalence from 62-95.7%. Subnormal maternal Vitamin D status was shown to have a significant association with Vitamin D deficiency in newborns and breastfeeding babies and subclinical Vitamin D deficiency is reported among exclusively breast fed infants with radiological rickets at 25OHD < 10 ng/ml. [8,55] These studies have revealed predisposition of lower 25(OH)D concentrations to neonatal hypocalcaemia and infantile rickets with biochemical evidence of hyperparathyroidism among infants manifesting with hypocalcemic seizures with Vitamin D deficiency.[56] Significant association between a deficient Vitamin D status (45.5%) and low birth weight has also been observed[57].

Table II: Prevalence studies among newborn and breast feeding group[41]

Author and Year	Location	Age Group	CB/ * HB	Area	Sample size	Prevalence (% age) (25(OH) D
Balsubramanian et al; 2006[8]	Chennai	Newborns	HB	Urban	50	26
Bhalala et al; 2007 [58]	Mumbai	Newborns-3 months	HB	Urban	38	80
Jain et al; 2010[56]	Delhi	10 weeks-6 months	HB	Urban	94	44.33
Nageshu et al; 2016[57]	Andhra Pradesh	Newborns	HB	Urban	121	13.8

*Community based/ Hospital based-(CB/HB) # 25(OH)D

VITAMIN D SUPPLEMENTS AND EFFICACY CONCERNS

Commercially, vitamin D2 is manufactured by ultraviolet irradiation of ergosterol from yeast. Vit D3 is produced by the ultraviolet irradiation of 7- dehydrocholesterol from lanolin. Both forms of vitamin D are available as vitamin D supplements. Several other forms of vit D supplements are also available. 1,25(OH)2D (Calcitriol) is indicated specifically for patients with renal diseases and 25(OH)D is useful when hepatic hydroxylation of vit D is impaired. Whether D2 or D3, is more efficacious in raising and sustaining serum 25(OH)D levels, remains controversial. Several studies have proved equivalence between the two forms. [59-62]

However, other studies reported that D3 is more effective than D2 in achieving and maintaining higher serum 25(OH)D levels.[63-66] Nevertheless, on a long term basis either form may be used, bearing in mind the

long half-life (2–3 weeks) of 25(OH)D in circulation. In India, supplements commonly available are—D3 (cholecalciferol), 1,25(OH)2D3 and 1 alpha hydroxy vitamin D3 (alfacalcidol). Some formulations have calcium too. Multivitamin formulations are also available and contain about 400 IU of D3. None of the pharmacists had heard of D2 supplements. D3 supplement of 60,000 IU is the highest selling one and is available in powder form in sachets or as oil-based capsules. Recommended dose on the label is once per week.[22]

Nano formulations of vitamin D are also available in Indian market with better bioavailability.[67] There is a high degree of variability in cholecalciferol content of commercially available preparations in the Indian market. Variation in the content from the printed range may have many clinical implications by either leading to under treatment or Vitamin D toxicity.[68] Various organizations and associations have recommended different doses of Vitamin D supplementation for different age groups as shown in Table III. But still there is no concise on the standard recommended dose of Vitamin D supplementation.

Table-III: Recommended doses of the vitamin D supplementation by various organizations

Association	Population	Recommended Dose of Vit D (Per Day)
American Academy of Pediatrics (**AAP**)[16]	All infants, children, and adults	**400IU**
European Society of Pediatric Gastroenterology Hepatology and Nutrition (**ESPGHAN**) [69]	Preterm infants	**800-1000IU**
World Health Organization (**WHO**) [18]	Low Birth Weight babies	**400-800IU**
Indian Academy of Pediatrics (**IAP**)[70]	Preterm infants	**400-800IU**

REVISED GUIDELINES FOR VITAMIN D RDA

There appears to be a discrepancy between vitamin D RDA (Recommended Dietary Allowance) based on recent research and current practice.[71] In 2003, the AAP Committee on Nutrition and section on Breastfeeding advocated 200 IU per day of vitamin D intake for children of all ages [72,73] but this amount was deemed to prevent the worst outcome of vitamin D deficiency i.e. rickets. But now the recommendation is 400 IU per day for all infants, children and adolescents[45, 73] till they are not getting this amount from alternative sources. It is likely that higher doses may be needed for dark skinned and preterm infants.[45]

Supplementation trials in infants and children have shown that 400- 1000 IU per day are needed to achieve serum level of 30ng/mL.[74] For children and adolescents (1 to 18 years of age), IOM [75] has specified estimated average requirements (EARs) and RDAs on the basis of serum 25(OH)D levels of 16 and 20 ng/mL, respectively. EAR and RDA for vitamin D, as per IOM review, are 400 IU/day and 600 IU/day respectively, while tolerable upper level of intake are 1000 IU/day for infants <6 months old, 1500 IU/day for 6-12 months old, 2500 IU/day for 1-3 years old, 3000 IU/day for 4-8 years old and 4000 IU/day for 9 years and above including pregnant and lactating mothers. The RDA estimates here have been made considering the minimal skin synthesis of vitamin D. The Indian Association of Pediatrics (IAP) guidelines on Vitamin D are given in Table IV.

IAP GUIDELINES ON VITAMIN D PREVENTION AND TREATMENT [47]

Table-IV: IAP Recommendations for Vitamin D doses for Prevention and Treatment

	Vitamin D			
Age	**Prevention**	***Tolerable upper limit**	**Treatment**	**Treatment with larger dose (oral route preferred)**
Premature neonates	400 IU/day	1000 IU/day	1000 IU/day	NA
Neonates	400 IU/day	1000 IU/day	2000 IU/day[$]	NA
1-12 months	400 IU/day	1000-1500 IU/day	2000 IU/day[$]	60000 IU wkly for 6 weeks (> 3months of age)

1-18 years	600 IU/day	3000 IU/day till 9 years, 4000 IU/day from 9-18 years	3000-6000IU/day[$]	60000 IU wkly for 6 weeks
At risk groups	400-1000 IU/day	as per age group	as per age group	as per age group

$For a minimum of 3 months; after treatment, daily maintenance doses need to be given; *Tolerable Upper Limit - the maximum level of total chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans.

TERM SMALL FOR GESTATIONAL AGE (SGA) BABIES

The term 'small for gestational age' (SGA) refers to the small size of the baby at birth, that is, when the birth weight is below the 10th percentile (Fig 2). Babies who are SGA may be[76]:

- Constitutionally small and at no greater risk than appropriate weight for gestational age (AGA) babies, or
- Small due to fetal/intrauterine growth restriction (FGR), a pathophysiological process occurring in-utero
- Differentiating between FGR (Fetal growth restriction) and SGA (Fetal growth restriction) remains an obstetric challenge [77]:
- Babies born SGA may not have FGR
- •Babies born AGA (Appropriate for gestational age) may have been affected by growth restriction and this may not have been detected antenatally[77]
- FGR may not be detected clinically before birth
- Babies who are SGA due to FGR are more likely to have problems during the newborn period and require specialized care, than SGA babies without FGR
- A SGA baby at term may not have a low birth weight (LBW) (i.e. less than 2500 g)

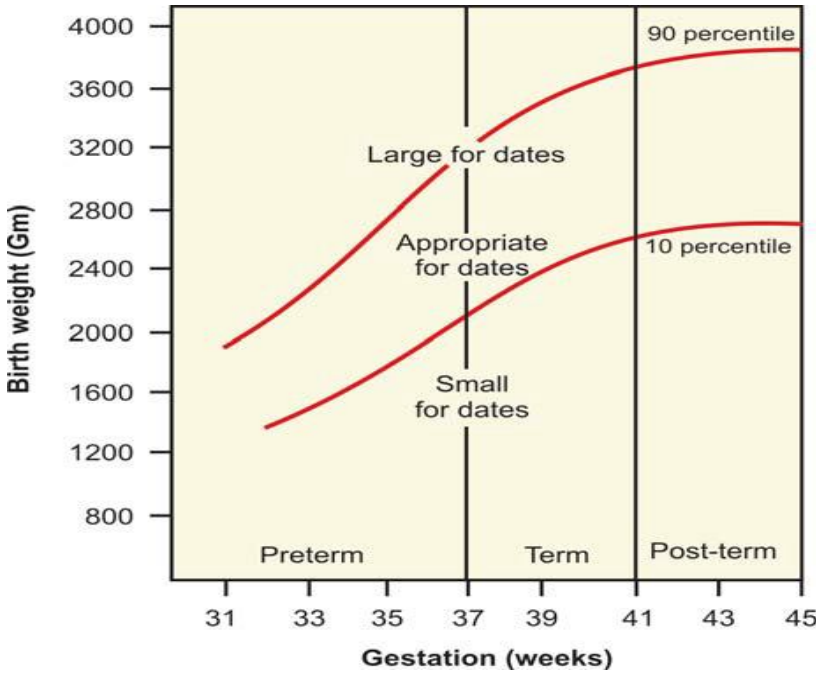


Figure-2: Fentons growth chart showing intrauterine growth curve[78] This helps in classifying neonates into three categories, viz. small-for-dates (SFD), appropriate-for-dates (AFD) and large-for-dates (LFD)

INCIDENCE OF SGA BABIES

The data on the incidence of SGA births are scarce in many countries because birth length and gestational age are rarely recorded in National databases. Based on the available data, it has been estimated that between

2.3 and 10% of all infants are born SGA.[79] India has a high incidence of low birth weight (LBW) and SGA babies [10-12]. The incidence of LBW in India is about 30% babies in contrast to 5-7% in developed countries [10]. A large percentage (approximately 70%) of LBW babies are SGA [80, 81] Kushwaha, *et al*.[82] studied 750 hospital deliveries (term singleton neonates) and found that 28.4% were SGA, which is almost similar to the incidence of 25% reported by Mehta, et al[83] Thus there is a huge burden of LBW and SGA in our country which needs to be addressed.

An Australian study reported perinatal mortality in term SGA babies was significantly higher than in term AGA babies. Perinatal mortality for SGA babies, which included more stillbirths than neonatal deaths, was reported at [84]:

- 3.5/1000 births at the 5th to less than 10th percentile, rising to 17.8/1000 births at less than the 1st percentile
- 0.89/1000 births at 37 weeks rising to 4.82/1000 births at 41 weeks

EPIDEMIOLOGY OF SGA BABIES

The causation of SGA is multifactorial. Fetal factors include chromosome abnormalities and genetic defects. Maternal factors involve age, weight and height, parity, chronic diseases, infections, impairment of nutritional status, and substance abuse. Placental factors include structural abnormalities and insufficient perfusion. Thus the ability to reach an optimal birth weight results from the interaction between the fetal growth potential (the fetal factors) and the environment (placental and maternal factors).[85] The definition of SGA does not take into account the background growth-modifying factors such as maternal size, ethnicity, and parity. These factors may help in understanding the mechanisms and implications of being born SGA. Narang, *et al*.[82] in 1997 concluded that idiopathic intrauterine growth retardation is the commonest cause of SGA in Indian babies, followed by pregnancy induced hypertension which is one of the most important risk factors for SGA/IUGR.

CONSEQUENCES OF BEING BORN SGA

Among SGA children who do not achieve catch-up growth by 2 year of age, the relative risk of short stature at 18 year of age is 5.2 for those born light and 7.1 for those born short. [86] Low birth weight due to fetal growth retardation, and SGA children who experience rapid catch-up growth during childhood have been linked to development of the metabolic syndrome with all its diverse components (referred to as insulin resistance syndrome) – type 2 diabetes, hypertension, obesity, and hyperlipidemia. Barker, *et al*. [87] observed that the risk of metabolic syndrome at the age of 50 yr was 10-fold greater in individuals with a birth weight less than 2.5 kg than in those whose birth weight exceeded 4.5 kg. In another study, there were statistically significant differences in all components of the metabolic syndrome at 22 yr of age between the SGA and the AGA groups. [88] They found that 2.3% of individuals born SGA develop metabolic syndrome according to Adult Treatment Panel III criteria, compared with only 0.3% of individuals born AGA. Furthermore, insulin resistance was significantly associated with other indicators of the metabolic syndrome, such as a high waist-to-hip ratio, hypertension, hypertriglyceridemia, and hyperglycemia Pubertal comorbidities in SGA are; higher risk for polycystic ovary syndrome, fertility problems, ovarian dysfunction, reduced fertility and early menopause.[89, 90]

STUDIES ON VITAMIN D DEFICIENCY AND SUPPLEMENTATION

Till few decades ago, vitamin D was thought of only in relation to bone health and Calcium homeostasis. Now, medical and nonmedical fraternities across the world are getting increasingly curious and realizing the potential role vitamin D plays in health and disease. There had been a rise in the rate of publication of peer reviewed articles on vitamin D in Pub Med from about 100 articles per year in 1975 to >1400 in 2007.[91] Time magazine has reported vitamin D as one of the top 10 medical breakthroughs of 2007.[92] New York Times has claimed vitamin D as a potential new miracle drug.[93] It stands at the frontline of current scientific endeavors, being a topic of greatest interest to medical researchers all over the globe. A growing body of evidence, implicating hypovitaminosis D as a risk factor for many diseases right from conception throughout lifespan, implies that awareness and management of widespread vitamin D deficiency may fetch profound future health benefits.

Narendra Rathi et al [94] reviewed that Vitamin D deficiency is highly prevalent throughout the world including India. Though some evidence suggests a role of hypovitaminosis D in pathophysiology of many clinical situations like autoimmune diseases, cardiovascular diseases, infections, cancers, fetal health, and exercise performance, some authorities feel there is a lack of unequivocal evidence in favour of nonskeletal health benefits of vitamin D. They concluded that widespread subclinical and pre-rachitic vitamin D deficiency in children should be diagnosed by serum 25(OH) D levels and these levels should be maintained > 20 ng/mL to obtain optimal health benefits.

Bhalala et al[59] did a longitudinal study on exclusively breastfed infants born at term with birth weight > 2.5 kg to normal, healthy mothers followed till 3 months. Serum calcium, phosphorous, heat labile alkaline phosphatase (HLAP) and 25(OH)D estimated in 42 mother / cord blood diads and in 35 (EBF) infants followed up at 3 months. Twenty five (OH)D < 15 ng/mL was considered low and 15 to 25 ng/mL low to normal. They found that Ca, P, HLAP were significantly higher in cord blood ($P < 0.001$) but mean 25 (OH)D, 19.36 ng/mL was comparable to maternal level of 22.9 ng/mL ($r = 0.82$, $P < 0.001$). At 3 months only HLAP was significantly higher compared to cord blood. Higher 25 (OH)D at 3 months correlated with higher 25 (OH)D values in cord blood ($r = +0.616$, $P < 0.001$) as well as higher antenatal maternal levels ($r = + 0.552$, $P < 0.001$). Serum 25 (OH)D values < 25 ng/mL was observed in 50 % mothers, 62 % cord blood specimens and 80 % infants at 3 months. They concluded that Subnormal maternal vitamin D status is associated with vitamin D deficiency in newborns and persists in exclusively breastfed infants.

Tergestina M. et al [95] did a prospective cohort study recruiting 111 preterm babies, 25 to 32 weeks' gestation from a tertiary care perinatal center in south India. Cord blood was assayed for serum calcium, phosphate, alkaline phosphatase, and 25-hydroxyvitamin D (25(OH)D). All of the babies were fed unfortified breast-milk and supplemented daily with calcium, phosphate, and 400 IU of vitamin D. At 6 weeks serum calcium, phosphate, alkaline phosphatase, parathyroid hormone, and 25(OH)D levels were estimated. A total of 90 (81%) of the preterm babies were followed up until 6 weeks. The median (interquartile range) vitamin D level in the preterm group was 34.7 (25.6-50.1) and 19.3 (13.9-27.1) ng/mL at birth and 6 weeks, respectively. Using a cutoff value of <20 ng/mL to determine vitamin D insufficiency (VDI), it was observed that 12.6% of the babies were vitamin D insufficient at birth. This increased to 52.2% at 6 weeks despite the recommended supplementation with vitamin D ($P < 0.001$). They concluded that the prevalence of VDI was not high at birth; however, a large proportion of preterm babies were vitamin D insufficient at 6 weeks despite being supplemented with vitamin D 400 IU/day. The recommended vitamin D supplementation of 400 IU appears to be inadequate to prevent VDI, and hence randomized controlled trials looking at higher doses of vitamin D supplementation are needed.

Zecca et al [96] did a study of neonates in which 400 IU per day of 25(OH)D3 were given to 126 preterm and 112 full-term, small for date newborn infants, while 1000 UI per day of Vitamin D2 were given to 18 preterm and 27 **full-term, small for date newborn infants**, in order to compare their effectiveness for the prevention hypocalcaemia in these neonates. 67 preterm and 67 full-term newborns were included in the control group. The incidence of late hypocalcemia was reduced from 16.4% to 0 in full-term babies and from 6% to 2.4% in preterm babies by the 25(OH)D3 but not by Vit D2 administration. The incidence of early hypocalcemia was not modified at all. The Authors suggested 25(OH)D3 administration to prevent the late hypocalcemia and, together with calcium support, to treat the early hypocalcemia in the low birth weight newborn.

STUDIES ON VITAMIN D DEFIECINCY AND SUPPLEMENTATION RELATED TO SGA BABIES

Yuan-Hua Chen [97] showed that there was a positive correlation between maternal serum 25-hydroxyvitamin D level and offspring birth weight ($r = 0.477$; $P < .001$). Study was done on 3658 pregnant ladies. Further analysis showed that 4.98% of neonates were LBW infants among the subjects with vitamin D deficiency (RR, 12.00; 95% confidence interval [CI], 4.37, 33.00) and 1.32% among the subjects with vitamin D insufficiency (RR, 3.18; 95% CI, 1.07, 9.48). After adjustment for confounders, the RR for LBW infants was 12.31 (95% CI, 4.47, 33.89) among subjects with vitamin D deficiency and 3.15 (95% CI, 1.06, 9.39) among subjects with vitamin D insufficiency. Moreover, 16.01% of neonates were SGA infants among subjects with vitamin D deficiency (RR, 5.72;95%CI, 3.80, 8.59) and 5.59% among subjects with vitamin D insufficiency

(RR, 1.99; 95% CI, 1.27, 3.13). After adjustment for confounders, the RR for SGA infants was 6.47 (95% CI, 4.30, 9.75) among subjects with vitamin D deficiency and 2.01(95%CI, 1.28, 3.16) among subjects with vitamin D insufficiency. They concluded that Maternal vitamin D deficiency during pregnancy elevates the risk of SGA and LBW infants in Chinese population

Alison D. Gernand [98] assayed serum samples at 12 to 26 weeks of gestation for 25(OH)D in a sample of participants in a multicenter clinical trial of low-dose aspirin for the prevention of preeclampsia in high-risk women (n=792). Multivariable log-binomial regression models were used to assess the association between 25(OH)D and risk of SGA (birth weight less than the 10th percentile of gestational age) after adjustment for confounders including maternal pre pregnancy obesity, race, treatment allocation, and risk group. Thirteen percent of infants were SGA at birth. Mean (SD) 25(OH)D concentrations were lower in women who delivered SGA (57.9 [29.9] nmol/L0 vs. non-SGA infants (64.8 [29.3] nmol/L, P=0.028). In adjusted models, 25(OH)D concentrations of 50-74 nmol/L and ≥75 nmol/L compared with <30 nmol/L were associated with 43% (95% confidence interval [CI] 0.33-0.99) and 54% (95% CI 0.24-0.87) reductions in risk of SGA, respectively. Race and maternal obesity each modified this association. White women with 25(OH)D ≥50 vs. <50 nmol/L had a 68% reduction in SGA risk (adjusted risk ratio [RR] 0.32, 95% CI 0.17-0.63) and non obese women 25(OH)D ≥50 vs. <50 nmol/L had a 50% reduction in SGA risk (adjusted RR 0.50, 95% CI 0.31-0.82). There was no association between 25(OH)D and risk of SGA in black or obese mothers. They concluded that maternal vitamin D status in the second trimester is associated with risk of SGA among all women, and in the subgroups of white and nonobese women

Yao Chen [99]A comprehensive literature search of PubMed, the Cochrane Library, Embase, and the Elsevier Science Direct library was conducted to identify relevant articles reporting prospective cohort studies in English, with the last report included published in February 2017. Pooled odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were used to evaluate the correlation in a random effects model. Results A total of 13 cohort studies were included in this meta-analysis with a sample of 28 285 individuals from seven countries. The pooled overall OR for babies born SGA was 1.588 (*95%* CI 1.138 to 2.216; p<0.01) for women with vitamin D deficiency. The prevalence of vitamin D deficiency during pregnancy varied from 13.2% to 77.3%. Subgroup analyses identified no significant differences in the association between vitamin D deficiency and SGA based on study quality, gestational week during which blood sampling was performed, cut-off vitamin D levels, sample size, adjustment for critical confounders and method for measuring vitamin D. This meta-analysis suggested that vitamin D deficiency is associated with an increased risk of SGA.

Natarajan et al [100] did a randomized double-blind trial, in which they allocated eligible infants to receive either 800 or 400 IU of vitamin D3 per day (n = 48 in both groups). Primary outcome was VDD (serum 25-hydroxyvitamin D levels, 20ng/mL) at 40 weeks' PMA. Secondary outcomes were VDD, bone mineral content, and bone mineral density at 3 months' corrected age (CA). The Prevalence of VDD in the 800-IU group was significantly lower than in the 400-IU group at 40 weeks (38.1% vs 66.7%; relative risk: 0.57; 95% confidence interval: 0.37–0.88) and at 3 months' CA (12.5% vs 35%; relative risk: 0.36; 95% confidence interval: 0.14–0.90). One infant (2.4%) in the 800-IU group had vitamin D excess (100–150 ng/mL). Bone mineral content (mean 6 SD: 79.6 6 16.8 vs 84.7 6 20.7 g; P = .27) and bone mineral density (0.152 6 0.019 vs 0.158 6 0.021 g/cm2; P = .26) were not different between the 2 groups. They concluded that Daily supplementation with 800 IU of vitamin D reduces the prevalence of VDD at 40 weeks' PMA and at 3 months' CA in preterm infants without showing any improvement in bone mineralization. However, there is a possibility that this dose may occasionally result in vitamin D excess.

These studies suggest that there is variable level of deficiency among pregnant mothers and neonates at birth in India. Also the prevalence of deficiency is different in different parts of the country. The role of daily Vitamin D supplementation to infants shows variable outcomes. The optimal dose of vitamin D supplementation in Indian population has not been widely tested.

We could not find a single study on Vitamin D supplementation exclusively on term SGA babies in the available literature. So we intent to study the effect of two different regimens (400 IU and 800IU) of oral Vitamin D supplementation in term neonates at birth and at 3 months following exclusive breast feeding. We

also try to evaluate the Vitamin D status of Mothers and their Neonates at birth in this part of the country. To the best of our knowledge no similar study has been done in this part of the country.

MATERIALS AND METHODS

Study Design: Single blinded randomized, controlled trial

Setting: Neonatology unit, Department of Pediatrics and Rajeev Gandhi Centre for Diabetes and Endocrinology, J.N.Medical College, A.M.U, Aligarh.

Time Period of Study: February 2016 to October 2017

Subjects: Hundred Term small for gestational age (SGA) new born babies admitted under Neonatology ward in the department of Pediatrics JNMC, AMU, Aligarh.

INCLUSION CRITERIA

Babies who have **ALL** of the following features

1. Healthy term small for date or small for gestational age (SGA) babies born of uncomplicated delivery (Birth weight <2500gms)
2. Babies who are likely to be exclusively breastfed till 3 months

(WHO guidelines for exclusive breastfeeding is that the infant should only receives breast milk without any additional food or drink, not even water)

EXCLUSION CRITERIA

Babies with **ANY ONE** of the following characteristics

1. Pre term babies (Gestation period up to 36 weeks or < 259 days)
2. Term babies with birth weight =/>2500gms
3. Mothers on long-term medications that affect vitamin D metabolism (except corticosteroids) such as anticonvulsants (phenobarbitone, phenytoin, carbamazepine, valproic acid), antifungals, antiretrovirals (ritonavir, sequinavir)
4. Mothers having any systemic diseases that can alter Vitamin D metabolism (Renal/Hepatic)
5. Non compliant Babies regarding Vitamin D supplementation or Exclusive breast feeding
6. Refusal to give consent

CONSENT FOR THE STUDY AND ETHICS APPROVAL

The study was submitted for Institutional Ethics Committee approval of Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh. Ethical committee clearance was obtained in February 2016 **(Annexure-1**). Informed parental consent was obtained from the parents before inclusion into the study.

Parent-patient information sheet in native languages was provided to all subjects before inclusion into the study **(Annexure 2).** The study has been also registered in Clinical Trial Registry India (CTRI) retrospectively in December 2016 with CTRI No CTRI/2016/12/007519

RANDOMIZATION

Randomization was done in blocks of 4. A total of 8 blocks were generated. Randomization sequence were generated by a computer program and maintained by a colleague not directly involved in this trial.

ALLOCATION CONCEALMENT

The allocated intervention was kept in a sealed opaque envelope (Fig 3), which was opened at the time of randomization. Subjects were allocated to either of the two interventions in order of their enrollment in the study.

Figure-3: Sample of opaque envelope used for randomization in the study

BRIEF PROCEDURE

This was a prospective randomized study done over a period of two years from Feb 2016 to Oct 2017 in Neonatology unit, (Fig 5) Dept of Pediatrics in collaboration with Dept of Endocrinology JNMC, AMU, Aligarh. One hundred and thirty new born term SGA Babies (Fig 4) after delivery were assessed for eligibility for the study. Of which 100 babies were found eligible and recruited as per inclusion and exclusion criteria. After taking written informed consent from the parent/legal guardian of the eligible subjects, a detailed history and physical examination finding were recorded on pre-designed Performa **(Annexure 3)**.

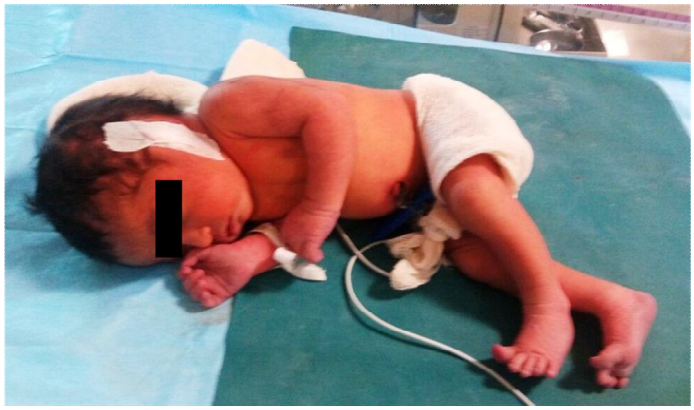


Figure-4: Showing a SGA baby

Figure-5: Showing the Neonatal ICU ward, JNMC, AMU, Aligarh

INTERVENTION

These 100 Eligible SGA babies included in this study were randomized to one of the two treatment allocations groups to receive either

Group 1: Vitamin D dose 400 IU/day daily orally for 3mths or

Group 2: Vitamin D dose 800 IU/day daily orally for 3mths

After randomization the Antenatal History, Postnatal history and Examination findings were recorded. The gestation age was determined by the Last menstrual period (LMP) as informed by mother or as per New Ballard score[101] and recorded in weeks. The birth weight, Length, Head circumference and Chest Circumference was documented at birth. About 3 ml of cord blood sample was drawn aseptically from the neonates within 48 hours of Delivery and refrigerated at about -20º in the department after labeling.

The mothers of both the groups are advised to exclusively breast feed the babies and inform any deviation from it. They were advised to give daily oral supplementation of 400 IU or 800IU of Vitamin D solution in the bottle as per the allotment. Vitamin D was supplemented as DEPURA KIDS (Sanofi Aventis pharma India) Vitamin D oral solution containing 400IU in 0.5ml. The mothers were instructed to give 0.5ml of solution for the group 1 and 1ml for the group 2 using the dropper. It was administered once daily to babies either directly or mixed with expressed breast milk. Compliance of the Vitamin D intake was checked with return of empty bottles at follow up and telephonically during intervention periods.

Figure-6: Showing the Vitamin D solution bottle and dropper used for the study

All the enrolled infants were followed up at 3 months when they visit for routine Immunization. Detailed history about any illness and breast feeding were recorded. Repeat examination was done at the follow up and Anthropometric measurements were recorded as done at enrolment. Another 3 ml of venous blood sample was be collected from Infants and refrigerated after labeling till analysis was done

Serum sample collection: Approximately 3ml of blood was drawn using a pre-refrigerated glass syringe via aseptic technique by peripheral venous phlebotomy. Nearly 1 ml of serum separated out of blood by centrifugation at 2000 rpm for 5 minute was transferred into screw-capped plastic vials. All samples were labeled and refrigerated immediately at -20°C in the Pediatric Department until they were tested. The laboratory technician conducting the test was unaware of the treatment allocation group of the patient and whether the levels were assessed pre- or post-supplementation with vitamin D. All These blood Samples will be subjected for estimation of serum vitamin D level. Total circulating 25(OH)-D were assayed on Access 2 immunoassay system (Beckman Coulter) using chemiluminescence in the laboratory of Rajiv Gandhi Centre for endocrinology, AMU (Fig 7). The assay measures total 25(OH) Vitamin D levels with equimolar measurement of 25(OH) Vitamin D2 and 25(OH) Vitamin D3.

Figure-7: Access 2 Immunoassay System (Beckman Voulter) for accessing serum 25(OH) Vitamin D

The normal reference range for vitamin D i.e. Serum25 (OH)D in the laboratory where test was conducted is 30-100ng/mL

The values of the serum Vitamin D levels was classified as follows[29]

- Deficiency: <20ng/mL
- Insufficiency: 20-30ng/mL
- Sufficiency: >30ng/mL
- Toxicity: >150ng/mL

OUTCOME MEASURES

The study used the following outcome measures to evaluate the differences between treatment groups:

- Primary outcome measure was mean change in serum 25(OH)-D levels
- Secondary measures were the change in the birth weight, Length, Head circumference and Chest Circumference

STATISTICAL ANALYSIS

Analysis of the data was done on per protocol population who completed the study without any major protocol violations. Ordinal data was compared using Chi-square test. Independent sample t-test and one-way analysis of variance (ANOVA) test were used to compare difference in serum Vitamin D levels between and within the intervention groups respectively. Mann Whitney U test was used to compare the percentage difference on supplementation between the groups. Significance was taken at P value of <0.05. The analysis was done using the SPSS software Version 22.0.0.0 (2015).

SAMPLE SIZE

Since no previous study was available in the literature to compare Vitamin D levels in SGA babies we calculated the sample taking the study of Natarajan et al 2014 as the study design and dose supplementation was comparable.[58] In their study the prevalence of vitamin D deficiency in 800 IU was significantly lower than the 400 IU group at 40 wks GA (38.1%v/s 66.7%); relative risk of 0.57%, At 3 months (12.5% v/s 35% relative risk :0.36). Assuming an estimate of prevalence of deficiency of 75%in 400 IU group we needed to enroll 35 babies per group to detect a decrease in prevalence of vitamin D deficiency after supplementation with 800 IU /day with a power of 90% and an alpha error of 0.05%. We planned to enroll total of 100 babies to account for loss to follow up and non compliance.

The sample size was calculated online using open epi (http://www.openepi.com/SampleSize/SSCohort.htm) by Kelsey method, considering significance level (1-alpha) of 90% and power (1-beta, % chance of detecting) of 80. Ration of sample size Unexposed/Exposed – 1, Percent of Unexposed with Outcome 73% and Percent of Exposed with Outcome 43%, Risk/Prevalence ratio 0.59.

OBSERVATIONS AND RESULTS

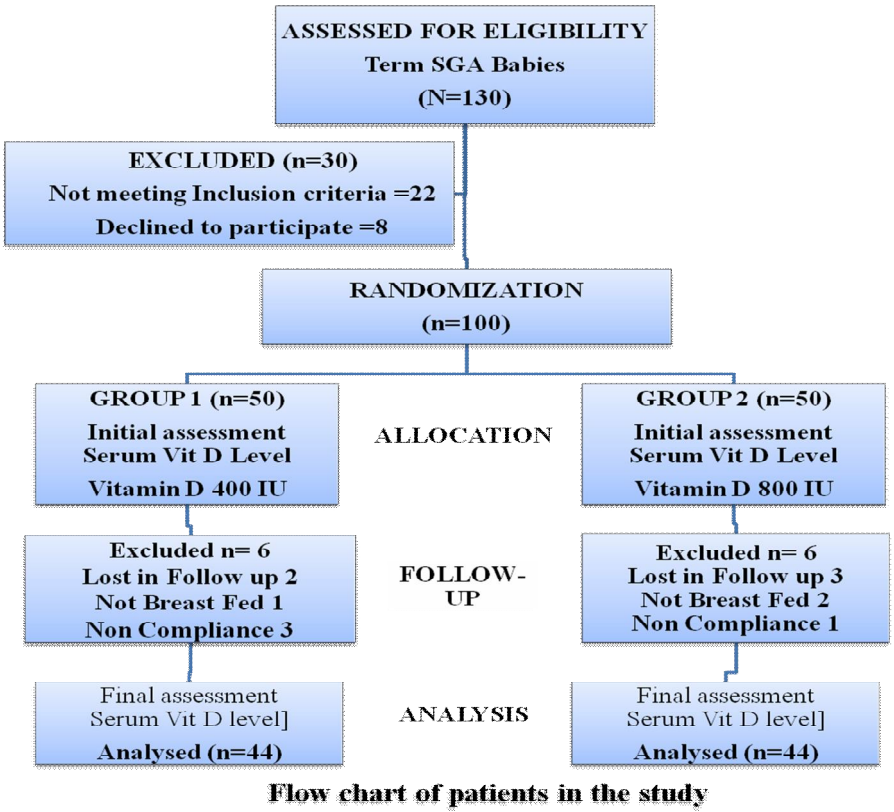
This randomized controlled trial to test the efficacy of daily supplementation of two different doses of oral vitamin D was done in the Neonatology unit in Department of Pediatrics and Rajiv Gandhi Centre of Diabetes and Endocrinology at Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh from February 2016 to October 2017.

During this study period 130 small for Gestational age new born babies were assessed for eligibility to participate in the trial. Thirty patients were excluded from the study as per exclusion criteria or not willing to participate in the study. Thus hundred neonates were enrolled in this trial. We intended to analyze our study on per protocol population considering only those patients who strictly adhere to the protocol.

Hundred patients were randomized to either intervention: group 1 (400IU of vitamin D) and group 2 (800 IU of Vitamin D) (Fig.8). These patients were followed for 3 months and reassessed at study completion. All children were evaluated at 3 months recording was made of their height, weight, Head circumference and Chest circumference. At study completion, the serum Vitamin D level was also repeated.

A total of five patients were lost to follow-up (two in group 1 and three in group 2). Three babies (one in group 1 and two in group 2) were not exclusively breast fed and were given fortified milk were excluded. Another four patients (Three in group 1 and one in group2) were non compliance in daily vitamin D intake and mothers have missed giving the Vitamin d drops during the study. So a total of twelve babies were excluded from the study at the final follow up.

Thus, a total of 88 babies completed the study, 44 patients in each group. Data of these 88 patients was analyzed and is being presented below.



Flow chart of patients in the study

Figure-8: Flow chart of patients in the study

BASELINE DEMOGRAPHIC AND ANTHROPOMETRIC CHARACTERISTICS

On analyzing the data of 88 subjects we found that, 53 (60.2%) were females and 35 (39.8%) were male babies. The ratio of male and females in both the groups is almost equal (Figure 9). The male is to female number was 26:18 in group 1 and 27:17 in group 2. The religion of mothers was Muslim in 60 (68.1%) and Hindu in 38 (31.9%) as shown in Figure 10. The number of Muslims and Hindus were 31:13 in group 1 when compared to 29:15 in group 2.

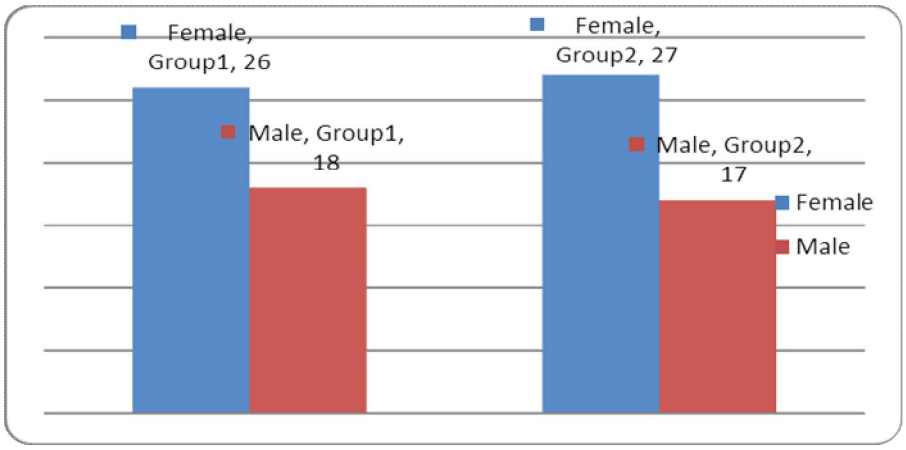


Figure-9: Sex distribution in both groups

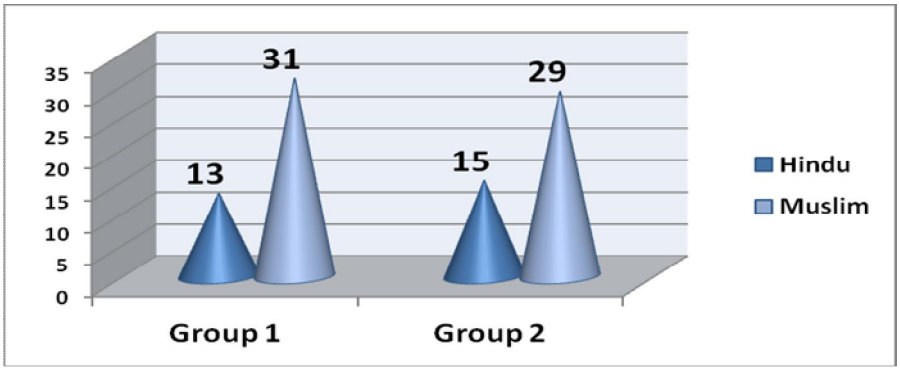


Figure-10 : Distriution of Religion

The Mean age of the mother at delivery in both the groups are comparable and not significant (figure 11). The mean age of mothers was 24.9±2.9 years in group 1 and 25.7±3.9 years in Group 2. In the type of delivery about 61.4% (n=54) had normal delivery and 38.6% (n=34) had caesarean section (LSCS). In group 1 we noticed that 23 babies (52.3%) were born by LSCS when and 21(47.7%) by normal delivery. Where as in group 2 we had more babies born of normal delivery 33(75%) while were LSCS was recorded in 11(25%) of mothers (Fig 12). The values are significant ($p<0.001$).

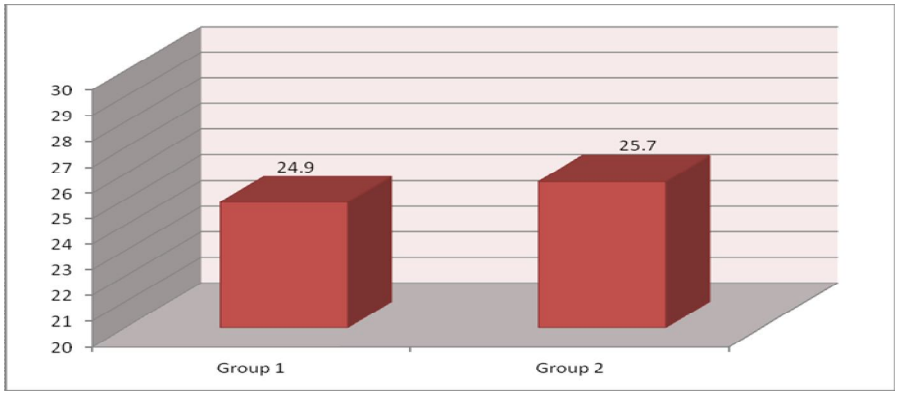


Figure-11: Mother age (mean) in both the groups

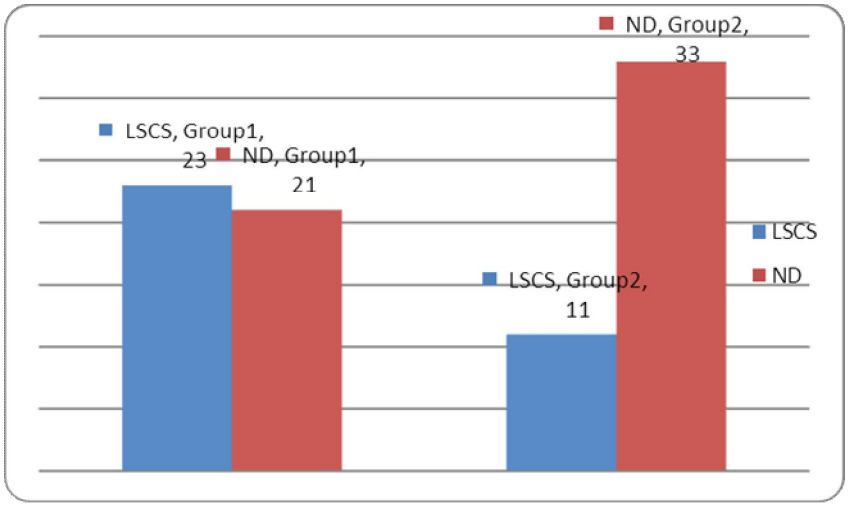


Figure-12: Showing the Type of Delivery

All the enrolled children were followed upto 3 months of age, when they visit for the routine 12 weeks immunization. The average follow up period was 94.9±3.6 (89 to 102) days in group 1 and 94.2±3.6 (88 to 104) days in group 2 (Fig 13). So the mean duration of supplementation of vitamin D was about 94 days in both the groups and is comparable. The various baseline demographic characteristics are summarized in Table V.

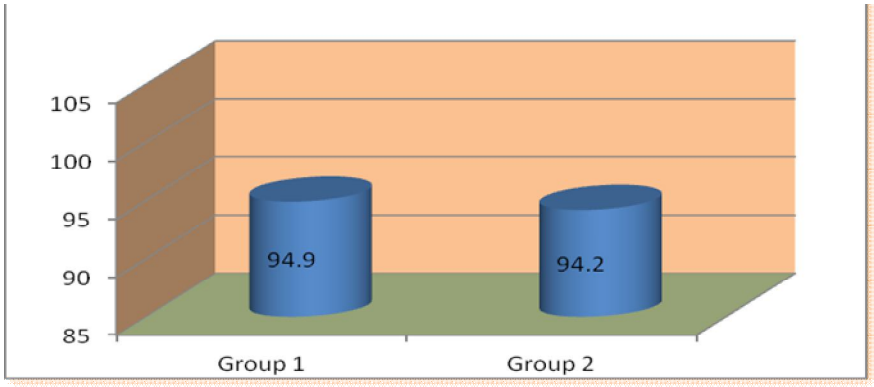


Figure-13: Follow up period (in Days)

Table-V: Clinical parameters of subjects at enrolment

	Group 1(n=44)	Group 2 (n=44)	P value
Sex - Male: Female	18:26	17:27	0.826
Religion - Hindu: Muslim	13:31	15:29	0.652
Type of Deliver- LSCS: Normal	23:21	11:33	**0.008**
Mother's age (years)	24.9±2.9	25.7±3.9	0.277
Follow up period (days)	94.9±3.6	94.2±3.6	0.364

CLINICAL AND DEMOGRAPHIC CHARACTERISTICS IN THE INTERVENTION GROUPS

The baseline clinical and demographic characteristics of new born babies at birth in the two interventions groups have been compared in Table V. The average birth weight in group 1 was 2.14 ± 0.28kgs which is comparable to group 2 2.12 ± 0.29kgs. The difference was not statistically significant. The average length of the babies measured at birth was 46.2 ± 2.9cms in group 1 and 45.6 ± 3.2cms in group 2. The difference is statistically insignificant.

The Head circumference and chest circumferences measured at birth were 31.4 ± 2.0cms & 29.6 ± 2.2cms in group 1 and 31.4 ± 2.4cms & 29.7 ± 2.3cms in group 2 respectively. These values also are not significant and comparable in both the groups.

Table-VI: Anthropometric parameters at Birth

	Group 1 (n=44)	Group 2 (n=44)	P value
Weight (in Kgs)	2.14 ± 0.28	2.12 ± 0.29	0.742
Length (in Cms)	46.2 ± 2.9	45.6 ± 3.2	0.351
Head Circumference (in Cms)	31.4 ± 2.0	31.4 ± 2.4	1
Chest Cicumference (in Cms)	29.6 ± 2.2	29.7 ± 2.3	0.835

At 3 months follows up after the Vitamin D supplementation. The average weight of babies in group 1 was 3.24 ± 0.31kgs and 3.44 ± 0.39kgs (Fig 14). In group 2 there was definite significant gain in the weight of babies at 3 months ($p<0.001$). The correlation of this increased weight gain in group 2 babies who received 800 IU of vitamin d supplementation is difficult to infer from this. Other factors like breast feeding, genetic factors, etc needs to be evaluated.

The average length of the babies measured at 3 months was 50.3 ± 3.3cms in group 1 and 48.6 ± 3.5cms in group 2. The difference is statistically significant and needs further evaluation as no direct correlation can be obtained by this data.

The Head circumference and chest circumferences measured at 3 months were 35.2 ± 2.4cms & 31.7 ± 2.3cms in group 1 and 34.3 ± 2.5cms & 32.1 ± 2.4cms in group 2. These values also are not significant and comparable in both the groups as shown in Table VII and Fig.15.

Table-VII: Anthropometric parameters at 3 months

	Group 1 (n=44)	Group 2 (n=44)	P value
Weight (in Kgs)	3.24 ± 0.31	3.44 ± 0.39	**0.009**
Length (in Cms)	50.3 ± 3.3	48.6 ± 3.5	**0.021**
Head Circumference (in Cms)	35.2 ± 2.4	34.3 ± 2.5	0.088
Chest Cicumference (in Cms)	31.7 ± 2.3	32.1 ± 2.4	0.427

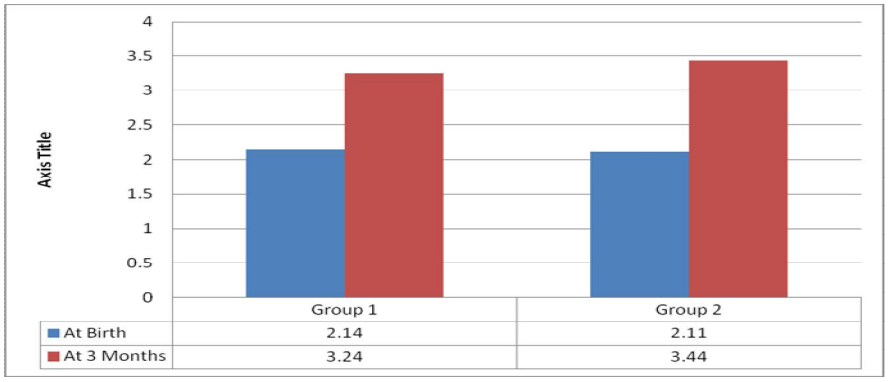


Figure-14 : Showing the comparision of weight of babies

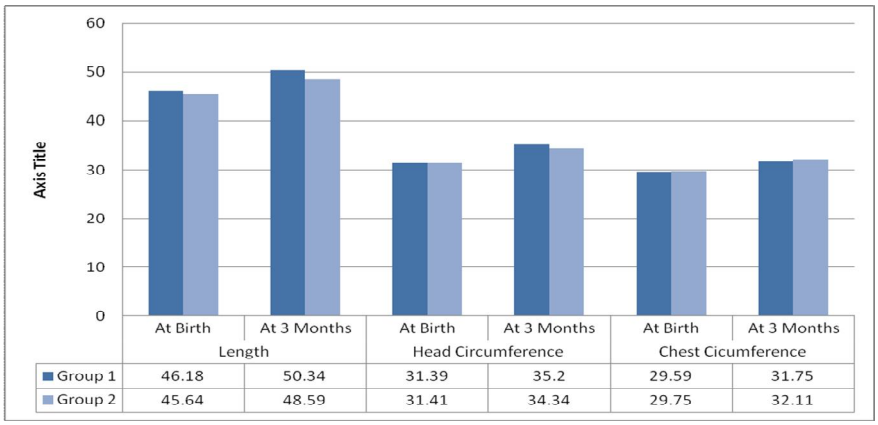


Figure-15: Showing the various anthropometric parameters

LABORATORY ASSESSMENT AT BASELINE

The Vitamin D levels of 85 babies (96.6%) analyzed in the study were <30ng/ml, three babies in group 2 had normal Vitamin D levels at the time of enrollment. 56 (63.7%) babies had vitamin D levels below 20ng/mL (deficiency) and 29 (32.9%) babies had values between 21-29ng/mL (insufficiency) and 3 (3.4%) babies had >30ng/mL (normal). The mean vitamin D levels of 88 babies at the time of enrollment were 17.7±6.9ng/mL.

POST-INTERVENTIONAL LAB PARAMETERS WITHIN GROUP CHANGE ON SUPPLEMENTATION

GROUP 1: The mean vitamin D level of 44 children in this group at enrollment was 17.81± 6.51 ng/mL. The mean vitamin D levels of these 44 children who completed the study were 31.43±6.60ng/mL. The mean increase in vitamin D levels in this group was 13.62±0.01ng/ml which was statistically significant (p=0.001).

GROUP 2: The mean vitamin D levels of 44 babies at the time of enrollment was 17.54 ± 7.46ng/ml.. The mean level of these 44 babies who completed the study was 34.06 ± 8.21ng/ml and the increase of 16.52±0.75 ng/ml was statistically significant (p=<0.001) as given in Table VIII.

Table-VIII: Change of Vitamin D levels within groups

	Baseline	Study completion	P value
Group 1 Serum Vit D levels	17.81± 6.51	31.43±6.60	P < 0.0001
Group 2 Serum Vit D levels	17.54 ± 7.46	34.06 ± 8.21	P < 0.0001

CHANGES BETWEEN GROUPS (GROUP 1 vs. GROUP 2):

The baseline parameters of both the groups were comparable at the time of enrollment as shown in Table V and VI. These parameters were comparable at the end of the study as well Table VII**.** The vitamin D levels of both the groups were comparable at the beginning and end of study (Table VIII)**.** The levels of vitamin D increase between both the groups were compared using Mann Whitney U test and the difference was found to be statistically insignificant (p>0.001) as shown in Box plot Fig 16 and 17. The vitamin D levels at 3 months was increased more in group 2 (800IU) than in group 1(400IU) but it was statistically not significant.

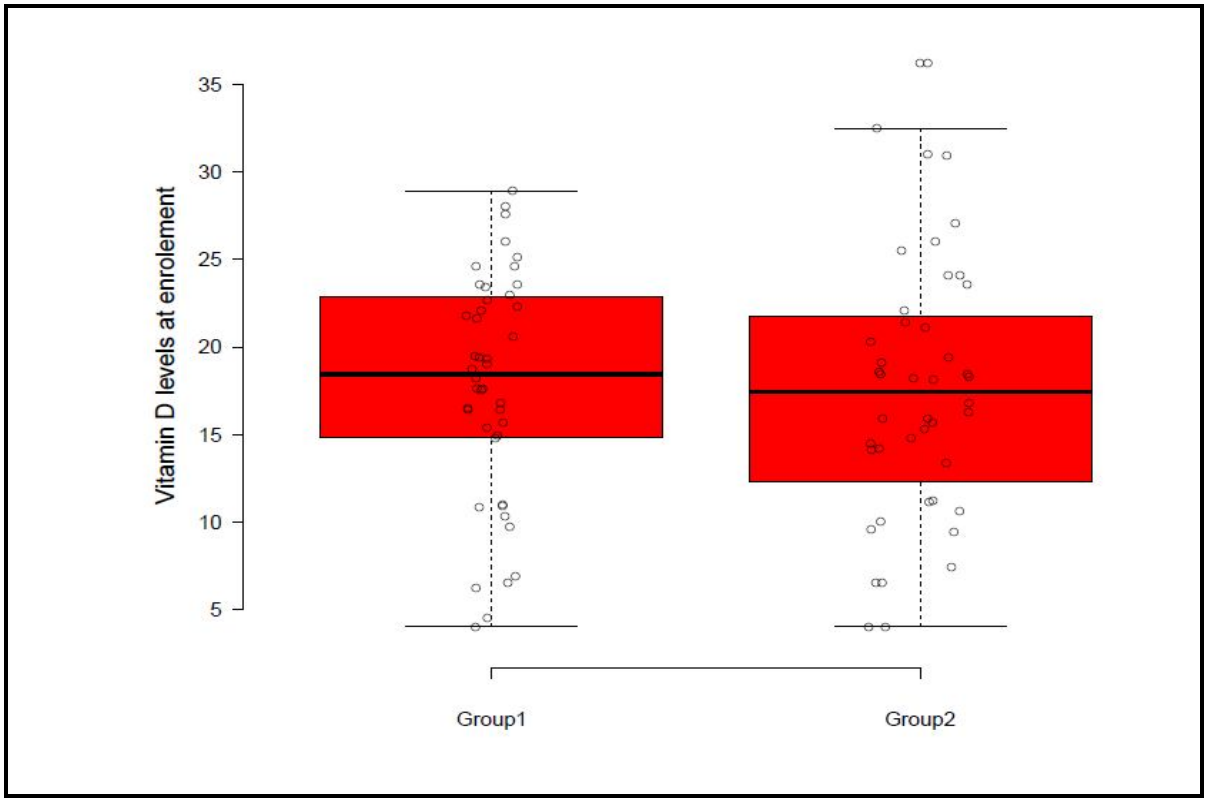


Figure-16: Boxplot showing baseline vitamin D values in the two intervention groups

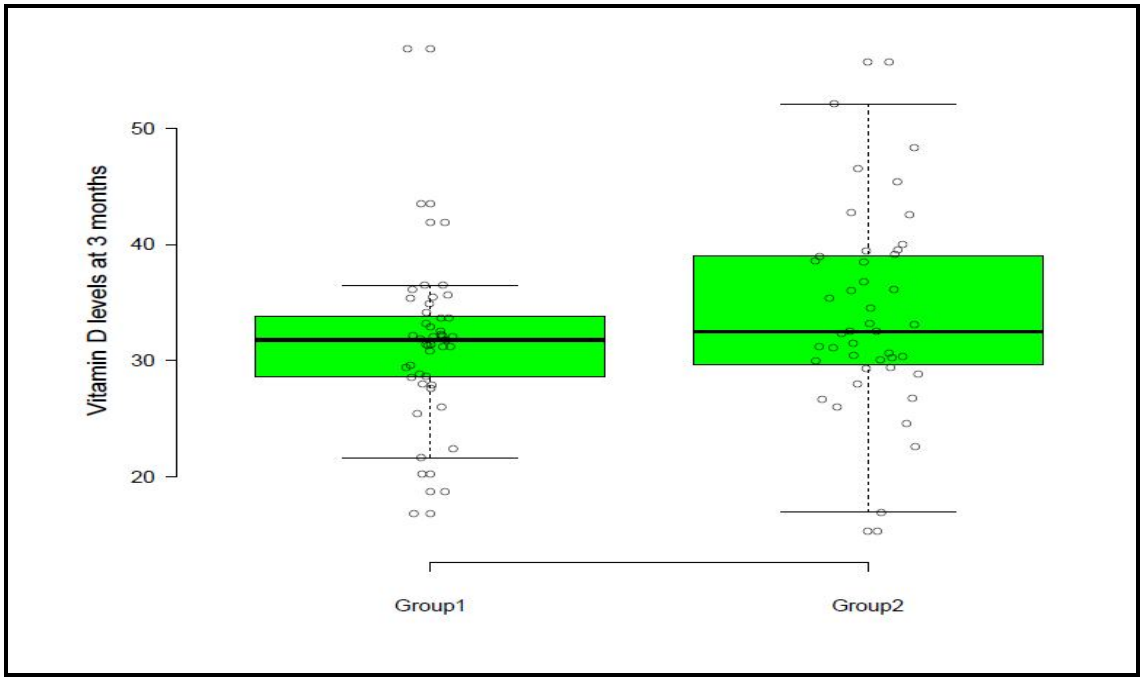


Figure-17: Boxplot showing vitamin D values at 3 months in the two intervention groups

On comparison of Vitamin D levels between the groups, the mean Vitamin D levels at birth were 17.81 ±6.51 and 17.54 ±7.46 ng/ml in Group 1 and Group 2 respectively. After the supplementation of Vitamin D for 3 months, the mean Vitamin D levels were 31.43 ± 6.60 in Group 1(400IU) and 34.06 ± 8.21 in Group 2(800IU). On comparing between groups there was some increase in value of Vitamin D but it was not statistically significant (p>0.001) Table IX and Fig 18. No patients in both groups had increased vitamin D levels leading to toxicity (>150ng/mL) during this study period.

Table-IX: Comparing Vitamin D levels between groups

	Group 1 (n=44)	Group 2 (n=44)	P value
At Birth	17.81 ± 6.51	17.54 ± 7.46	0.856
At 3 Months	31.43 ± 6.60	34.06 ± 8.21	0.101

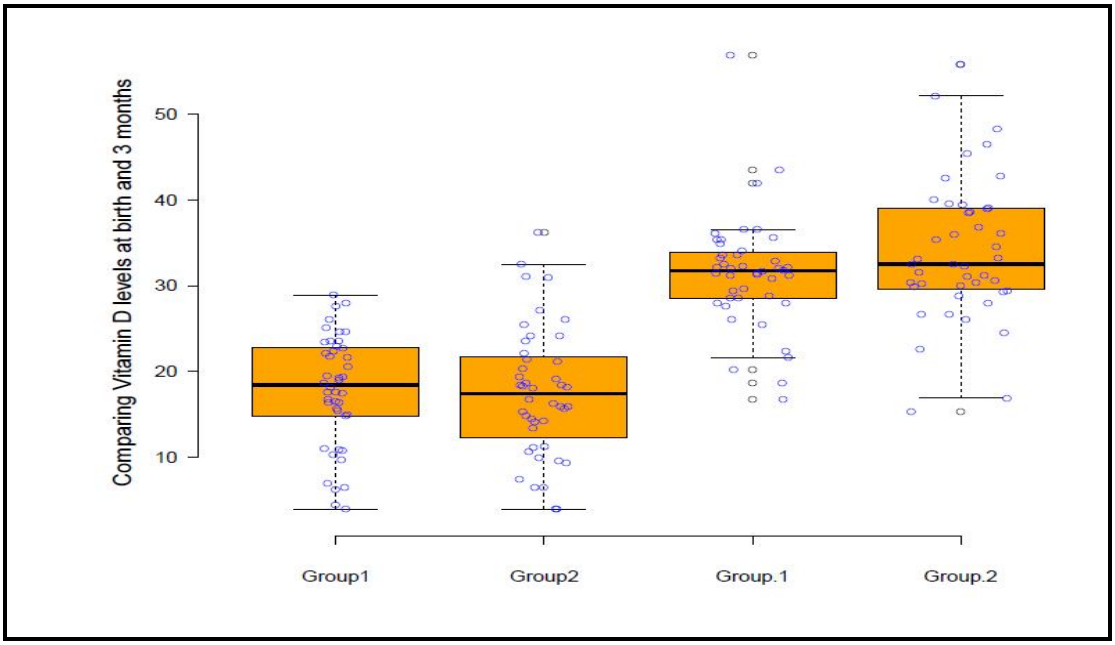


Figure-18: Box plot showing the change of Vitamin D levels between the groups

CHANGE IN VITAMIN D STATUS WITHIN AND BETWEEN GROUPS

The vitamin D levels of the 85 babies at enrollment were below normal (<30ng/mL). Three patients in group 2 had vitamin D levels >30ng/mL at birth itself. None of the children had clinical features of rickets at enrollment. No patients showed any toxicity at the end of study (Table X and Fig 20)

GROUP 1: There were 27 (61.4%) babies who had vitamin D deficiency (levels <20ng/ml) and the rest 17 (38.6%) had vitamin D insufficiency (21-29ng/ml) at birth. On completing the study, 28 (63.7%) children attained sufficiency, 14 (31.8%) had insufficiency and 2 (4.5%) babies remained deficient (Fig 19). Chi square test was used to compare the change in vitamin D status pre and post supplementation. The change was found to be statistically significant (p<0.001).

GROUP 2: At enrollment 29 (65.9%) babies had vitamin D deficiency, 12 (27.3%) had insufficiency and 3 (6.8%) babies had normal values. On completing the study we recorded that 27 (61.4%) babies had attained sufficient vitamin D levels (>30ng/mL), while 15 (34.1%) babies had insufficiency and 2 (4.5%) babies continue to remain deficient (Fig 19). The change in vitamin D status post supplementation was statistically significant (p<0.001).

The effect of supplementation was compared in between the allocation groups. It was not found to be statistically different (p>0.001)). Supplementation using either dose regimen improved the vitamin D status of study subject, so that while most patients in either intervention group were deficient at baseline (Fig.8), they moved up to insufficient levels following vitamin D supplementation (Fig.9).

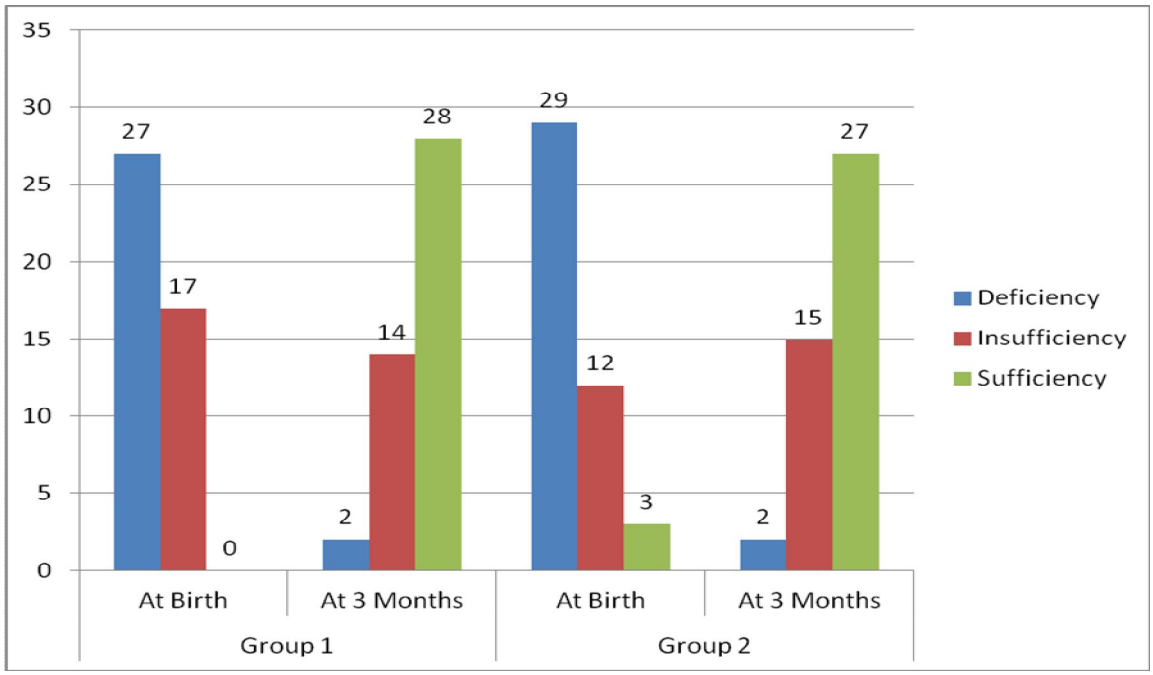


Figure-19 : Showing the distribution of Vitamin D levels in both groups

Table-X: Change in Vitamin D status within and between groups

	Group 1 (n=44)*		Group 2 (n=44))*	
	At Birth#	**At 3 Months#**	**At Birth#**	**At 3 Months#**
Deficiency	27 (61.4%)	2 (4.5%)	29 (65.9%)	2 (4.5%)
Insufficiency	17 (38.6%)	14 (31.8%)	12 (27.3%)	15 (34.1%)
Sufficiency		28 (63.7%)	3 (6.8%)	27 (61.4%)

* P=0.662 on comparison between two groups at enrollment.

* P=0.824 on comparison between two groups at study completion.

# P<0.001 on comparison within both groups.

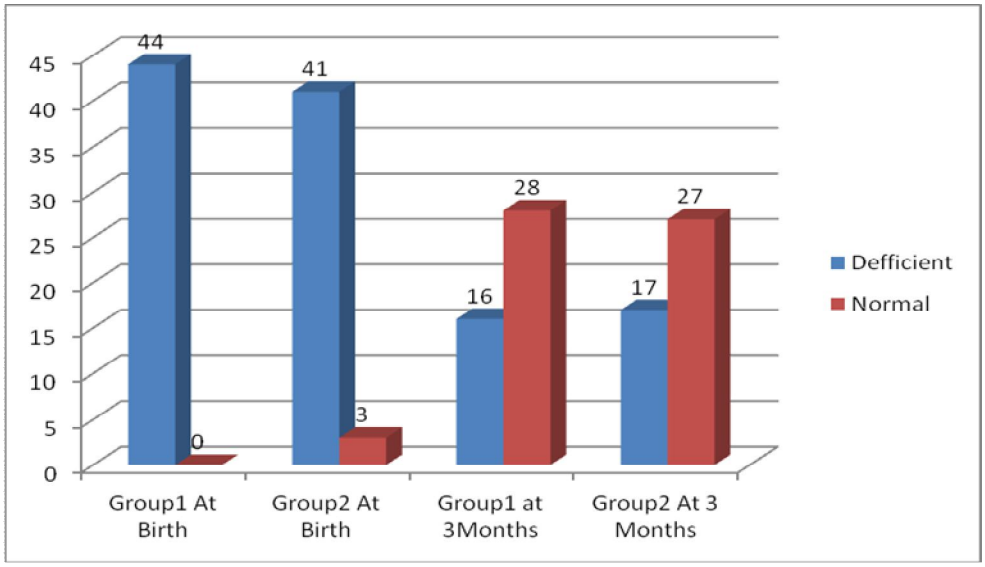


Figure-20: Showing the change of Vitamin D in both groups

DISSCUSION

This randomized controlled single blinded trial was conducted in Aligarh, Uttar Pradesh, India on 88 term SGA new born babies admitted in Neonatology unit Pediatric of Jawaharlal Nehru Medical College (JNMC), AMU, Aligarh. The study was conducted over a time period of one and half years (February 2016 to October 2016). Eighty eight babies were allocated into either of two intervention groups; 44 babies in group 1 (400IU of vitamin D) and another 44 babies group 2 (800 IU of vitamin D). Baseline parameters were noted and vitamin D levels were done at enrollment. These babies were given daily oral supplementation of Vitamin D solution as per allotted group with exclusive breast feeding. The babies were followed up for a mean duration of 94 days. At end of study, the vitamin D levels were repeated and the clinical parameters were compared both before and after supplementation within groups and between groups.

India is a vast tropical country that lies to the north of the equator between 6° 44' and 35° 30' north latitude and 68° 7' and 97° 25' east longitude. There is plenty of sunshine here and vitamin D deficiency was thought to be non-prevalent in this population. But it has been found that vitamin D deficiency prevails in epidemic proportions all over the Indian subcontinent, with a prevalence of 70%–100% in the general population.[22] The average serum levels of 25(OH)D levels in healthy children from northern India was 11.8±7.2ng/mL.[50]

Aligarh lies at a latitude of 27.88°N and longitude of 78.08°E and 178m above sea level. There is ample of sunshine throughout the summer days. Yet, the apparently healthy pediatric population including new born babies and pregnant mothers in this area is deficient in vitamin D as suggested by unpublished studies conducted in our institution. Along with racial, genetic factors, cultural, environmental and dietary factors also influence the levels of vitamin D levels in mothers and children.[22-24]

SMALL FOR GESTATIONAL AGE BABIES AND VITAMIN D STATUS

Data from rural Nepal and South India were taken compare the prevalence of small-for-gestational-age (SGA) and neonatal mortality risk associated with SGA using different birth-weight-for-gestation in 46 reference populations in low, middle, and high income countries.

The prevalence of SGA ranged from 10.5% to 72.5% in Nepal, and 12.0% to 78.4% in India, depending on the reference population. Females had higher rates of SGA than males. SGA prevalence was lowest when using reference populations from low-income countries. Infants who were both preterm and SGA had much higher mortality risk than those who were term and appropriate-for-gestational-age. Risk ratios for those who are both preterm and SGA ranged from 7.34–17.98 in Nepal and 5.29–11.98 in India, depending on the reference population.[102]

A recent meta-analysis to determine association of maternal vitamin D deficiency during pregnancy and small for gestational age (SGA) involving a total of 13 cohort studies with a sample of 28 285 individuals

from seven countries. The pooled overall odds ratio for babies born SGA was 1.588 (*95%* CI 1.138 to 2.216; p<0.01) for women with vitamin D deficiency. The prevalence of vitamin D deficiency during pregnancy varied from 13.2% to 77.3%. Subgroup analyses identified no significant differences in the association between vitamin D deficiency and SGA based on study quality, gestational week during which blood sampling was performed, cut-off vitamin D levels, sample size, adjustment for critical confounders and method for measuring vitamin D. This meta-analysis suggested that vitamin D deficiency is associated with an increased risk of SGA.[100]

PREVIOUS STUDIES ON SUPPLEMENTATION OF VITAMIN D IN TERM SMALL FOR GESTATION AGE BABIES

Our study is comparable to following studies[97, 101] in many aspects. We have done a randomized study similar to other studies but single blinded. The daily vitamin d supplementation doses compared are also similar i.e. 400 IU vs 800IU. The duration of study is 3 months in our study also and in many other similar studies. In their study[101] one patient had vitamin d excess (100-150ng/mL) in the 800IU group, but in our study we did had any babies who had vitamin levels > 100ng/mL in both the groups at the completion study.

Zecca et al[97] did a study of neonates in which 400 IU per day of 25(OH) D3 were given to 126 preterm and **112 full-term, small for date newborn infants**, while 1000 IU per day of Vitamin D2 were given to 18 preterm and 27 **full-term, small for date newborn infants**, in order to compare their effectiveness for the prevention hypocalcaemia in these neonates. 67 preterm and 67 full-term newborns were included in the control group. The incidence of late hypocalcemia was reduced from 16.4% to 0 in full-term babies and from 6% to 2.4% in preterm babies by the 25(OH)D3 but not by Vit D2 administration. The incidence of early hypocalcemia was not modified at all. The Authors suggested 25(OH)D3 administration to prevent the late hypocalcemia and, together with calcium support, to treat the early hypocalcemia in the low birth weight newborn.

In this study 112 full term SGA babies were given 400IU and 27 babies were given 1000IU of Vitamin D supplementation. Our study is comparable to the above study in terms of number of SGA babies included in the study. We have done a randomized study and have included 88 term small for gestation age babies (SGA), In our study both the groups have same number of babies 44 in each group. The dose of daily oral vitamin D supplementation given in their study was 400IU and 1000IU is comparable to our study i.e 400IU and 800IU. As there were no definite guidelines recommended for the vitamin D supplementation in SGA babies we have given 800 IU in the group 2 to avoid risk of toxicity.

Natarajan et al[101] did a randomized double-blind trial, in which they allocated eligible infants to receive either 800 or 400 IU of vitamin D3 per day (n = 48 in both groups). Primary outcome was VDD (serum 25-hydroxyvitamin D levels, 20ng/mL) at 40 weeks' PMA. Secondary outcomes were VDD, bone mineral content, and bone mineral density at 3 months' corrected age (CA). The Prevalence of VDD in the 800-IU group was significantly lower than in the 400-IU group at 40 weeks (38.1% vs 66.7%; relative risk: 0.57; 95% confidence interval: 0.37–0.88) and at 3 months' CA (12.5% vs 35%; relative risk: 0.36; 95% confidence interval: 0.14–0.90). One infant (2.4%) in the 800-IU group had vitamin D excess (100–150 ng/mL). Bone mineral content (mean ± SD: 79.6 ± 16.8 vs 84.7 ± 20.7 g; P = .27) and bone mineral density (0.152 ± 0.019 vs 0.158 ± 0.021 g/cm2; P = .26) were not different between the 2 groups. They concluded that Daily supplementation with 800 IU of vitamin D reduces the prevalence of VDD at 40 weeks' PMA and at 3 months' CA in preterm infants without showing any improvement in bone mineralization. However, there was a possibility that this dose may occasionally result in vitamin D excess.

NEED FOR OUR STUDY

The literature on supplementation of vitamin D in small for gestation age babies is not conclusive enough to reach a consensus on the optimal dose of vitamin D for supplementation in SGA babies. To the best of our knowledge we could not find a single study comparing two different doses of vitamin D supplementation in term SGA babies in the literature. Most of the studies have been conducted on pre term babies or term babies.[22] In these studies also the optimal dose required for the babies could not be concluded. Apprehensions of potential vitamin D toxicity could have prevented researchers from using higher dose of the vitamin.

Our study was a randomized controlled trial that had mainly focused on the changes in levels of vitamin D following supplementation. We followed up these children for a period of 3 months which is in comparison to most previous studies[101], Based on reports from healthy children of this region[50], as well as our own observations at this center we had expected most of our patients to be vitamin D insufficient or deficient, more so because of the low awareness of importance of vitamin D and consequences of its deficiency by the people. Also the pregnant mothers are also risk of low vitamin D levels due to poor nutrition, non supplementation, low exposure to sun light and various other factors.

Therefore we compared RDA of vitamin D in SGA babies with two different doses. This was based on the recommendations by the WHO and IAP that recommend that preterm infants should be given 400-800 IU of vitamin D supplementation. Since there was no standard recommendation for SGA babies we used the same doses of 400IU and 800 IU for our study. We found that among the 44 babies who received the higher doses of vitamin D, none of them had signs of vitamin D toxicity.

VITAMIN D STATUS AT ENROLLMENT

We enrolled all our 88 subjects who are full term SGA babies and we found that vitamin D levels of majority of babies (81; 96.5%) were in either deficient or insufficient. i.e. levels <30ng/mL. All the patients in Group 1 had vitamin D levels below normal at enrollment. Three babies (3.5% of 88 babies) in group 2 had sufficient vitamin D levels at enrollment. This may be attributed to either supplementation vitamin D by pregnant mothers without their knowledge in some form of fortified food. As on re enquiring these mothers again denied taking any vitamin di supplementation during pregnancy. So this may be mere co incidence and since we had only 3 patients and <5% of subject population it is not statistically significant. The vitamin D status of our study population was comparable to that of prevalence of vitamin D in Indian population in various age groups including new born babies.[22]The mean vitamin D levels of 88 babies at the time of enrollment were 17.7±6.9ng/mL. Our values are comparable to that of the serum vitamin D levels recorded in the various studies as per the Ritu G et al study. Our values were also comparable to that of healthy newborn population in our area though we did not set them as controls in our study.

VITAMIN D STATUS AT STUDY COMPLETION

All the 88 patients enrolled for the study with 44 babies in Group 1 (Received 400IU of Vitamin D) and 44 patients in Group 2 (Received 800 IU) were analyzed on completion of study. We found a statistically significant improvement in the levels of vitamin D in both the groups following supplementation ($p<0.001$ in both groups). The vitamin D levels of both the study groups changed from that of deficiency to that of insufficiency or sufficiency. The mean Vitamin D levels at birth were 17.81 ±6.51 and 17.54 ±7.46 ng/ml in Group 1 and Group 2 respectively. After the supplementation of Vitamin D for 3 months, the mean Vitamin D levels increased to 31.43 ± 6.60 in Group 1(400IU) and 34.06 ± 8.21 in Group 2(800IU).

On completing the study, 28 (63.7%) children attained sufficiency, 14 (31.8%) had insufficiency in group 1 and 27 (61.4%) babies had attained sufficient vitamin D levels (>30ng/mL), while 15 (34.1%) babies had insufficiency in group 2. More than 60% of patients in both the groups had sufficient vitamin D levels (> 30ng/mL) at the end of the study. This is comparable to other studies on Indian population as per Ritu et al. Two patients in both the groups who had defiency at birth still remained deficient after 3 months of vitamin D supplementation.

COMPARISON BETWEEN THE TWO DOSES OF VITAMIN D

We have noted a statistically significant rise in the levels of vitamin D in both the interventional groups from baseline levels ($p<0.001$). However the increase was more in group 2 i.e., 800IU (by 2.63±1.61ng/mL) compared to group 1 (400IU) which was statistically not significant ($p>0.001$). The clinical significance of such a marginal difference however may be questionable. The percentage change in vitamin D levels on supplementation between both the groups, was not found to be statistically significant ($P>0.001$).

In spite of supplementing a higher dose of vitamin D for 3 months duration, we could not attain levels of sufficiency in most patients, although >60% of patients did attain sufficiency (28 babies in Group 1 and 27 babies in group 2. None of the children in our study showed any clinical features of vitamin d deficiency at the end of study. We expected the serum levels of vitamin D to rise higher and reach levels of sufficiency on

supplementing 800 IU of vitamin D for long duration. Intake of vitamin may not have a major role to play in determining vitamin levels beyond the RDA doses. It could be interesting to test what doses given regularly, after achieving vitamin D sufficiency at the beginning of therapy, is able to maintain such levels. Since all our babies were exclusively breast fed for study period and we have not taken mothers vitamin D status into consideration it may have enlighten more information about the true status of Vitamin D in these SGA babies.

LIMITATIONS OF STUDY

Like most other clinical trials, this trial too was not free of limitations.

1. The trial was not blinded, raising the possibility of outcome bias. However, this possibility was unlikely since the primary objective of this study was a laboratory measurement and not a subjective clinical criteria or score. Also the person assessing vitamin D levels was blind to the treatment allocation group.
2. We did not measure the vitamin D levels of the mothers due to financial constraints. Although this was not the main intention of our study. This could have revealed the actual difference in effect between the two supplementation regimens of vitamin D. As the vitamin D levels of the new born babies depend on the vitamin D levels in their mothers.
3. We did not consider the possibility of vitamin D supplementation by the mother during the study period. Since all the babies were exclusively breast fed for 3 months, the vitamin D levels could have been affected by mother's vitamin D supplementation. However, we believe subjects in both groups belonged to similar socio-economic background with similar dietary intakes. Moreover, randomization could have reduced the effects of possible differential intake.
4. Since vitamin D is also produced by exposure to sunlight, we have not taken this factor in our study. However as our population is newborn babies' majority of the people in this part of country will not consider for exposing the babies to sun due to lack of knowledge and social factors
5. Our study follow up was of short duration 3 months only. Since it was a time bound study we had to keep short duration only. Also most of other studies have given supplementation for 3 months only.

Despite these limitations, the strength of our study lies in the fact that it was a randomized controlled trial of a good number sample size and was adequately powered to detect a clinically meaningful difference in vitamin D levels. Patients enrolled in the study had been registered at our Neonatology clinic for routine Immunization in OPD, this ensured regularity of follow-up. Additionally, drug compliance was ensured both telephonically and by keeping a count of empty wrappers at each visit. Moreover vitamin D was measured using chemiluminescence, which is more sensitive and reliable rather than ELISA. Thus, it is reasonable to conclude from this study that both the doses of vitamin D daily supplements of 400 IU and 800IU for seems not sufficient to attain normal levels given for 3 months duration in this region. Although there is significantly improvement in their serum vitamin D status after regular supplementation for 3 months from below normal level at birth. The improvement seems more in the 800 IU compared to 400 IU but it is not statistically significant. However, the clinical significance of this rise is difficult to comment.. It can be confirmed, however, that such higher daily doses of vitamin D could be given to term small for gestational age (SGA) babies over long periods of time without significant adverse effects.

SUMMARY

This study titled "Vitamin D levels of the term Small for Date newborns at birth and at 3 month of age after vitamin D supplementation with 2 different doses 400IU v/s 800IU- A Randomized controlled trial" was conducted in Neonatology unit, J.N.Medical College & Rajiv Gandhi Center for endocrinology, AMU, Aligarh, Uttar Pradesh, India during the time period from February 2016 to October 2017. Eighty eight term small for gestational age (SGA) newborn babies were enrolled in the trial; 44 babies were allocated to intervention group 1 (400IU of vitamin D) and another 44 babies to intervention group 2 (800IU of vitamin D). The vitamin D supplementation was given daily upto 3 months for all babies in both groups. Baseline anthropometric measurements, clinical parameters and serum Vitamin D levels were recorded at enrollment. The enrolled children were followed up for a mean duration of about 94 days. Investigations were repeated at study completion. A summary of our observations and results are given below.

- On analyzing the data of 88 subjects we found that, 53 (60.2%) were females and 35 (39.8%) were male babies. The male is to female ratio was 26:18 in group 1 and 27:17 in group 2.
- The religion of mothers was Muslim in 60 (68.1%) and Hindu in 38 (31.9%). The number of Muslims and Hindus were 31:13 in group 1 when compared to 29:15 in group 2.
- The mean age of mothers was 24.9±2.9 years in group 1 and 25.7±3.9 years in Group 2.
- In the type of delivery about 61.4% (n=54) had normal delivery and 38.6% (n=34) had caeseration section (LSCS). In group 1 we had 23 babies (52.3%) born by LSCS and 21(47.7%) by normal delivery. Where as in group 2 we had more babies born of normal delivery 33(75%) while were LSCS was recorded in 11(25%) of mothers. The values are significant ($p<0.001$).
- The average follow up period was 94.9±3.6 (89 to 102) days in group 1 and 94.2±3.6 (88 to 104) days in group 2.
- The average birth weight in group 1 was 2.14 ± 0.28 kgs which is comparable to group 2 2.12 ± 0.29 kgs. After the Vitamin D supplementation the average weight of babies in group 1 was 3.24 ± 0.31kgs and 3.44 ± 0.39 kgs. In group 2 there was definite significant gain in the weight of babies at 3 months ($p<0.001$).
- The average length of the babies measured at birth was 46.2 ± 2.9 cms in group 1 and 45.6 ± 3.2 cms in group 2. At 3 months it was 50.3 ± 3.3cms in group 1 and 48.6 ± 3.5cms in group 2. The difference is statistically significant
- The Head circumference and chest circumferences measured at birth were 31.4 ± 2.0cms & 29.6 ± 2.2cms in group 1 and 31.4 ± 2.4cms & 29.7 ± 2.3cms in group 2 respectively. At 3 months it has increased to 35.2 ± 2.4 cms & 31.7 ± 2.3cms in group 1 and 34.3 ± 2.5cms & 32.1 ± 2.4 cms in group 2. These values also are not significant and comparable in both the groups
- The Vitamin D levels of 85 babies (96.6%) analyzed in the study were <30ng/ml, three babies in group 2 had normal Vitamin D levels at the time of enrollment.
- 56 (63.7%) babies had vitamin D levels below 20ng/mL (deficiency) and 29 (32.9%) babies had values between 21-29ng/mL (insufficiency) and 3 (3.4%) babies had >30ng/mL (normal). The mean vitamin D levels of 88 babies at the time of enrollment were 17.7±6.9ng/mL.
- The mean Vitamin D levels at birth were 17.81 ±6.51 and 17.54 ±7.46 ng/ml in Group 1 and Group 2 respectively. After the supplementation of Vitamin D for 3 months, the mean Vitamin D levels were 31.43 ± 6.60 in Group 1(400IU) and 34.06 ± 8.21 in Group 2(800IU). On comparing between groups there was some increase in value of Vitamin D but it was not statistically significant ($p>0.001$)
- No patients had increased vitamin D levels leading to toxicity (>150ng/mL).
- In group 1 at birth there were 27 (61.4%) babies who had vitamin D deficiency (levels <20ng/ml) and the rest 17 (38.6%) had vitamin D insufficiency (21-29ng/ml) at birth. On completing the study, 28 (63.7%) children attained sufficiency, 14 (31.8%) had insufficiency and 2 (4.5%) babies remained deficient.
- In group 2 at enrollment 29 (65.9%) babies had vitamin D deficiency, 12 (27.3%) had insufficiency and 3 (6.8%) babies had normal values. At 3 months 27 (61.4%) babies had attained sufficient vitamin D levels (>30ng/mL), while 15 (34.1%) babies had insufficiency and 2 (4.5%) babies continue to remain deficient.
- The change in vitamin D status post supplementation was statistically significant ($p<0.001$) in both the groups
- The effect of supplementation on the vitamin D status was compared between the two allocation groups. It was not found to be statistically significant ($p>0.001$). Supplementation of both the doses of vitamin D improved the mean status of study subject from deficiency to that of insufficiency in more than 90% patients at 3 months.
- There was definite increase in Vitamin D levels in group 2 (800IU) when compared to group 1(400IU) at 3 months by 2.63±1.61ng/mL in term SGA babies, but this rise was not significant.

CONCLUSIONS

Our study comparing the efficacy of two different daily doses of vitamin D (400 IU *vs.* 800IU) in term small for gestational age (SGA) babies who are exclusively breast fed for a period of 3 months could not establish superiority of higher doses of vitamin D. Vitamin D deficiency is prevalent in our study population. Both the doses caused a significant rise in the vitamin D levels in SGA babies. There was significant increase in weight and length of the babies who received 800 IU of vitamin D which needs further evaluation for correlation. Higher doses of vitamin D did not cause any toxicity in these babies after supplementation of vitamin d for 3 months. To the best of our knowledge this study was first of its kind on vitamin d supplementation in term SGA babies. However further study taking larger population, long duration and higher doses of vitamin d may bring more light on the optimal dose of vitamin d requirement in these patients.

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About the Book

Vitamin D deficiency and its supplementation have been increasingly catching the researchers in past few years. There are no definitive guidelines for the effective dose of vitamin D supplementation in newborns, especially in Indian population. The study published in this book will enlighten the comparison of two doses (400IU vs 800 IU) of vitamin D supplementation in term small for gestation age babies.

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