"PREVALENCE OF VITAMIN D DEFICIENCY AND ITS ASSOCIATION AMONG PRETERM NEONATES WITH RESPIRATORY DISTRESS SYNDROME"

BY

REGISTER NUMBER: BM0121020

Dissertation

Submitted to the KLE Academy of Higher Education and Research, Belagavi, Karnataka

In Partial Fulfilment

of the Requirements for the Degree of

M.D. (Doctor of Medicine)

in

PEDIATRICS

DEPARTMENT OF PAEDIATRICS
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELAGAVI, KARNATAKA

DECEMBER 2024/JANUARY 2025

KLE Academy of Higher Education and Research
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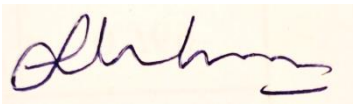

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"PREVALENCE OF VITAMIN -D DEFICIENCY AND IT'S ASSOCIATION AMONG PRETERM NEONATES WITH RESPIRATORY DISTRESS SYNDROME"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee.

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LIST OF ABBREVIATIONS

VitD	–	Vitamin D
VitDD	–	Vitamin D deficiency
VitDBP	–	Vitamin D Binding protein
VDRE	–	Vitamin D Responsive element
VDR	–	Vitamin D Receptor
RXR	–	Retinoid X Receptor
DHCR	–	Dihydrocholestrol Reductase
PTH	–	Parathyroid hormone
GH	–	Growth hormone
IGF	–	Insulin like Growth Factor
ALP	–	Alkaline phosphatase
DBD	–	DNA Binding domain
LBD	–	Ligand Binding Domain
RANKL	–	Receptor Activator of nuclear factor kappa – B Ligand
CAMP	–	Cathelicidin antimicrobial peptide
APC	–	Antigen presenting cells
TNF	–	Tumor necrosis factor
GM-CSF	–	Granulocyte Macrophage Colony stimulating factor
RDS	–	Respiratory distress syndrome
BPD	–	Bronchopulmonary Dysplasia
VLBW	–	Very low birth weight
MHC	–	Major histocompatibility complex
NCoR	–	Nuclear receptor co-repressor
SRC	–	Steroid receptor co-activators

TIF	–	Transcriptional intermediary factor
RAC	–	Receptor-activated co-activators
RNA	–	Ribonucleic acid
LPS	–	Lipo polysaccharide
GR	–	Glutathione Reductase
COX	–	Cyclo-oxygenace
MAPK	–	Mitogen-activated protein kinase
MKP	–	Mitogen-activated protein kinase phosphatase

ABSTRACT

BACKGROUND AND AIMS:

Vitamin D is an important steroidal hormone with a well-known effect on bone mineralisation. Apart from the effects of Vitamin D on bone and mineral metabolism, Vitamin D has other extraskeletal effects through its action on Vitamin D receptors (VDR) which are present in many organs in the body. One such action is the effect of Vitamin D on the preterm Respiratory distress syndrome (RDS) where we had the primary objective to assess if Hypovitaminosis D could be a risk factor for the development of RDS. Also, we intended to find the prevalence of Vitamin D deficiency among the preterm neonates and to find the association between Vitamin D deficiency and duration of hospital stay, Neonatal sepsis and HNNE (Hammersmith Neonatal Neurological Examination) scores at 40 weeks PMA.

MATERIALS AND METHODS:

A prospective observational study was done in the NICU of KLES Dr.Prabhakar Kore Hospital and MRC from April 2023 to February 2024 among preterm neonates born >28 and ≤ 34 weeks of gestation. 70 neonates were enrolled in the study. Vitamin D levels were measured within 1 hour of life by Electro Chemiluminescence Immuno Assay along with blood culture which was sent before the initiation of antibiotic therapy and when clinical suspicion of sepsis set in during the hospital stay. The neonates were considered to have Vitamin D deficiency and insufficiency if 25-hydroxy Vitamin D levels were <12ng/ml and 12-20ng/ml respectively. Neonates were followed for the development of RDS. Diagnosis of RDS was based on Chest X-ray findings and clinical grading of respiratory distress was

done by Silverman Andersen scoring. The Neonates were followed up for the requirement and the duration of the respiratory support received respectively, duration of hospital stay, and presence of neonatal sepsis, and were compared across Vitamin D levels. HNNE score was documented at 40 weeks postmenstrual age during the baby's follow-up at a High-risk baby clinic and was in turn compared with Vitamin D levels.

RESULTS:

A statistically significant association was found between inadequate Vitamin D levels (Vitamin D deficiency + insufficiency) and RDS ($p = 0.046$). Prevalence of Vitamin D deficiency was 37.14%. 71.43% of the study group had inadequate Vitamin D levels. Preterm neonates with inadequate vitamin D levels required at least one form of respiratory support during the hospital stay with a significant p-value of 0.033. No association was found between inadequate vitamin D levels and Neonatal sepsis (p value:0.680). No significant association was found between Vitamin D deficiency and duration of hospital stay, HNNE at 40 weeks PMA.

CONCLUSION:

The development of RDS was high in babies with Inadequate Vitamin D levels suggesting that Vitamin D deficiency could be a risk factor for the development of RDS warranting additional investigations.

KEYWORDS: Vitamin D deficiency, Respiratory distress syndrome, neonatal sepsis, HNNE.

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INTRODUCTION

The exciting story of VitD's discovery, which took centuries and involved the work of many scientists, is one of scientific curiosity and perseverance. Physicians looked into the possible therapeutic effects of cod-liver oil in the late 17th and early 18th centuries after learning about the early cases of rickets, a bone disorder brought on by paucity of VitD. But it wasn't until the early 20th century that researchers like Elmer McCollum and Edward Mellanby discovered that VitD was the active component in cod-liver oil and was essential for preventing rickets [1].

Additional research revealed the identification of different forms of VitD, such as VitD2 (ergocalciferol), which comes from plants, and VitD3 (cholecalciferol), which comes from animals or is synthesized by the body in reaction to sunlight exposure. Dietary recommendations and the development of VitD supplements helped make sure that individuals had sufficient amounts of this crucial nutrient as our understanding of VitD expanded.

VitD is a fat-soluble vitamin that has a pivotal role in Bone metabolism. Besides its renowned effect on bone health, the extraskeletal importance of VitD especially its crucial effect on immunological functions, and its effect as a nutrient and prohormone is being increasingly recognized through its effect on VitD receptor [2] which is available close to all organ systems in the body.

VitDD principally causes Rickets which was unbridled in the United States and Northern Europe during the early 20th century.

In affluent nations, rickets is still an ongoing issue, despite being mostly resolved by public health initiatives that give access to sufficient VitD. Many cases are still related to avoidable nutritional deficiencies in VitD.

The skin converts 7 dehydrocholesterol to VitD3 (cholecalciferol), the body's natural form of VitD, during exposure to radiation. When 7-dehydrocholesterol is exposed to sunshine, it forms pre-VitD3, which then changes three double bonds in a temperature-sensitive manner to become VitD3. The primary source of VitD is the skin's synthesis, which is reliant on UV radiation intensity, which varies with latitude and season.

VitDD (VitD deficiency) is emerging as a regular problem worldwide, specially in exclusively breastfed newborns [3,4,5]. VitDD is regarded as a global public health problem with studies carried out in different continents revealing inadequate VitD status among the respective inhabitants. VitDD prevalence rates, which are defined as 25 hydroxy-D <30 nanomol/L (or 12 ng/ml), are 5.9 percentage in the United States [6], 7.4% in Canada [7], and 13% in European inhabitants [8], according to reports. 25hydroxy-D levels <50 nanomol/L, or 20 nanogram/ml, are estimated to be prevalent in 24% of US & with 37% of Canada, and 40% of European inhabitants.

In 2016–18, the “Ministry of Health and Family Welfare, Government of India”, performed the Comprehensive National Nutrition Survey (CNNS), which included around 35 thousand children from all over the Indian subcontinent, ages one to nineteen. According to the definition of VitDD, which is defined as serum 25hydroxy-D < 12 ng/mL, the prevalence was reported to be “14% in children 1-4

years old, 18% in school-aged children (5-9 years old), and 24% in teenagers (10-19 years old) [9]".

Enough vitamin D is needed for the fetus's skeleton to develop normally in pregnancy. Deficient Maternal VitD levels during pregnancy correlate with a reduction in bone mineral acquisition in infants [10].

A study done to estimate the prevailing VitDD among infants, children, and adolescents found the highest prevalence of VitD in neonates from the Middle East. Data from individual studies estimated VitDD <12ng/ml and <20ng/ml to be 61% and 99% respectively among Indian infants [11].

Maternal VitDD is an essential hazard for VitDD at birth where maternal VitD levels positively correlated with cord blood VitD levels. Neonatal levels of VitD at birth are largely linked to maternal VitD levels [12]. There have been reports from India of a substantial amount of physiological relevant hypovitaminosis D in pregnancy and their term babies [13]. This deficiency may occur whenever the mother is deficient in VitD due to inadequate production or intake.

In tropical nations such as India, VitDD is unanticipated because of year-round access to enough sunlight, Naik KD, et al [14] found the average cord blood VitD level of term healthy babies to be 11.36 ±4.75 ng/mL in Kerala, India which showed that neonatal VitD was also found in tropical climates which in turn is influenced by various factors.

There is a dearth of information on the VitD status of premature Indian newborns which this study will focus more on. The prevalence of VitDD in VLBW babies at birth varied in different studies. According to a study conducted in a higher-

education hospital in Odisha, India, 67% of VLBW newborns were predicted to have VitDD, and 45% of infants had severe VitDD [15].

VitDD influences the development of the foetus, influences the metabolism of bones, is involved in cell and embryogenesis, and aids in the maturation of the fetus's lungs [16,17].

VitD is involved in the development of immune systems that are innate as well as adaptive. VitD has been studied in various areas due to its extraskeletal effects, which include immunomodulator, prodifferentiative, proapoptotic, and antiproliferative effects [18,19,20,21,22,23]. Due to its capacity to bind to the VitD receptor and to its ability function as a transcriptional factor-TF, it also controls gene expression and has extensive immunomodulatory effects.

The development and prognosis of various diseases have been linked to variations in the VitD and VitD receptor polymorphisms such as Alzheimer's disease [24], cardiovascular disease [25], multiple sclerosis [26], carcinoma [27], lower respiratory tract infection [28], type 1 diabetes [29], autoimmune diseases via cell proliferation and immune function [30,31,32].

Over the last few decades, a growing recognition of the significance of VitD for foetal and childhood health has been recognised. Research on the effects of VitD on early pulmonary development and maturation as well as early-life pulmonary disorders has only recently started. Reduced placental development is linked to hypovitaminosis D during pregnancy, which raises the possibility of preterm birth, which in turn raises the risk of respiratory distress syndrome and bronchopulmonary dysplasia. Furthermore, it has been proposed that VitD regulates lung maturation in

the fetus and has a function in cellular proliferation and differentiation, including embryogenesis. Hypovitaminosis D may exacerbate early neonatal lung disorders [33].

Many animal & laboratory studies have noted the +ve effect of VitD on Alveolar type II pneumocytes, fibroblasts proliferation, surfactant production, and alveolarization [34,35,36,37] hence its effect on the neonatal RDS.

"The concept that hypovitaminosis D is an avoidable risk factor for RDS arose from our understanding of the beneficial effect of VitD on foetal lung maturation and function".

RDS is a leading cause of death among premature babies. Because of inadequate surfactant production, the syndrome is characterised by the presence of signs and symptoms of respiratory distress and an increase in oxygen demand soon after birth. The etiology of RDS is believed to be caused by lung immaturity and surfactant deficit [38]. VitD regulates the formation of surfactant, which contributes to RDS, by upregulating the alveolar Type II pneumocyte expression.

VitDD during pregnancy can lead to decreased placental development and subsequent risk of preterm birth [39,40] which in turn can lead to RDS in the premature neonate. Understanding the various effects of VitD, and studying its effect on other co-morbidities and clinical outcomes among preterm infants is an emerging field of research with very limited studies available and this study will focus more on the clinical health outcomes of preterm newborns along with the neurological outcome of preterm babies admitted to the NICU.

OBJECTIVES:

PRIMARY OBJECTIVES:

1) To know the association between VitDD and Respiratory distress syndrome in preterm neonates.

SECONDARY OBJECTIVES:

1) To know the prevalence of VitDD in Preterm neonates.
2) To know the association between VitDD and the outcome of preterm neonates admitted to NICU in terms of
    - Duration of Hospital stay
    - Sepsis
    - Neurological outcome at 40 weeks Postmenstrual age.

REVIEW OF LITERATURE

"VitDD is one of the major public health problems and its prevalence is reported worldwide in many studies" [6,7,8,11,41,42,43] however data representing particular age groups are lacking, especially in the young.

PREVALENCE OF VITDD – WORLDWIDE:

The prevalence of VitDD among various age groups like infants, children & adolescents was estimated globally by Palacios C, Gonzalez L [11] with the help of numerous available articles worldwide. The study concluded that VitD was a global public health problem [11] in all age groups.

Oktaria et Al [44] did a systematic review among South East Asian children to describe the VitDD prevalence with the aid of 21 studies from 5 different countries. The VitDD prevalence (<50nmol/L) was estimated to be between 0.9% and 96.4%, with more than 50 % of the newborns having VitDD and severe VitDD ranging from 0 – 55.8%.

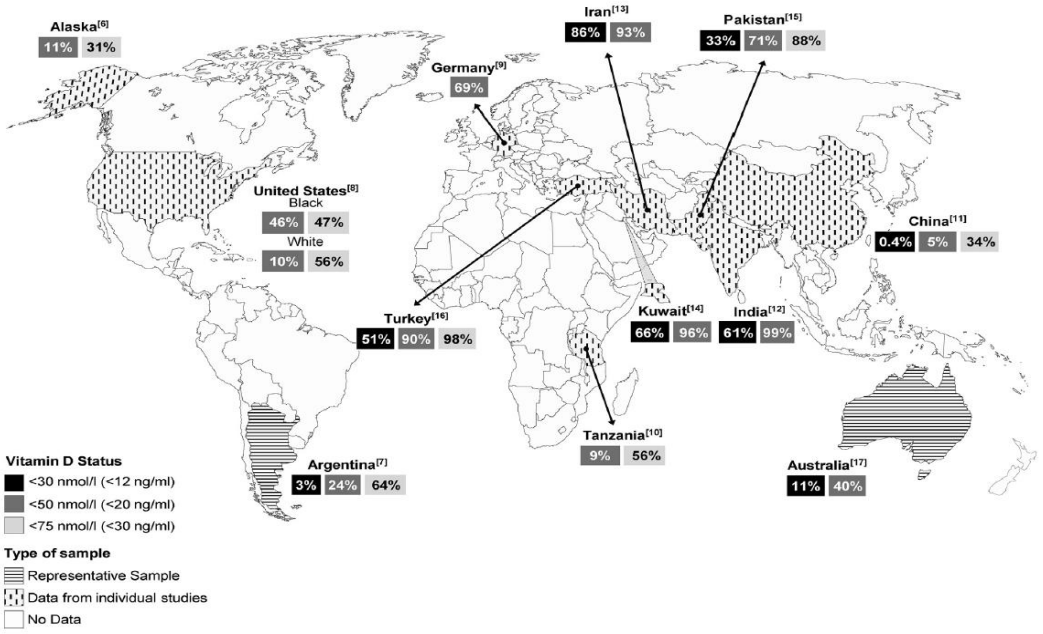


Fig 1: Low VitD prevalence status in infants worldwide. Palacios et Al [11]

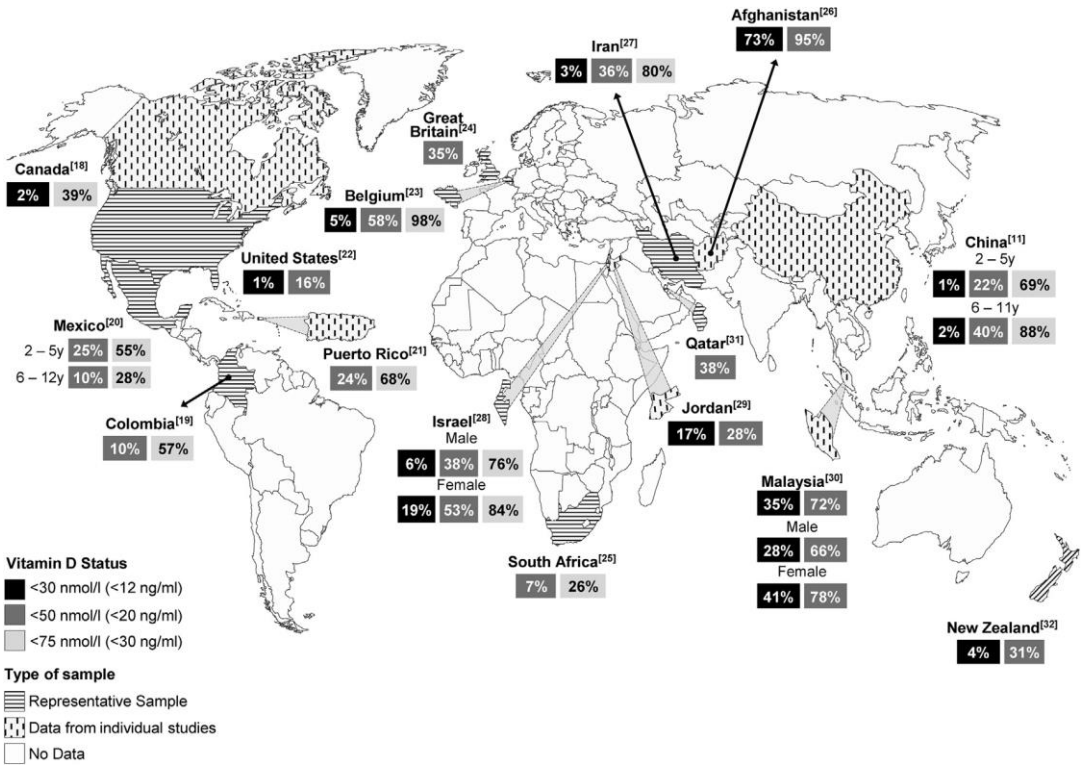


Fig 2: Low VitD prevalence status in children worldwide. Palacios et Al [11]

PREVALENCE OF VitDD IN INDIA:

Multiple studies were done estimating the VitD status over the last decade in several countries including India. Even though India is a tropical country receiving abundant sunlight on most days of the year, both higher & lower social strata, all the age groups, all genders, & individuals in a variety of occupations are affected by VitDD.

In 2011, "Kadam et al. carried out a study in school going children (sample size = 214) in Pune. It revealed a 34.2% prevalence of VitDD[45]. Another investigation was conducted in Himachal Pradesh in the schools of Kangra and Kullu districts by Kapil et al [46]. in 2017 among 1222 children aged 6 to 18. The respective prevalences of VitDD were 81% and 80%" [46]. The prevalence of VitD insufficiency was reported in both investigations using the U.S. Endocrine Society criteria.

Table 1: Prevalence of VitDD in India- Hospital-based studies

Study conducted by and year of study	place	Sample size	Study participants	%of VitDD
Farrant et Al in 2009 [47]	Mysore	559	Pregnant women	66.5
Marwaha et Al in 2011 [48]	Delhi	521 342 342	Pregnant women Lactating mothers EBF infants	96.3 99.7 98.8
Dasgupta et Al in 2012 [49]	Guwahati	50	Pregnant females	42
Angurana et Al in 2014 [50]	Chandigarh	338	3months-12years	40.2
Basu et Al in 2015 [51]	Kolkatta	310	1-16years	52.9
Kumar et Al in 2015 [9]	Bengaluru	106	Mothers in labor Umbilical cord blood of a newborn	70.7 83
Sharma et al in 2016 [52]	New Delhi	418	Pregnant women	93.5

VitDD PREVALENCE IN PREGNANCY AND NEWBORN – WORLDWIDE AND INDIA:

"According to a US NHANES - National Health and Nutrition Examination Survey in 2011–2014 on VitD levels, 5.7% of women and 17.8% of women, respectively, had VitD insufficiency (12–20 ng/mL) and VitDD (<12 ng/ml). Among adults aged 20–39, the rates of deficiency and insufficiency were 7.6% and 23.8%, respectively. VitDD prevalence differed by race and ethnicity: non-Hispanic Blacks made up 17.5% of the population, non-Hispanic Asians made up 7.6%, Hispanics made up 5.9%, and non-Hispanic White people made up 2.1%. The status of VitD in the US stayed constant between 2003 and 2004 and 2011–2014[53]."

Between 2005 and 2007, "cross-sectional study conducted in Boston showed that 35.8% of the maternal population and 58% of the newborns had VitDD (< 20 nanogram/mL), and 23.1% of the mothers and 38.0% of the neonates had severe VitDD (< 15 ng/mL). Maternal VitDD (adjusted odds ratio [Odds ratio]: 5.28 [95% CI: 2.90–9.62]), winter birth (aOdds ratio: 3.86 [95% CI: 1.74–8.55]), African-American race (aOdds ratio: 3.36 [95% CI: 1.37–8.25]), and maternal BMI of 35 (aOdds: 2.78 [95% CI: 1.18–6.55]) were risk factors for neonatal VitDD[54]."

During the winter, a Canadian study found that 25% of women aged 18 to 35 had VitDD, which took serum VitDD as levels < 40 nmol/l [17]. Deficiency of VitD is equally widespread in Europe & the Middle East. Serum VitD < 50 nanomol/L, or 20 nanogram/mL, is the threshold for VitDD, and it affects 6–33% of people in Northern Europe, 30–60% in Western, Southern, & Eastern Europe [55], and 30–90 percentage in the Middle East. In >10% of Europeans, there is a severe deficiency (serum 2hydroxyD < 30 nanomol/L or 12 nanogram/mL) [55].

Hospital-based Studies regarding the prevalence of VitDD were limited in infants and especially in preterm neonates in the Indian country which was one of the main purposes of this study.

Physiologically significant VitDD among pregnant women and their newborns was reported in the following studies in India.

"VitDD was seen in 84 percentage of pregnant women and 96 percentage of infants in the Asian continent as reported in 2009 by the International Osteoporosis Foundation."

In a tertiary care centre in northern India, Chacham et Al. conducted a prospective observational study [56] from January 2017 to December 2018. Mothers and infants under one-year-old were included in the research. Two hundred mothers and their newborns were registered. Of the infants in the study, twenty percent were older than the neonatal stage and eighty percent were neonates. In newborns, the incidence of VitDD was 74%, whereas in mothers, it was 85.5%.

In the study by Kumar et al [57] at a tertiary care hospital at Bengaluru, VitD levels in expectant mothers and their babies were measured. The mean (SD) VitD level in the mother's cord blood was 12.8 (8.5) nanogram/mL, while the mother's mean VitD level was 16.3 (10.3) nanogram/mL. Hypovitaminosis-D was present in 75 mothers (70.7%) and 88 infants (83%). Low VitD levels were found in 70 (93.3%) babies of hypovitaminosis-D mothers. Newborn and mothers VitD levels were highly correlated ($p < 0.001$).

All studies done in the past 2 decades for identifying the prevalence of VitDD emphasize the need for VitD in all age groups not only as an important Vitamin in the

aspect of bone and mineral health but also its effects on other organ systems for which further studies are required. Studies related to preterm newborns and the impact of VitD on various dimensions of newborn clinical outcomes were reviewed in this study.

ETIOLOGY OF VitDD:

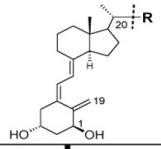
VitDD prevalence is quite prevalent from the above-mentioned studies. Due to the aforementioned causes, there is a combination of insufficient intake and in adequat-e cutaneous synthesis that leads to VitDD. Transplacental transfer of VitD typically provides enough VitD for the first 2 months of life unless the mother has severe VitDD. Breast milk has low VitD levels and the infants are dependent on cutaneous synthesis or vitamin supplements for its requirement. The amount of exposed skin, skin pigmentation, and latitude are some of the factors that influence the synthesis of VitD from sunlight. Many widespread cultural and societal practices in Asian continent and the Middle East that restrict young women's and adolescent girls' exposure to sunlight are the cause of VitDD.

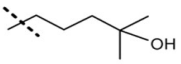
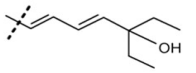
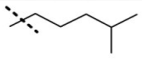
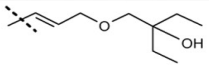
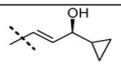
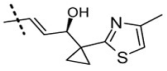
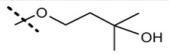
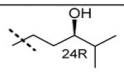
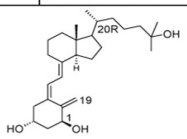
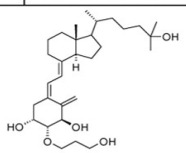
VitD insufficiency is exacerbated by increased skin pigmentation, less time spent outside due to urbanisation, and increased pollution [13]. Pregnant women with low serum VitD levels due to these conditions will have their newborns VitD deficient as VitD levels in the mother correlate with the VitD levels of the newborn. These children are at greater risk of developing rickets.

VITD METABOLITES, ANALOGS:

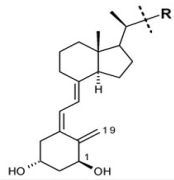
The two primary forms of VitD are VitD2 (or ergocalciferol) which is produced by plants and fungi, and VitD3 also known as Cholecalciferol or calcitriol [58]. VitD3 can be generated internally by skin exposure to sunlight or through diets high in eggs, cod liver oil, and oily seafood. Plants produce VitD2, which is different from VitD3 due to a methyl group in Carbon24 and the presence of a double bond in Carbon22–Carbon23. The VitD receptor mediates the biological effects of calcitriol and its synthetic analogs.

Vitamin D Analogs



Compound	R	Compound	R
1α,25-$(OH)_2D_3$ (Calcitriol)		1α,25-$(OH)_2$-22,24-diene-24a,26a,27a-trihomo-D_3 (EB 1089)	
1α-$(OH)D_3$ (Alfacalcidol)		1α,25-$(OH)_2$-22-ene-25-oxa-D_3 (ZK 156718)	
1α,24-$(OH)_2$-24-cyclopropyl-D_3 (Calcipotriol)		25-(4-methylthiazol-2-yl)-calcipotriol (ZK 191732)	
1α,25-$(OH)_2$-22-oxa-D_3 (Maxacalcitol)		1α,24R-$(OH)_2D_3$ (Tacalcitol)	
 1α,25-$(OH)_2D_3$ (Calcitriol)		 ED-71 [1α,25-$(OH)_2$-2β-(3-hydroxypropyl)D_3]	

"20-Epi Vitamin D Analogs"



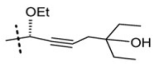
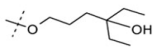
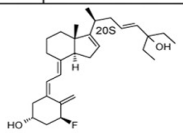
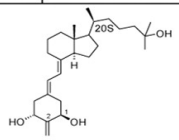
Compound	R	Compound	R
20-epi-22-ethoxy-23-yne-24a,26a,27a-trihomo-1α,25-$(OH)_2D_3$ (CB 1093)		20-epi-1α,25-$(OH)_2D_3$ (KH 1060)	
 1α-fluoro-25-(OH)-16,23E-diene-26,27-bishomo-20epi-cholecalciferol (Ro-26-6228, BXL-628, RS-980400)		 2-methylene-19-nor-(20S)-1α,25-$(OH)_2D_3$ (2MD)	

FIG:3 VitD ANALOGS [60]

VitD METABOLISM:

7-dehydrocholesterol, an intermediary product in cholesterol synthesis, is converted by the skin to VitD3. Ultraviolet radiation is required to penetrate the top layers of the skin. The most significant source of VitD is its production in the skin,

which in-turn depends on the intensity of the ultraviolet irradiation. When the skin is exposed to ultraviolet B (UVB) light, which has a wavelength ranging from 290 to 315 nm, electrocyclic rearrangement of the ring occurs at the Carbon9–Carbon10 position, producing pre-VitD (Pre.D3). After Pre.D3 has been produced, a hydrogen atom is shifted from C19 to C9 during thermal isomerization to produce VitD3. These are temperature sensitive rearrangement of bonds which finally aids in the formation of VitD3 [61].

The activity of 7-Dehydrocholesterol Reductase (DHCR7) determines the concentration of 7-DHC, which determines the production of Vitamin D3. The enzyme catalyses the reversible conversion of 7-DHC to cholesterol.

For the biologically active vitamin to be produced, VitD3 has to be hydroxylated twice. First at the carbon 25-position in the liver by 25 hydroxylase into 25-hydroxyVitD3 [25(OH)D3] – calcidiol [62]. Measurement of 25(OH)3 levels is a reliable marker of VitD status. Several cytochrome P450 (CYP) isoforms (including the mitochondrial CYP27A1 and the microsomal CYP2R1, CYP3A4, and CYP2J3) [63] accomplish this hydroxylation step, but CYP2R1 is thought to be having high-affinity for 25-hydroxylase [63]. Once Calcidiol is transferred to the mitochondria of the proximal tubule (PT) of the kidney through the bloodstream, it is hydroxylated at the 1α-position to form calcitriol (1α,25-dihydroxycholecalciferol), by the enzyme 25-hydroxyVitD-1α hydroxylase (CYP27B1), the levels of which are elevated by parathyroid hormone secreted by the parathyroid gland.

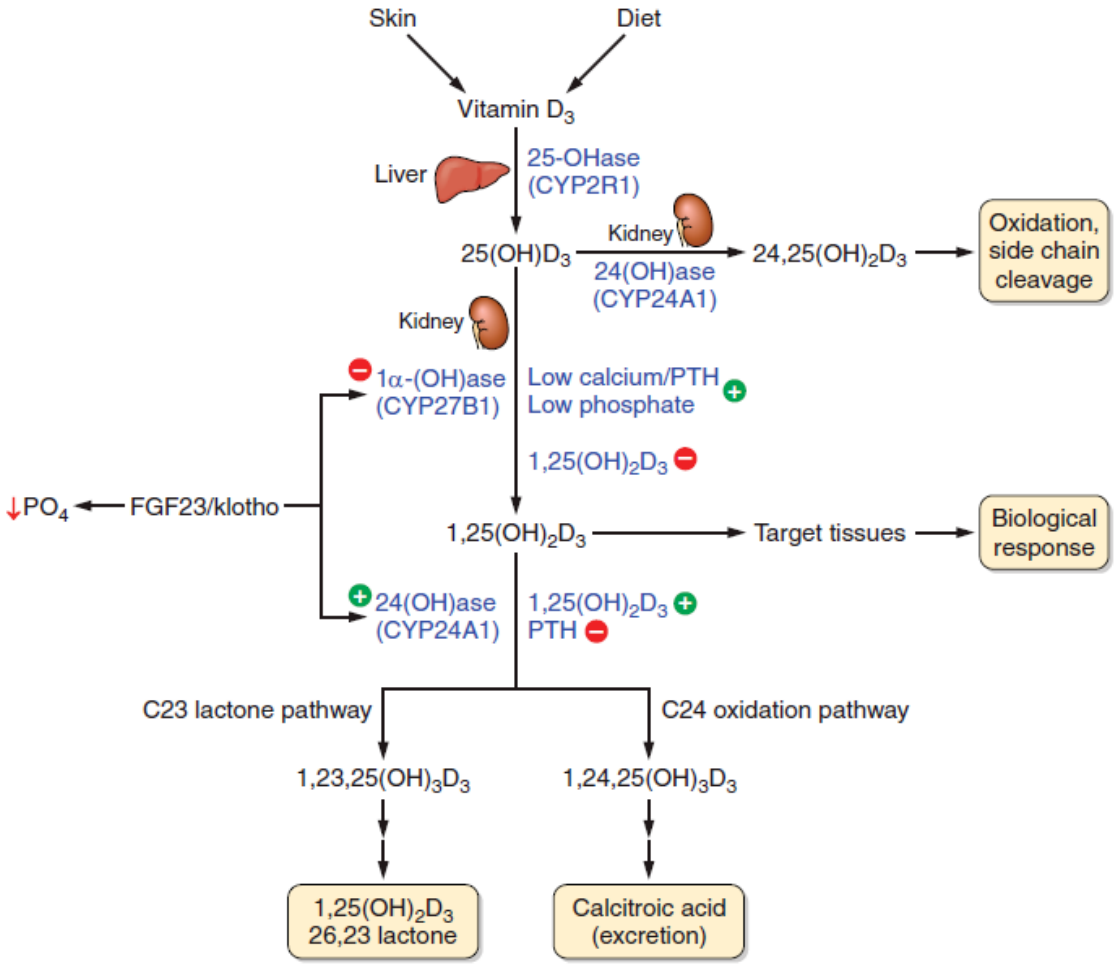


Fig :3 VitD metabolism [62]

The human CYP2R1 gene, which codes for a 501-amino acid protein, is found on chr.11p15.2(15.5 kb) and has five highly conserved exons. 25(OH)D levels in CYP2R1 knockout mice are decreased but not abolished, indicating the presence of other 25-hydroxylase enzymes.

Variants in genes involved in the generation of cholesterol ("the 7 dehydrocholesterol reductase gene DHCR7/NADSYN1"), 25-hydroxylation ("CYP2R1"), VitD transport ("the VitD Binding Protein gene GC"), and degradation

("the 24 hydroxylase gene CYP24A1") have been detected to affect the serum VitD concentration in white Europeans, according to genome-wide association research [64].

Adequate 25 hydroxy VitD levels in the circulation is needed for 1,25(Dihydroxy)D3 regulation of many physiological processes apart from its usual play in bone mineral metabolism.

The expression of CYP27B1 in renal tubules is induced by hypophosphatemia, hypocalcemia, prolactin, GH, IGF-I, PTH, and calcitriol, and inhibited by FGF23 and calcitriol [110]. The expression of CYP27B1 in monocytes and macrophages is not controlled by Ca2+, phosphate, PTH, or calcitriol concentrations, but can be stimulated by inflammatory cytokines such as interferon-γ [110]. Hyperphosphatemia & Hypercalcemia reciprocally inhibit CYP27B1 expression directly [65].

Calcitriol through its action on VitD receptor has an inhibitory effect on CYP27B1 expression. It also prevents the parathyroid gland from producing PTH, which indirectly decreases the production of CYP27B1[110]. A reciprocal regulatory system is established for these chemicals whereby calcitriol enhances FGF23 expression and FGF23 decreases 25 hydroxy VitD-1α hydroxylase activity [66]

Both 25OHD & $1,25(OH)_2D$ are hydroxylated by CYP24A1[111] resulting in the production of $24R,25(OH)_2D$ and $1,24,25(OH)_3D$, respectively [67]. The compounds are further hydroxylated by the same enzyme in several steps, producing a series of 24-& 23-hydroxylated derivatives. The end derivatives are 26–23 lactone and inactive calcitroic acid, which are expelled with bile or urine. FGF23 and calcitriol upregulate CYP24A1, but it is inhibited by hypocalcemia and PTH.

PHYSIOLOGICAL ACTIONS OF VITD:

By controlling the gene (expression) which codes for the "calcium transporters -calbindin; calcium channels -TRPV5 and TRPV6 which are predominantly expressed in the kidneys and intestinal tract respectively; bone matrix proteins -osteocalcin, osteopontin, type I collagen, alkaline phosphatase; and osteoclast activators - RANKL", Calcitriol primarily regulates intestinal, skeletal, and renal function [110]

Calcitriol increases the uptake of Ca^{2+} in the intestines, bones, and kidneys through the nuclear VitD Receptor. With the help of calbindin 9k, alkaline phosphatase-ALP, Ca^{2+} ATPase, calmodulin & other proteins, calcitriol enhances the calcium absorption from the intestinal lumen in duodenum and proximal small intestine. This is achieved via raising the amount of TRPV6 in enterocytes, facilitating its passage across the cytoplasm, and its passage into the basal lateral membrane and into the bloodstream. Employing a transcellular mechanism that makes use of the type 2 Na^{+}-HPO_4^{2}-cotransporter NPT2a present on the luminal enterocyte surface [110], calcitriol also stimulates the absorption of phosphate in the jejunum and ileum. Forty percent of dietary Ca^{2+} and eighty percent of dietary $PO4^{+}$ could be absorbed when VitD reserves are sufficient. Rapid growth, pregnancy, and lactation all result in even better mineral absorption efficiency. One of calcitriol's key functions is to keep blood calcium and phosphate concentrations high enough to support osteoid mineralisation [110].

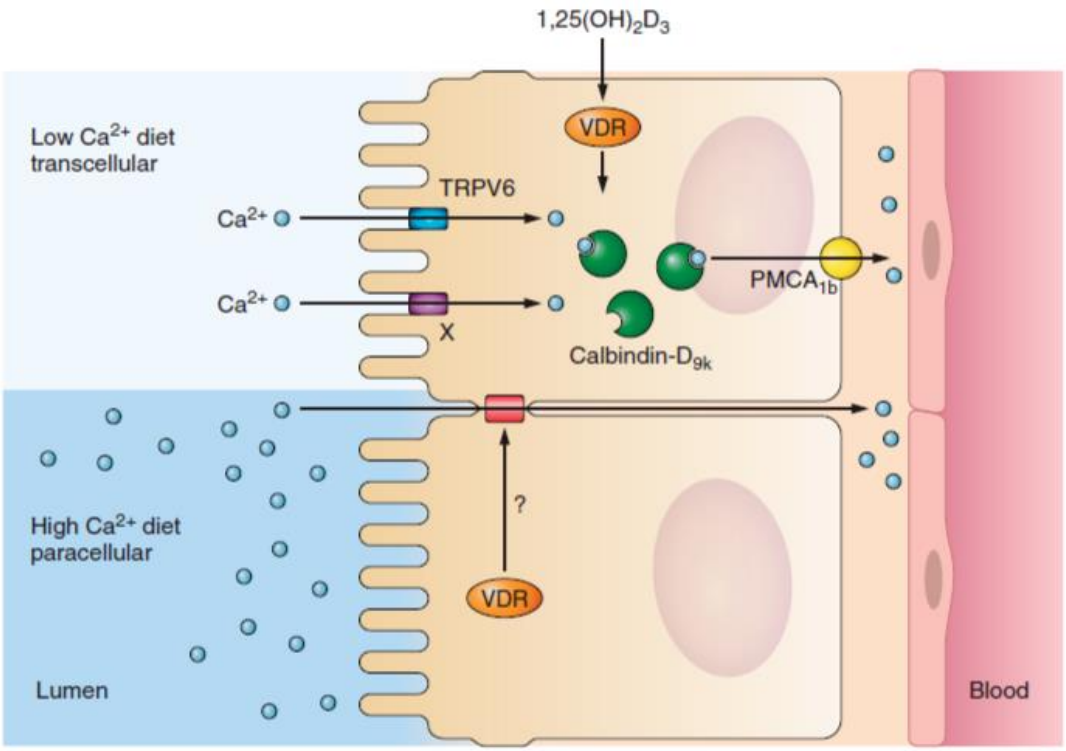


Fig:4 : Intestinal actions of Calcitriol [62]

The upregulation of calbindin-D9k and TRPV6 is a mechanism by which calcitriol promotes transcellular calcium transport in the intestine. The calcium extrusion through the basolateral membrane is caused by PMCA1b. This mechanism is increased in cases where there is insufficient dietary calcium intake. Nevertheless, studies on mouse genetics show that other calcium transporters are also present. When calcium intake is high, the paracellular calcium transport mechanism takes over; however, studies suggest that calcitriol may also play a role in the control of this system.

At the proximal tubules, sixty-five % of the calcium filtered is reabsorbed passively in a Calcitriol-independent manner. Since most of the calcium is absorbed again in the PT-proximal tubule and distal tubule, just one to two percent of the

calcium that has passed through the glomerulus will eventually be passed in the urine. However, PTH and Calcitriol are required for the absorption of calcium in the distal tubular system. In contrast to the mechanism of calcium uptake in the intestine which occurs through a dynamic transcellular process like in the distal tubule, calcium reuptake in the proximal tubule occurs passively and is dependent on a sodium-Na+ gradient. Calcium entry via TRPV5, calcium ejection via plasma membrane Ca2+ pump 1b and sodium/calcium exchanger, and calcium transfer in the cytoplasm by binding to calbindin-D28k and calbindin-D9k are all included in the model. One step that limits the rate of renal calcium reabsorption is the uptake of calcium by TRPV5.

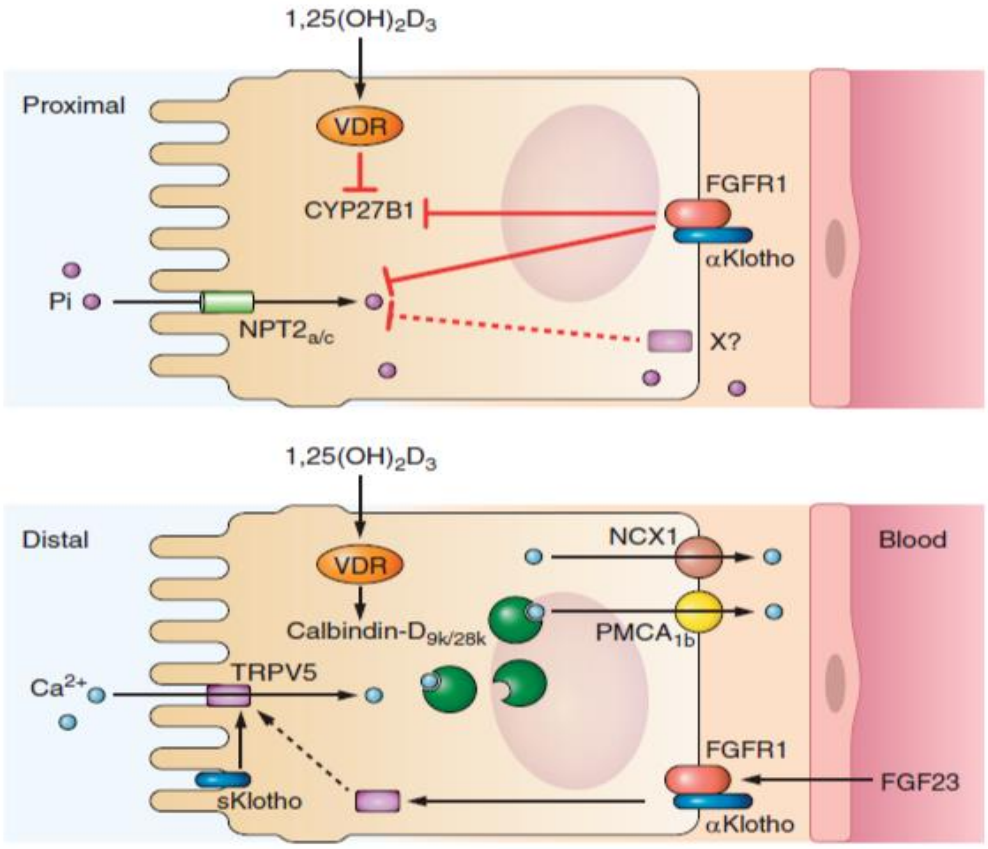


Fig:5 Renal VDR actions.[62]

1,25(OH)2D3 (Calcitriol) and FGF23 inhibit CYP27B1 expression in the proximal tubule cells. By reducing apical membrane phosphate transporters expression, FGF23 also promotes phosphate excretion. The expression of calbindin-D28k, calbindin-D9k, &TRPV5 is increased by Calcitriol.PMCA1b and NCX1 mediate the basolateral side calcium expulsion.

Two hypothesised scenarios explain how FGF23 & Klotho control the expression of TRPV5: "1) secreted Klotho hydrolyses sugar residues from TRPV5's glycan chains, improving TRPV5 entrapment in the apical membrane; and 2) FGFR1 – Klotho basolateral complex bound FGF23 promotes TRPV5's intracellular trafficking to the plasma membrane."

VITD ON BONE HEALTH:

One of calcitriol's key functions is to keep blood calcium and phosphate concentrations high enough to support the mineralization of osteoid. Ironically, when there is insufficient calcium in the body, calcitriol stimulates the production of osteoblast/stromal cells that produce osteoclast-activating factors like RANKL, which in turn causes monocytic stem cells to differentiate into osteoclasts. In times of low calcium, calcitriol can accelerate bone resorption by increasing osteopontin production by osteoblasts, which is a noncollagenous protein found in the bone matrix that binds to integrin receptors on the surface of osteoclast cells to facilitate bone resorption. In addition, osteoblasts are stimulated by calcitriol to create osteocalcin, osteoprotegerin, and alkaline phosphatase unique to bone.

VitD BINDING PROTEIN (DBP)

Serum containing vitamins D3 and D2, as well as their metabolites, is carried by "VitD-BP(binding protein) (DBP), a polymorphic form of serum α2-globulin that is produced by the liver." DBP has a strong affinity and capability for binding vitD and its hydroxylated metabolites [69]. The proximal tubule cells of the kidney receive the 25-hydroxyVitD/VitD binding protein complex from serum, which is then bound to megalin, a transmembrane protein, and acted upon by mitochondrial 25-hydroxyVitD-1α-hydroxylase. The binding of calcitriol to the VitD-receptor requires the presence of its 1α-hydroxyl group.

The biologically active portion of circulating calcitriol is its free fraction, while the majority of it is bound to D- binding protein. "The calcitriol pool that enters the cell and controls gene transcription [70] is unaffected by the serum amount of DBP." The serum contains about 0.4% of calcitriol and 0.04% of calcidiol in its free form. Human studies have revealed that D-binding protein is largely polymorphic, with 3 widely known variations (GC1S, GC1F, and GC2) that have been demonstrated to impact the function of the protein. 2 SNPs in GC, rs4588 and rs7041, define the 3 common variants with significance to VitD metabolism. Due to the changes in the D-binding protein amino acid sequence, DBP's binding affinity for VitD ligands appears to be altered, with Gc1F exhibiting the highest affinity and Gc2 the lowest for VitD metabolites [71}.

VitD, VitD RECEPTOR AND ITS MULTI-TARGET MYSTERY

Research points to several functions for VitD and its active metabolites across a wide range of organs through its action on VitD receptors. The biological action of Calcitriol is mediated by binding to "Retinoid X receptor-a (RXR-a)" and VitD receptor in the nucleus of a variety of cells in the body [73].

The steps of translation, transcription and post-translational changes with the encoded proteins, storage & secretion take place in numerous cytosolic compartments, resulting in an effects of calcitriol on nucleus for hours to days. Additionally, the VitD Receptor binds to caveolae, which are flask shaped membrane invaginations in the plasma membrane made of sphingolipids & cholesterol." Once the VitD receptor binds to the ligand, it can interact with intracellular signal transduction systems to trigger quick, functional cell responses." By attaching to the nuclear VitD receptor, a steroid receptor superfamily member, Calcitriol can exert its effects. In reaction to Calcitriol, the VitD receptor, a ligand-dependent transcription factor, modifies the expression of a few target genes [74].

"The VitD Receptor is a 427 Amino acid nuclear transcription factor that is widely expressed in the intestine, distal renal tubule, osteoblast, keratinocyte, hair follicle, fibroblast, smooth and cardiac muscle, lung, bladder, thyroid, parathyroid, pancreas, adrenal cortex and medulla, pituitary, placenta, uterus, ovary, testis, prostate, activated T and B lymphocytes, macrophages, monocytes, spleen, thymus and tonsil, brain, spinal cord, and sensory ganglia."

The 2 primary operational domains of the VitD receptor are the more variable carboxyhydroxylase-terminal ligand binding domain (LBD) and the highly stable

NH2-terminal DNA binding domain (DBD). The LBD is made up of three beta sheets (S1-3) and at least twelve alpha helices. The DBD & the LBD are linked together by a hinge region. The DBD is a zinc finger region that is rich in cysteine. It has two zinc fingers, both of which has a single zinc atom arranged tetrahedral formation with 4 invariable cysteine residues.

The VitD Receptor - ligand complex along with the "retinoid-X receptor" forming a heterodimer that binds VitD-responsive elements (VDREs) which is found mostly in the promoter regions of target genes [75].

"The majority of members in the nuclear receptor transactivating factor superfamily pair as homodimers to attach to the target gene's hormone response elements." However, the calcitriol-VitD Receptor complex collaborates with its obligatory partner, unliganded RXR alpha, via its E (ligand binding) domain to form a heterodimer that subsequently attaches to VitD response elements. 9-cis retinoic acid is the endogenous ligand for RXRalpha. The majority of the VDR is cytoplasmic when it is unliganded; when calcitriol binds to the VDR, it heterodimerizes with "RXRα & translocates the tripartite complex to the nucleus [110,76]."

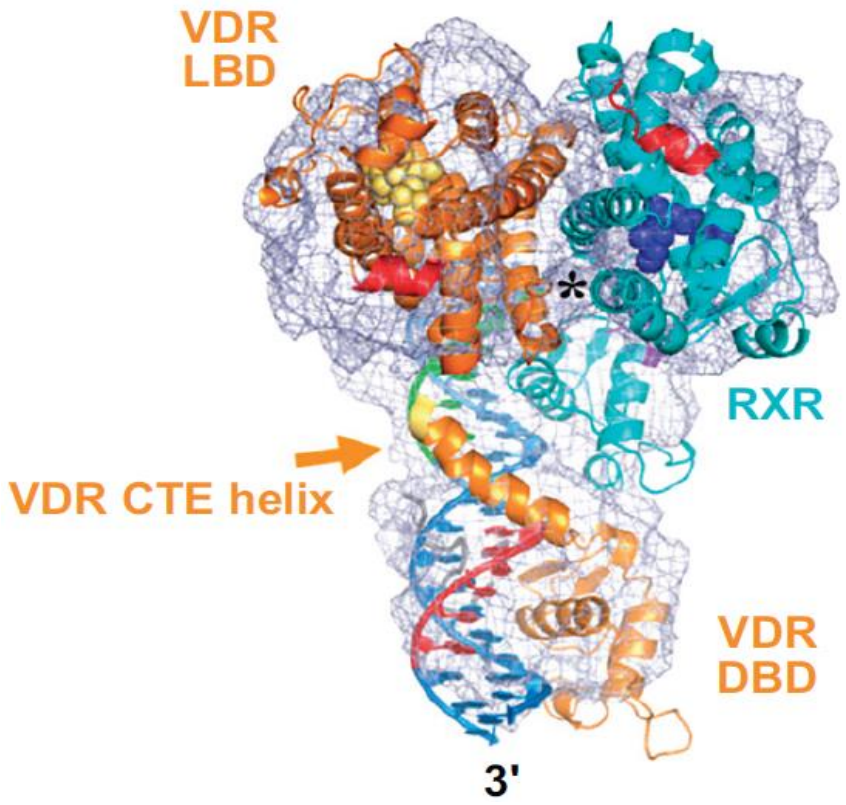


Fig:6 RXR/VitD human receptor heterodimeric complex structure along with target DNA[62].

The hormone signal is transduced into modified gene transcriptional events by the heterodimer produced by the RXR and the Calcitriol-VitD receptor complex. The Calcitriol-VitD Receptor complex either stimulates or inhibits the transcription of a large number of genes in conjunction with transcription factors and coregulatory proteins [77].

The human genome may include more than 2 thousand VitD Response Elements, suggesting that the VitD system may have vast effects. Through the VitD Receptor, calcitriol induces the transcription of genes encoding PTH, PTH related Peptide, 25-hydroxyVitD-1α hydroxylase, osteocalcin, osteopontin, calcium transport proteins (TRPV5 and TRPV6) and bone resorption factors (RANKL). It represses the

transcription of genes encoding the Parathyroid hormone. By adversely interacting with transcription factors that promote the transcription of several cytokines, including interferon-γ, interleukin-2 and GM-CSF, the calcitriol-VitD Receptor complex also decreases their production [110].

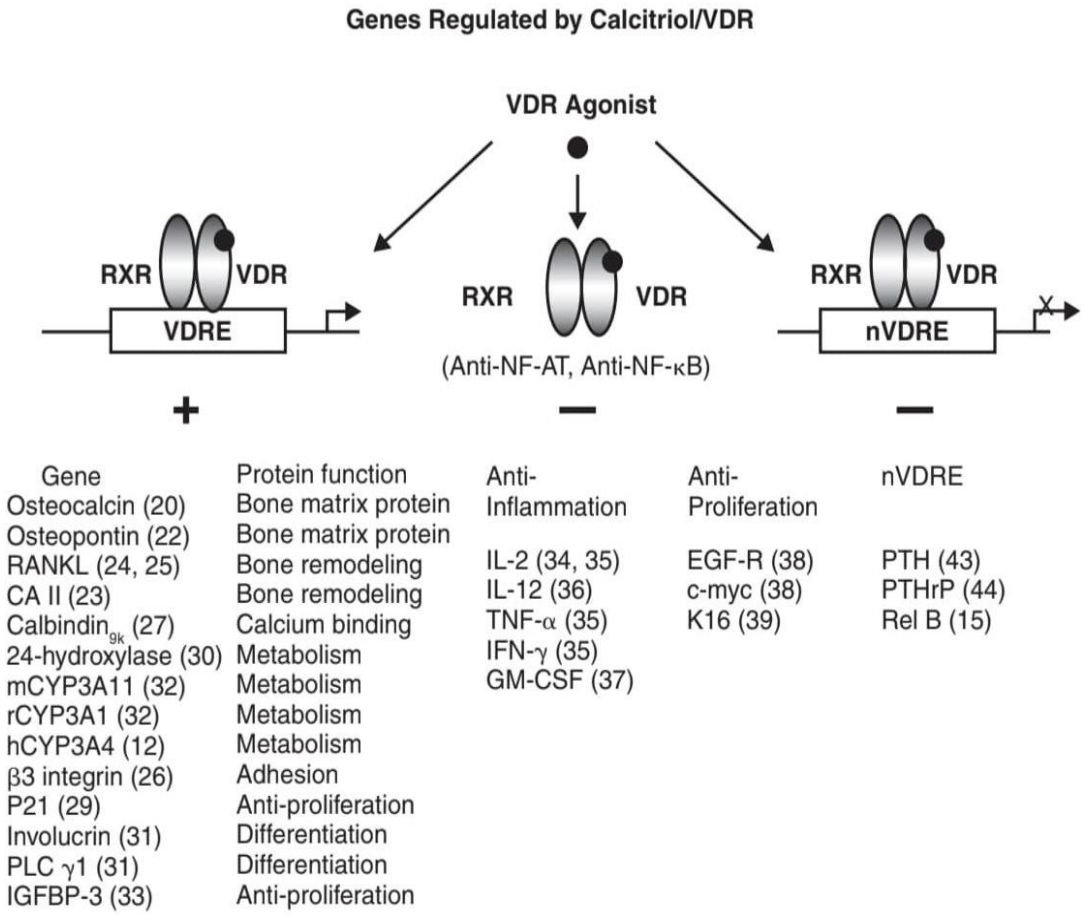


Fig: 7: Genes regulated by Calcitriol/VDR [60]

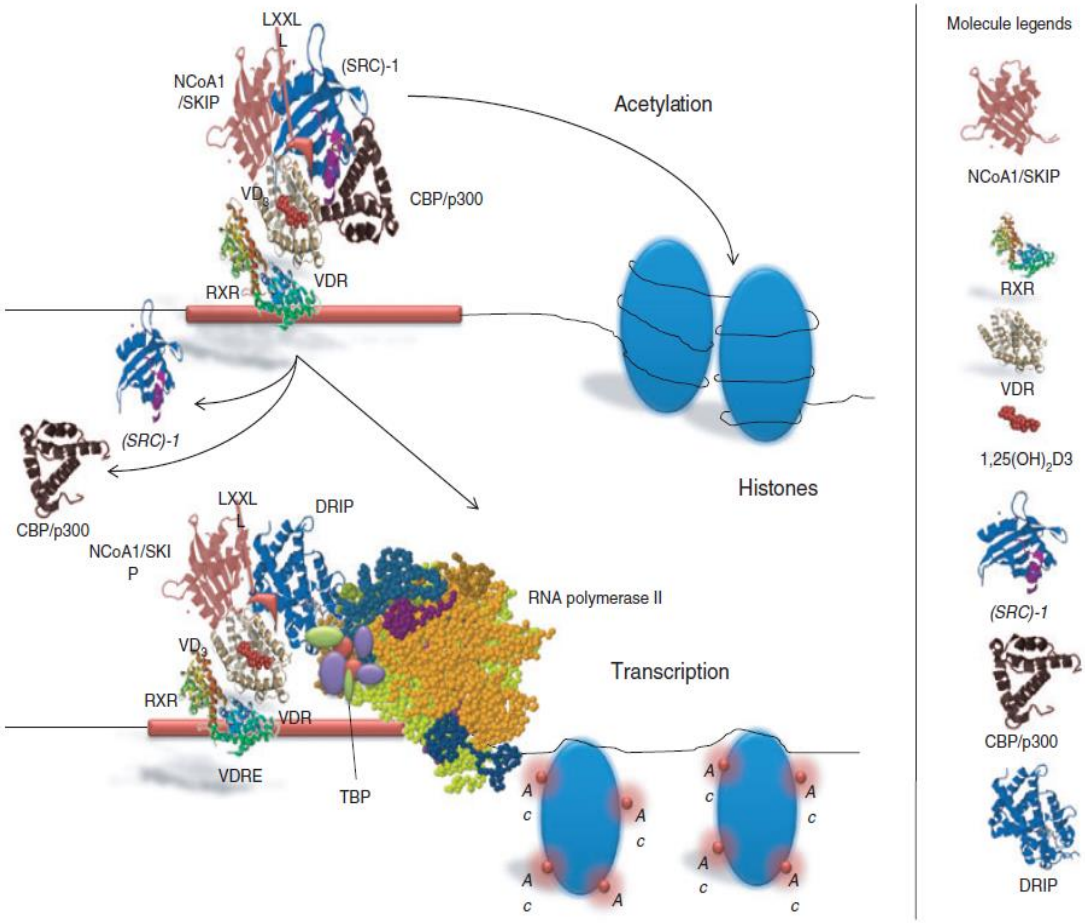


Fig:8. Biological actions of Calcitriol [2]:

By heterodimerizing with RXRs, the ligand-bound VitD receptor stimulates transcription and it is also required for the increasd affinity DNA binding to the corresponding VitD response elements. When ligands cause conformational changes in VitD receptor–RXR heterodimers, nuclear receptor co-repressor (NCoR) dissociates. "This process also facilitates interaction with other members of the p160 and CBP/p300 co-activator families, including steroid receptor co-activators-1 (SRC-1), transcriptional intermediary factor 2 (TIF2), and receptor-activated co-activators-3 (RAC3) [2]." VitD receptor-interacting protein and other co-regulatory proteins that regulate chromatin remodelling, transcriptional initiation, RNA polymerase II binding, and histone alterations are drawn to this activated complex [2].

IMMUNOMODULATORY FUNCTIONS OF VITD:

VitD has an influence on acquired as well as innate immune system. Increasing evidence has emerged to support this physiological role of the VitD system in immunological function, including maintenance of self-tolerance and defence. Most of the immune system cells have VitDR (receptors) and hydroxylase enzymes are necessary for the hydroxylation of VitD on the 1α and 25 sites. Immune cells as a result cause activation of Calcitriol from Calcidiol providing within the immune system a paracrine presence of Calcitriol. VDR is expressed by both monocytes and macrophages, however, the latter does more so than the former. It has been shown that Calcitriol promotes monocyte differentiation and proliferation, whereas the overall effect on activated macrophages reduces the inflammatory response. Calcitriol prevents the release of pro-inflammatory factors such as tumour necrosis factor (TNF), IL-6, IL-1, RANKL, and cyclooxygenase-2 and increases the synthesis of IL-10 ,the anti-inflammatory cytokine [78]. The following signalling pathways are thought to be involved in the anti-inflammatory effect: (i) LPS/p38 inhibition and upregulation of MKP and MAPK; (ii) COX2 inhibition caused by thioesterase superfamily member 4 alteration; (iii) "cathelicidin antimicrobial peptide (CAMP) induction causing direct antimicrobial effect" (iv) Reduced ROS and elevated glutathione reductase (GR) causing an antioxidative effect.

VitD also suppresses the acquired immune system by enhancing regulatory T cells, lowering T-Helper 1 and 17 cell activity, and lowering MHC class II & co-signaling molecule expression on antigen presenting cells. The outcome is to support T cells' protective and regulatory nature.

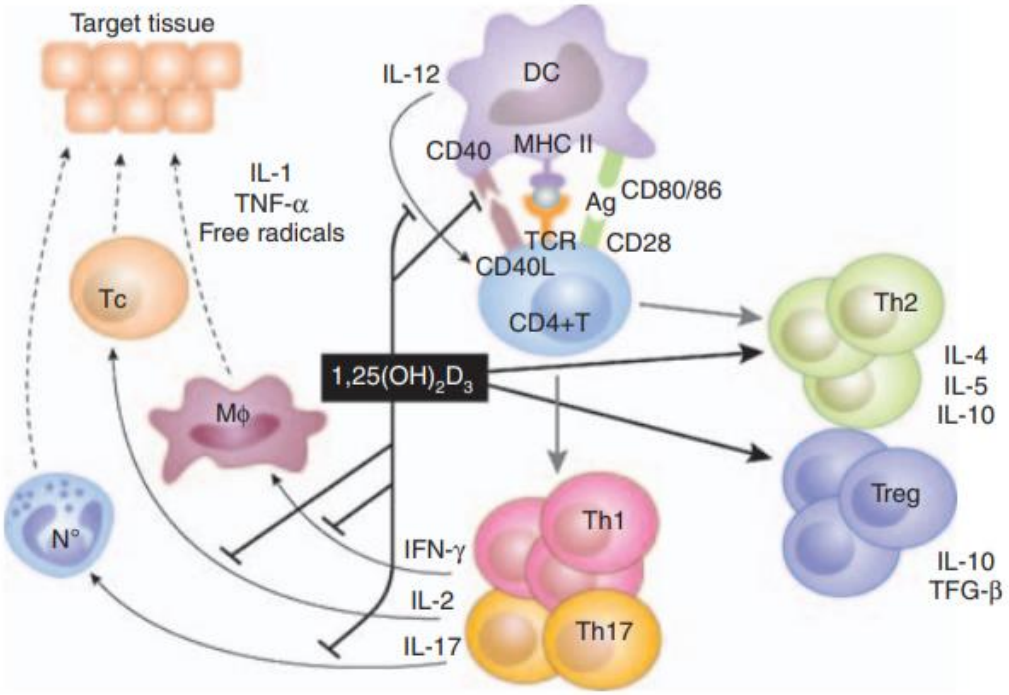


Fig:9 Immunomodulatory effects of Calcitriol [78]

Over the cell surface of antigen-presenting cells(APC), such as dendritic cells, Calcitriol inhibits the costimulatory molecules (CD80, CD40 and CD86) and major HSC-class II expression." It also inhibits the production of inflammatory cytokines, such as interleukin-12 (IL-12). By this, Calcitriol indirectly shifts T-cell polarization from an inflammatory T-helper 1 to a protective T-helper 2 and regulatory T-cell phenotype (Treg)[78]". In this way, Through the activation of T-helper 2 cytokines and the suppression of inflammatory T-helper 1 and T-helper17 cytokines, Calcitriol also directly influences T-cell responses. These combined effects of Calcitriol on the immune system could potentially prevent autoimmune diseases.

Vit D AND FETUS

Pregnancy increases a woman's need for VitD due to many changes in her metabolism of the vitamin. If sun exposure and VitD intake remain unchanged during normal pregnancy, maternal plasma levels of VitD don't usually fluctuate. On the contrary, the circulating active Calcitriol levels raise multiple times from early pregnancy and remain elevated until the end of pregnancy [79].

The foetus depends on the transfer from the mother and does not produce 25(OH)D on its own. While 2hydroxy-D crosses the placenta, Calcitriol-1,25(hydroxy)$_2$D3, which is only found in the mother's bloodstream, is thought to be produced both in the developing fetus as well as within the placenta. The existence of the less biologically active or the inactive C3-epimer of 25(Hydroxy)D throughout pregnancy & in early infancy complicates the serum 25(hydroxy)D levels assessment [80].

The C3-epimer doesn't undergo placental transfer, unlike 25(Hydroxy)D3 which crosses the placenta effectively. Both newborns and pregnant women frequently have hypovitaminosis D. Since the placental transfer of 25(hydroxy)D primarily happens during the third trimester of pregnancy, preterm newborns are especially vulnerable to VitDD.

PLACENTA AND VitD:

The placenta, apart from the kidney, is a key location for the conversion of Calcidiol to Calcitriol. Both maternal decidua and foetal trophoblasts in the placenta express CYP27B1. "The role of Calcitriol in the placenta was not recognised, even though it was identified as a significant site of extrarenal synthesis of CYP27B1".

Early in pregnancy, placental expression of CYP27B1 mRNA has been shown to peak in the 1st trimester [81].

According to recent research, the placenta's manufacture of calcitriol may be crucial in regulating the placenta's reaction to infection. Reduced cytokine generation, including TNF, GM-CSF, and IL-6, is observed in 1,25-(Hydoxy)$_2$D3 or 25 (Hydroxy)D3 [82] treated human decidual cells. Further evidence of the significance of Calcitriol as a regulator of immunomodulatory action in the placenta comes from the increased antimicrobial peptide- cathelicidin expression in trophoblasts & decidual cells in response to the hormone. "Cyp27b1 mRNA was increased in the mouse placenta when the TLR4s ligand Lipopolysaccharide was administered in vivo, suggesting that placental Calcitriol production is likewise susceptible to immunological challenge in vivo [83]."

According to reports, the placenta's CYP24A1 inhibition brought on by high methylation may be a factor in the placenta's higher bioavailability of 1,25(OH)$_2$D3 in humans [84]. All of these results point to the significance of placental CYP27B1 as a paracrine/autocrine regulator of innate and acquired immune responses throughout the early stages of fetoplacental life.

VitD, FOETAL LUNG MATURATION, AND RESPIRATORY- DISTRESS SYNDROME:

Respiratory distress syndrome -RDS, is a condition occurring primarily in preterm newborns < 34 weeks of gestation. RDS is characterized by "symptoms and signs of respiratory distress like tachypnoea, grunting, and rising oxygen requirements shortly after birth occurring primarily as a result of deficiency in the

surfactant levels." The incidence of RDS is inversely related to weight and the gestational age of the newborn at birth. RDS rates have decreased since the introduction of antenatal corticosteroids, but it remains a predominant cause of mortality and morbidity among the neonates. In addition to prematurity, other factors that contribute to increased risk of RDS are maternal diabetes, Perinatal asphyxia, cesarean section, and absence of preenatal steroid intake. The primary hallmark is the surfactant insufficiency.

PATHOPHYSIOLOGY OF RDS:

Neonates with RDS undergo generalised atelectasis, hypoxemia, ventilation-perfusion mismatch, and respiratory acidosis as a consequence of unstable, collapsing alveoli with insufficient (or malfunctioning) surfactant. Shear stress develops in the terminal bronchioles and alveoli while breathing (either spontaneously or with assistance) as a result of the overdistention of open alveoli and the repeated reopening of collapsed alveoli. These forces can rapidly harm the sensitive architecture of the lungs, causing proteinaceous debris (i.e., hyaline membranes) to leak into the airways. The effectiveness of what little surfactant is there may be compromised by this debris, which could accelerate the development of respiratory symptoms and if left unchecked, respiratory failure.

The phase of repair often starts the next following birth with the emergence polymorphonuclear cells and macrophages, if supportive care is successful. The injured epithelium regenerates when debris is phagocytosed. When lymphatics are activated by interstitial fluid, RDS enters its diuretic phase, which is marked by increased urine production. By the end of the 1st week following delivery, the neonates' condition improves with uncomplicated RDS. Nevertheless, infants with

severe RDS who need high oxygen concentrations and positive pressure ventilation may experience inflammation, increasing lung injury, and inappropriate lung repair, which could result in bronchopulmonary dysplasia.[85,86].

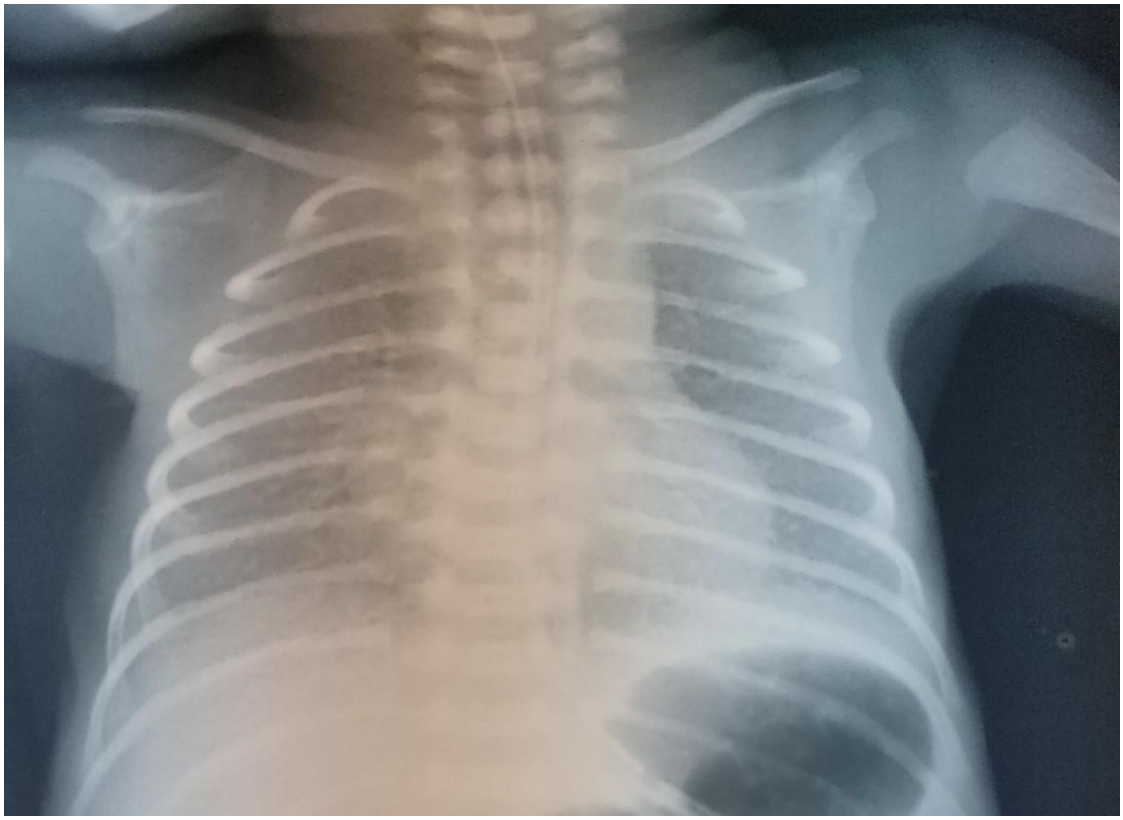


FIG:10 Presence of respiratory Distress Syndrome on Chest X-ray. Note the Ground glass opacity of the lungs. (Xray of a preterm newborn from KLES. Dr.P.K. Hospital adapted with permission)

Studies on animals and in the lab revealed that "VitD had a significantly favourable impact on the type II cell in the alveoli, fibroblast proliferation, alveolarization and surfactant generation. This evidence provides credence to the theory that hypovitaminosis D is a common and adjustable risk factor for RDS."

One of the important aspects of VitD is its effect on preterm Respiratory distress/ respiratory distress syndrome through its effect on the foetal lung maturation and development which is the primary objective of this study.

According to animal studies [87,88,89], VitD Receptor is primarily present in the lung during the later stages of pregnancy, when Alevolar Type II cells differentiate, surfactant production start, and the glycogen content in the lung declines. The overexpression of the receptor and the stimulation of proliferation and differentiation in both Alveolar Type II cells and fibroblasts were seen upon incubation with 1,25 $(hydroxy)_2D3$. 1,25 $(hydroxy)_2D3$ accelerated a reduction in glycogen content and enhanced surfactant related phospholipid production and secretion by Alveolar-Type II cells isolated from foetal rat lungs, according to Marin et al [90,91]. Notably, dexamethasone did not cause surfactant exocytosis when $1,25(OH)_2D3$ was present, nor did it have any further effects on surfactant concentration when $1,25(OH)_2D3$ was present.

A common and scientifically supported method of preventing RDS in pregnancy is to give the expectant mother dexamethasone or other glucocorticoids when there is a risk of an early birth[102]. According to animal research, VitD might be a further, if not better, choice than glucocorticoids for preventing RDS in mothers whose premature birth poses a risk.

Nguyen et al [17] provided additional evidence of the "physiological function of 1,25 $(OH)_2D3$ by demonstrating a correlation between VitD Receptor expression and the various phases of pulmonary maturation in rat foetuses." "The discovery of elevated F1,6BP mRNA expression by 1,25 $(OH)_2D3$ in human Alveolar Type II cells suggests that F1,6-BP activation could be involved in the effects of 1,25

$(OH)_2D3$ on surfactant formation through the gluconeogenesis pathway of gluconeogenesis." In addition to its effects on gluconeogenesis, Fructose1,6-BP may help the developing lung for surfactant synthesis [92].

VitD has been shown to have an impact on several physiological pulmonary maturation processes in mice, according to laboratory investigations [93]. "VitD can enhance the production and secretion of surfactant related phospholipids in human Alveolar Type II cells, as shown by Rehan et al. [94]. "The effects of Calcitriol on the expression of SP-B mRNA in human Alveolar Type II cells and the expression of SP-A mRNA in human foetal lung tissue and isolated Alveolar Type II cells were demonstrated by Phokela et al [93]." 1,25 $(OH)_2D3$-induced rising SP-B microRNA and decreases in SP-A microRNA are consistent with findings in the presence of RA and glucocorticoids, suggesting a complex regulation of foetal surfactant production and lung maturation. In addition, Phokela et al [93] confirmed the identifications of Nguyen et al [17] in human Alveolar Type II cells by showing that 1,25 $(OH)_2D3$ increases VitD Receptor in human-isolated Alveolar Type II cells and foetal lung tissue. Considering these manifestations of VitD on foetal lung maturation, VitD therapy may be preventive against the development of RDS.

A prospective study on the relationship between VitDD and Respiratory distress syndrome was conducted in a tertiary care centre by Dogan P et al. [95] 72 preterm babies ≤ 32 gestational weeks who were admitted to the NICU over one year made up the study. Of the patients, 49 had RDS, while 23 did not. Neonates with RDS had decreased mean 25 (OH)D levels ($p = 0.04$). Patients with greater 25 (OH)D levels may be able to prevent developing RDS, according to multivariate analysis (OR 0.89 with $p = 0.04$). They concluded that VitDD is an independent risk factor for RDS

and that more research was needed to establish if there was any relationship between RDS and VitDD.

In a cohort study at the Neonatal Health Research Centre, Jafari et al [96] (2011) considered low VitD levels in preterm infants as exposure and respiratory problems with associated interventions as outcomes. "Of the 113 preterm babies that were included, 65 (58%) had VitD insufficiency (group I), while 48 (42%) had normal VitD levels (group II)." RDS was considerably higher in a group with VitD insufficiency than in a group with normal VitD (P = 0.036). They discovered that on comparison to the normal group, newborns in the VitDD group required greater oxygen requirement and mechanical ventilation (P = 0.390 and P = 0.549, respectively). "20 cases (41.7%) in group II and 40 cases (61.5%) in group I had respiratory distress syndrome (RDS) and required surfactant therapy (P = 0.036)".

"Furthermore, non-invasive ventilation (NIV) was required for 46 newborns (70.8%) in the first group and 22 newborns (45.8%) in the second group (P = 0.007). VitD status and RDS (OR, 95% CI = 2.840 (1.083–7.447), P = 0.034), need for surfactant (OR, 95% CI = 2.840 (1.083–7.447), P = 0.034), and requirement for NIV (OR, 95% CI = 3.929 (1.526–10.113), P = 0.005) were significantly correlated, according to multiple logistic regression analysis."

"VitD levels in preterm newborns ≤ 34 weeks gestation were measured on the day 01 of life in a case control study by A.M. Hegazy et al[97]. The study included 65 preterm babies with gestational age less than 34 weeks and 40 newborns with RDS, and 25 newborns without RDS".

Compared to controls, preterm babies with RDS had a mean serum 25 (hydroxy)D level that was substantially lower (1037.6 Vs 13.9 ng/dl). While not reaching a significant level (p value¼.08), "neonates with severe 25-(OH)D deficiency developed more bronchopulmonary dysplasia- BPD than those with moderate deficiency (29.4 Vs 8.7%)". A correlation between serum 25 - (Hydroxy)D level and mechanical ventilation duration was not found. A low serum 25 - (Hydroxy)D level has been found as an independent risk factor for RDS by logistic regression analysis.

"A study conducted by Fettah et al to explore the relationship between serum 25(OH) VitD levels and the development of respiratory distress syndrome in preterm neonates less than 32 gestational weeks [98]."

The 25 (hydroxy)VitD levels were measured in the umbilical cord blood of these neonates. The range that was specified in other studies, where VitDD was defined as levels < 20 nanogram/millilitre, was not the same as the cut-offs used in this study, which defined VitDD as ≤ 15 nanogram/millilitre in Group I and normal level as > 15 nanogram/millilitre in Group II. Additionally, patients in Group I were subdivided into 2 groups: mild deficiency (Group Ib, 5–15 nanogram/millilitre) and severe deficiency (Group Ia, 5 nanogram/millilitre).

57 babies in Group 1 and Group 2 of this study exhibited decreased 25 (Hydroxy)D levels (median 8.0 nanogram/millilitre [interquartile range 05–10] and 21 nanogram/millilitre [Interquartile 19–24.7] respectively). Group Ia (N = 18 with 32.7percentage) and Group Ib (N = 34 with 61.8 %) had significantly greater RDS rates than Group II (N = 3, 5.4 %) (p¼0.001). Between Group 1a (94.7%) and Group 1b (89.5%), there was no change in RDS (p ¼ 0.512). Higher levels of 25 (hydroxy)D

have been shown through multivariate analysis to be protective against establishment of RDS (OR, 0.6, 95% CI (0.5–0.8) , p¼0.001) [98].

VitDD AND OTHER CLINICAL OUTCOMES:

Very few studies in India have been done that correlated the effect of VitDD with the clinical outcomes of preterm newborns. Considering there are studies stating the other immunological functions of VitD, articles were searched regarding its effect on clinical outcomes other than RDS too, like neonatal sepsis and Retinopathy of Prematurity.

One such study was conducted by Srinivasan N et al [99] wherein "80 preterm newborns under 34 weeks gestation (mean gestation age 29±2 weeks and BW: 1210±350 grams) had their cord blood levels measured at the neonatal intensive care unit of a tertiary care hospital in Kerala, Southern India". A daily dosage of 400 IU or 800-1000 IU of VitD was given to the infants. Serum VitD levels were correlated with the clinical outcome 2 - 3 weeks after supplementation of VitD.

The mean level of VitD in cord blood was 12±8.5 nanogram/ml. Infants who developed sepsis and compared without sepsis, and those with ROP and without ROP had VitD levels: of 13.5±6 (nanogram/ml) versus 30.5±10 (nanogram/ml) ($p < 0.01$) and 15.7±11 (nanogram/ml) versus 34±18 (ng/ml) ($p < 0.03$) respectively. Infants receiving 800-1000 IU VitD exhibited levels of 46±17 (nanogram/ml) ($p < 0.001$), while those supplemented with 400 IU VitD showed values of 17±8.6 (ng/ml).

"At a tertiary care neonatal facility in Odisha, India, a prospective observational study was conducted to determine the prevalence of VitDD and its co-morbidities in VLBW neonates [15]. The study included all VLBW infants admitted

during the first 24 hours of birth to the NICU. 40 VLBW newborns with a mean gestational age of 30.60 ± 2.274 weeks and a mean birth weight of 1133.95 ± 208.487g were included in the study. "

The mean VitD level was 16.906 ± 12.708 ng/dl and the prevalence of VITDD was 67.5%. Babies with VitDD have increased risk of sepsis and respiratory distress syndrome (RDS).

"In the neonatal intensive care unit of a tertiary care teaching institution in central India," Agrawal A et al[100] carried out a case-control study. Cases included full-term neonates with LOS that had been confirmed by culture. Demographics about mothers and newborns, clinical assessments, and investigations were documented. It was investigated whether vitamin-D insufficiency (<20 ng/ml) and LOS were correlated.

"There were 225 term neonates in total of which 175 were cases and 50 were controls. Comparable demographic profiles applied to mothers and newborns. Comparing the mean VitD level between the cases (12.2866.11 ng/ml) and controls (14.8867.2 ng/ml), a significant difference was observed (p¼0.002). Of the 175 cases, 151 (86.29%) and 37 (74%) of the 50 controls were found to be vitamin-D deficient (p¼0.00003). Multiple regression analysis revealed a strong correlation (p¼0.00003) between VitD insufficiency and neonatal sepsis. This study reveals that neonates with VitDD may be more susceptible to LOS."

"Chinmay Kumar Behera et Al [101] carried out a prospective cohort study in the NICU of a tertiary care teaching hospital in Odisha, Eastern India, between January 2015 and December 2016. The study group consisted of 40 newborns with

culture-positive sepsis. Mothers and newborns in both groups had their VitD levels assessed."

The study group's neonatal VitD level (12.71 ± 2.82 nanogram/ml) was considerably lesser than the control group's (25.46 ± 7.02 ng/ml). For neonates with VitDD/insufficiency and culture-positive sepsis, the odds ratio was calculated as 273 (95% CI 30.39–2451.6). The 25hydroxy -VitD level of mothers of septic newborns was considerably lower (20.92 ± 3.92 ng/ml) than that of mothers of healthy newborns in the control group (27.31 ± 6.83 nanogram/ml). The odds ratio calculated for culture (+ve) sepsis in infants born to women with VitDD/insufficiency was 4.71 (95% CI 1.69–13.1).

Sepsis was more common in newborns with VitDD/insufficiency than in those with adequate VitD levels. A higher risk of sepsis in newborn babies is associated with mothers' lower VitD levels.

MATERIALS AND METHODS

PLACE:

The study was carried out in the NICU of KLES Dr. Prabhakar Kore Hospital & Medical Research Centre, Belagavi.

DESIGN OF THE STUDY:

Single centre, Hospital based, Prospective Observational study.

STUDY PERIOD:

From April 2023 to February 2024

SOURCE OF DATA:

All preterm neonates born between 28 weeks to 34 weeks of gestational, admitted to NICU and fulfilling the inclusion & exclusion criteria.

INCLUSION CRITERIA:

All preterm neonates born between 28 weeks to 34 weeks period of gestation and admitted to NICU.

EXCLUSION CRITERIA:

1. Infants with congenital anomalies, cyanotic congenital heart disease.
2. Infants with chromosomal anomalies.
3. Maternal history of chorioamnionitis.
4. Duration of NICU stay for < 48 hrs.

ETHICAL CLEARANCE:

Institutional ethical clearance was obtained from the KLE Academy of Higher Education & Research.

SAMPLE SIZE:

Minimum sample size required based on the prevalence rate is

- **Sample size** = $\frac{X^2 N p (1-p)}{d^2 (N-1) + X^2 p (1-p)}$

  Where X^2 is the Chi-square value corresponding to 95% confidence (3.84) and for 1 degree of freedom,

  d = error = 0.05 (margin of error)

  From the previous study p = 0.425

  X^2 = 3.84, N = 65,

  Minimum Sample size required = 56

INFORMED CONSENT:

The parents of children who fulfil the eligibility criteria were explained about the nature of the study. Informed consent was taken and then the baby was enrolled in the study.

STUDY PROTOCOL:

All neonates more than 28 weeks and less than 34 weeks gestation born in KLES Dr. Prabhakar Kore hospital and meeting the inclusion & exclusion criteria were included in the study.

DATA PROCESSING AND ANALYSIS:

The descriptive analysis of the data from the study was analysed using mean and standard deviation for quantitative variables. For categorical variables, data was analysed by using frequency and proportion. Data representation was done using bar graphs, pie charts and box plots.

Cross-tabulation and comparison of percentages was used for assessing the association between explanatory variables and categorical outcomes. Statistical significance was evaluated by using Chi-square test.

The relation between quantitative explanatory variables and categorical outcomes was assessed by independent sample t-test for 2 groups) and for more than two groups ANOVA was used to assess the statistical significance.

IBM SPSS version 22 was used and P value < 0.05 was considered statistically significant.

The parents/guardian of the baby, who fulfilled the eligibility criteria were briefed about the study. After obtaining written informed consent, the baby was enrolled for the study. The consent forms were prepared in English as well as major regional languages used in the region – Kannada, Marathi, and Hindi (ANNEXURE 1). Then the necessary details were recorded in a proforma (ANNEXURE 2). All the data collected were statistically analysed.

The details of the newborn like Gestational age, gender, birth weight resuscitation details, APGAR scores, Ballard scoring, Antenatal steroid intake, maternal risk factors like PROM, Diabetes mellitus, pre-eclampsia, Thyroid disorder, and maternal demographic details with LMP, and EDD were entered in a structured proforma.

VITAMIN D ESTIMATION

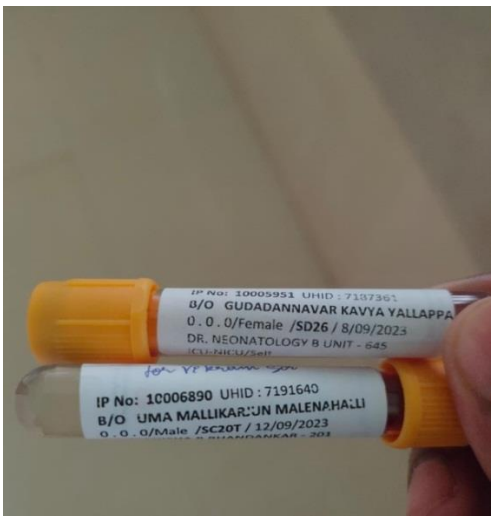
SPECIMEN COLLECTION:

Within 1 hour of admission to the NICU, about 2ml of blood was collected in a yellow-topped vacutainer. The samples were labelled for identification and were transported to the Hi-Tech lab of the hospital for estimation of VitD. The sample was centrifuged in Eppendorf Centrifuge 5702R centrifuge machine for 10 minutes at 3500RPM to separate the serum.

SYSTEM USED:

Elecsys VitD total III – Cobas e801.

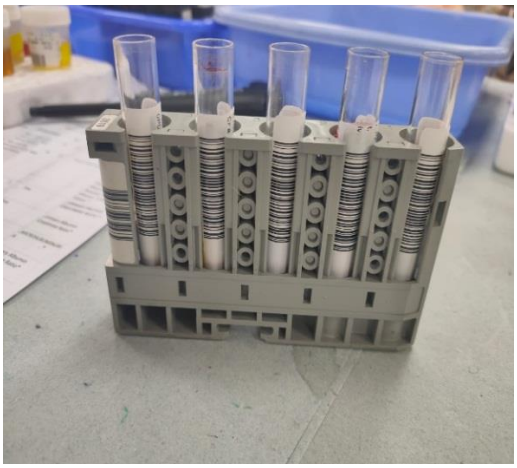
Quantitative estimation of total 25-hydroxyVitD in the plasma was done by the **electrochemiluminescence binding assay** intended for use on **cobas e** immunoassay analyser.



The samples collected are labelled for identification and transported to the laboratory immediately.

The sample is centrifuged for 10 mins at 3500 rpm

The serum is separated after centrifugation



After serum separation the samples are arranged in sample rack for placing the rack in the analyser for estimation

Elecsys VitD total III – Cobas e 801 for estimation of VIt

The Elecsys VitD total III assay employs a VitD binding protein labelled with ruthenium complex as a capture protein to bind 25-hydroxyVitD3 and 25-hydroxyVitD2. The product of 25-hydroxyVitD catabolism by 24-hydroxylase is 24,25- dihydroxy VitD. A specific monoclonal antibody blocks the cross reactivity to 24,25- dihydroxyVitD.

TEST PRINCIPLE:

Total duration of the test: 27 mins by competition principle

- First incubation: By incubating the sample (9 microlitre) with pretreatment reagents 1 and 2, bound 25‑hydroxyVitD is released from the VitD binding protein.
- Second incubation: The pre-treated sample is incubated with the ruthenium labelled VitD binding protein, and a complex between the 25‑hydroxyVitD and the ruthenate VitD binding protein is formed. 24,25‑dihydroxyVitD is bound by a specific unlabelled antibody which inhibits the cross-reactivity to this VitD metabolite.
- Third incubation: After adding streptavidin-coated microparticles and 25‑hydroxyVitD labelled with biotin, the unbound ruthenium labelled VitD binding proteins become occupied. A ruthenylated VitD binding protein and the biotinylated 25‑hydroxyVitD complex are formed and bound to the solid phase through biotin and streptavidin interaction.

The microparticles are magnetically attracted to the electrode surface of the measurement cell upon aspiration of the reaction mixture into it. ProCell II M is then used to remove the unbound materials. A photomultiplier is used to measure the chemiluminescent emission that is induced when a voltage is applied to the electrode. The calibration curve, which is an instrument particularly created via two-point calibration, and the master curve obtained from the Cobas link are used to determine the results.

BLOOD CULTURE:

After wearing sterile gloves, the area of puncture is disinfected with the help of 70% isopropyl alcohol followed by the application of povidone-iodine moving outward from the centre applied in concentric circles, followed again by 70% isopropyl alcohol. Once the skin is air dried, about 1 millilitre of blood was collected from a fresh venipuncture site under sterile aseptic precautions in a BACTEC blood culture vial containing 10 ml of culture medium at the time of NICU admission before antibiotics initiation and the collection was repeated in the same manner when necessary if clinical evidence/suspicion of sepsis sets in.

The BACTEC blood culture vials are inspected for cracks or signs of contamination such as excessive cloudiness. The cap is removed and the septum is disinfected with the help of 70% isopropyl alcohol before blood collection from the patient.

Blood cultures give a confirmative determination of bacteraemia and is a gold standard for Neonatal Sepsis diagnosis. Early onset sepsis, defined commonly as neonatal sepsis onset before 72hrs of birth usually manifests as asymptomatic

bacteraemia, pneumonia, and/or meningitis. The clinical evidence of sepsis is usually apparent in the 1st hours of life and by 24 hrs of life majority are symptomatic. The most frequent initial symptom is respiratory distress, which can range in severity from mild tachypnoea and grunting—with or without the need for additional oxygen—to respiratory failure. Irritability, tiredness, temperature instability, poor perfusion, and hypotension are among other less specific symptoms. Severe septic shock instances may result in DIC. The symptoms of the gastrointestinal system include ileus, vomiting, and poor eating. Seizures, low sensorium, and apnea are possible symptoms of meningitis.

Similarly, lethargy, a rise in the frequency or severity of apneic episodes, feeding intolerance, temperature instability, and/or an increase in the respiratory support are risk factors for late onset sepsis, which is typically defined as sepsis that develops beyond 72 hrs of life.

Apart from blood cultures sent during the time of admission, blood cultures are repeated when signs of neonatal sepsis mentioned above occur in the neonate.

The BACTEC vials are transported to the Hi-Tech lab of the hospital and transferred into the blood culture instruments to detect the growth of pathogenic organisms.

INSTRUMENT USED:

BD BACTEC FX blood culture system.

The BACTEC vials are inserted into the BD BACTEC instrument for incubation and periodic reading. Each vial contains a chemical sensor that can detect

increases in CO2 produced by the growth of microorganisms. The sensor is monitored by the instrument every ten minutes for an increase in its fluorescence, which is proportional to the amount of CO2 present. A positive reading indicates the presumptive presence of viable microorganisms in the vial. Detection is limited to microorganisms that will grow in a particular type of medium.

TEST PRINCIPLE:

If microorganisms are present in the test sample inoculated into the **BD BACTEC** vial, CO2 will be produced when the organisms metabolize the substrates present in the vial. Increases in the fluorescence of the vial sensor caused by the higher amount of CO2 are monitored by the **BD BACTEC** Instrument. Analysis of the rate and amount of CO2 increase enables the **BD BACTEC** instrument to determine if the vial is positive; i.e., that the test sample contains viable organisms.

REAGENTS USED:

The **BD BACTEC** culture vials have the following active ingredients :

Processed Water, Soybean-Casein Digest Broth, Yeast Extract, Animal Tissue Digest, Sucrose, Hemin, Menadione, Pyridoxal HCl, Sodium Bicarbonate, Sodium Polyanetholsulfonate

The BD BACTEC FX series instrument will identify the positive vials. Although there won't be a visible difference between the sensor in positive and negative vials, the BD-BACTEC FX series equipment can detect a change in fluorescence. The growth if present as indicated by the instrument, is then subculture onto Blood and Mac Conkey agar for the identification of particular microorganisms.

BD BACTEC FX Blood culture device

DIAGNOSIS OF RDS:

The diagnosis and severity of Respiratory distress syndrome will be on the basis of Chest X-ray findings of the neonate.

HMD -1:

- Reticulo-granular pattern,
- A reduction in lung transparency with no discernible variation from normal findings, No air bronchogram

HMD -2:

A soft reduction in transparency with an air bronchogram that crosses across the heart (is invariably indicative of an alveolar lung reaction)

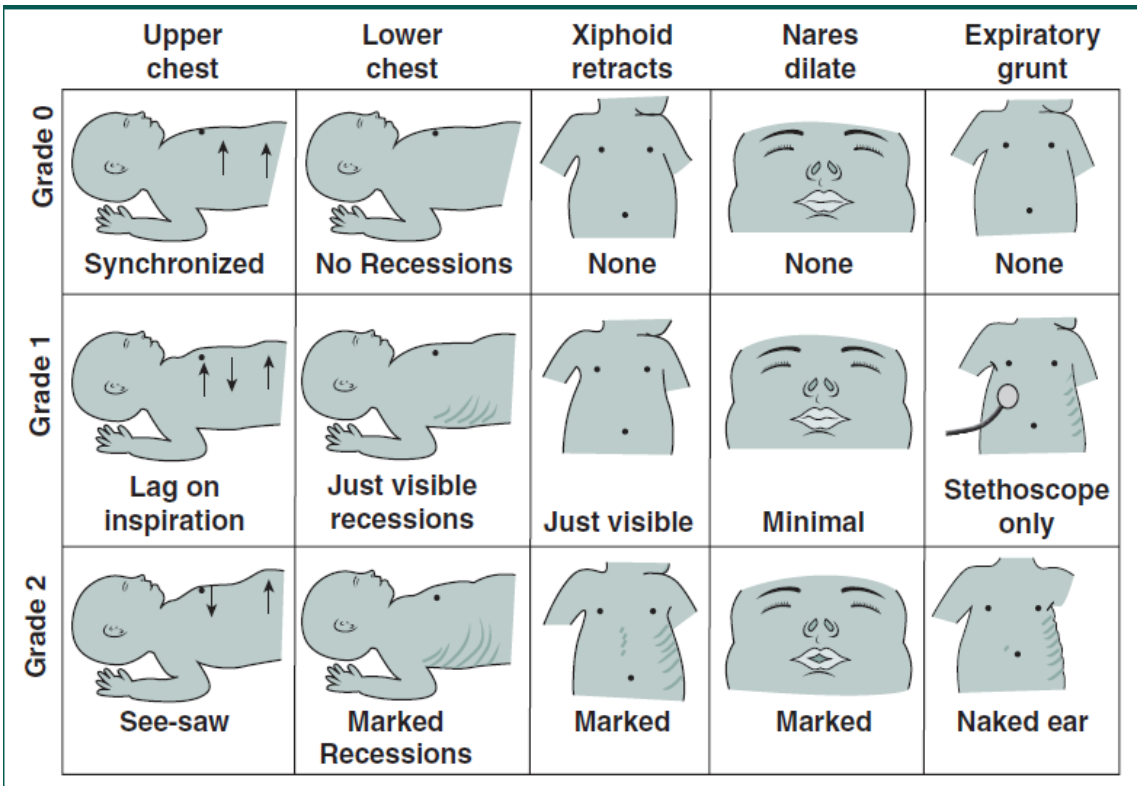
HMD -3:

Similar to stage 2, but with an increasingly marked decline in transparency and an indistinct heart and diaphragmatic silhouette.

HMD- 4:

White lung with homogenous opacities in the lung.

Clinical grading of the respiratory distress was done based on Silverman -Anderson scoring



Source: Silverman WA, Andersen DH. A controlled clinical trial of effects of water mist on obstructive respiratory signs, death rate and necropsy findings among premature infants. *Pediatrics* 1956;17:1–10.

Neonates with Silverman Anderson scoring <4 were started on oxygen by nasal prongs/ observed for worsening of respiratory distress. Neonates having SAS between 4-7 and > 7 were started on CPAP and Mechanical Ventilation respectively.

The decision of escalation and de-escalation of the respiratory support was based on the decision of the neonatologist. Surfactant was administered for all neonates with HMD ≥ 3. All neonates with respiratory distress were followed up for the requirement and duration of respective respiratory support received.

METHOD OF SURFACTANT ADMINISTRATION:

The preterm neonate requiring surfactant administration is intubated under all aseptic precautions with an appropriately sized ET tube and the breath sounds are confirmed over B/L lung fields for equality. The neonate is strictly monitored for saturation and heart rate throughout the procedure. The surfactant – Neosurf is warmed before administration and is given at a dose of 5ml/kg is delivered through a feeding tube inserted through the ET tube in 3-4 aliquots and the neonate is connected back to the ventilator. Suctioning is avoided strictly for at least 2 hours post surfactant administration.

OTHER RECORDED PARAMETERS FOR ANALYSIS:

1) Duration of hospital stay.
2) Day of starting enteral nutrition
3) Day of achieving complete enteral nutrition
4) Grading of HMD based on X-ray findings.
5) Neurological outcome at 40 weeks postmenstrual age assessed by Hammersmith Neonatal neurological examination[104].

   Hammersmith Neonatal neurological examination is done at 40 weeks postmenstrual age for the baby when the baby is called for a follow-up to the high-risk baby clinic.

HAMMERSMITH NEONATAL NEUROLOGICAL EXAMINATION

POSTURE	arms & legs extended or very slightly flexed	legs slightly flexed	leg well-flexed but not adducted	leg well flexed & adducted near to abdomen	abnormal posture: opisthotonus a) arms flexed, b) legs extended
ARM RECOIL	arms do not flex	arms flex slowly not always; not completely	arms flex slowly; more complete	arms flex quickly and completely	arms difficult to extend; snap back forcefully
ARM TRACTION	arms remain straight; no resistance	arms flex slightly or some resistance felt	arms flex well till shoulder lifts, then straighten	arms flex to approx. 100° & maintained as shoulder lifts	flexion of arms <100°; maintained when body lifts up
LEG RECOIL	No flexion	incomplete or variable flexion	complete but slow flexion	complete fast flexion	legs difficult to extend; snap back forcefully
LEG TRACTION	legs straight - no resistance	legs flex slightly or some resistance felt	legs flex well till bottom lifts up	knee flexes & remains flexed when bottom up	flexion stays when back & bottom up
POPLITEAL ANGLE	180°	≈ 150°	≈110°	≈90°	<90°
HEAD CONTROL (1)	no attempt to raise head	infant tries: effort better felt than seen	raises head but drops forward or back	raises head: remains vertical	
HEAD CONTROL (2)	no attempt to raise head	infant tries: effort better felt than seen	raises head but drops forward or back	raises head: remains vertical; it may wobble	head upright or extended; cannot be passively flexed
HEAD LAG	head drops & stays back	tries to lift head but it drops back	able to lift head slightly	lifts head in line with body	head in front of body
VENTRAL SUSPENSION	back curved, head & limbs hang straight	back curved, head ↓, limbs slightly flexed	back slightly curved, limbs flexed	back straight, head in line, limbs flexed	back straight, limbs above body

1	.5	2	.5	3	.5	4	.5	5	
3	0	9	6	60	9	12	0	1	25-27w
1	0	6	2	61	16	12	1	1	28-29w
2	0	4	2	65	17	8	0	2	30-31w
0	0	0	2	81	4	9	0	4	32-34w
0	0	0	0	6	3	90	1	0	Full term

3	1	9	9	44	9	23	2	0	25-27w
1	1	3	4	42	15	33	0	1	28-29w
1	0	8	3	42	10	36	0	0	30-31w
0	0	2	2	54	15	25	0	2	32-34w
0	0	5	2	22	3	67	1	0	Full term

3	0	17	5	51	10	14	0	0	25-27w
7	1	14	7	45	8	18	0	0	28-29w
7	2	15	4	51	7	14	0	0	30-31w
6	2	25	0	59	4	4	0	0	32-34w
0	0	1	0	22	8	69	0	0	Full term

3	0	14	4	18	5	52	0	4	25-27w
0	0	5	2	24	5	62	0	2	28-29w
0	0	10	2	34	2	50	0	2	30-31w
0	0	9	0	38	2	49	0	2	32-34w
0	0	3	1	4	1	91	0	0	Full term

3	1	17	6	35	6	27	1	4	25-27w
1	1	17	2	36	6	35	1	1	28-29w
2	0	21	8	38	5	25	0	1	30-31w
0	4	29	10	43	2	10	0	2	32-34w
0	0	0	1	12	12	72	0	3	Full term

3	0	22	8	46	6	14	0	0	25-27w
5	1	16	5	48	7	17	1	0	28-29w
2	0	15	10	53	5	15	0	0	30-31w
2	0	26	4	49	4	13	0	2	32-34w
0	0	5	5	19	20	51	0	0	Full term

3	0	17	4	46	9	21	0	0	25-27w
0	0	13	5	46	12	24	0	0	28-29w
3	0	14	2	48	13	20	0	0	30-31w
4	0	15	4	55	4	18	0	0	32-34w
0	0	0	6	26	12	56	0	0	Full term

3	0	3	5	57	11	21	0	0	25-27w
1	2	6	4	50	13	24	0	0	28-29w
1	0	2	2	63	11	21	0	0	30-31w
0	0	4	2	77	2	15	0	0	32-34w
0	0	0	4	29	15	52	0	0	Full term

3	3	27	13	36	3	15	0	0	25-27w
3	3	18	7	40	14	15	0	0	28-29w
7	3	16	5	46	7	16	0	0	30-31w
4	0	21	4	56	0	15	0	0	32-34w
0	0	9	4	44	12	31	0	0	Full term

0	0	21	11	38	11	15	4	0	25-27w
3	0	25	8	44	8	10	0	2	28-29w
3	0	22	8	47	5	14	1	0	30-31w
2	0	17	2	56	2	19	0	2	32-34w
0	0	4	5	47	16	28	0	0	Full term

TONE PATTERN ITEMS

FLEXOR TONE (compare arm and leg traction)		arm flexion < leg flexion	arm flexion = leg flexion	arm flexion > leg flexion; difference ≤1 column	arm flexion > leg flexion; difference > 1 column
FLEXOR TONE (resting posture)			arms and legs generally flexed	strong arm flexion with strong leg extension *intermittent*	strong arm flexion with strong leg extension *continuous*
LEG TONE (leg traction and popliteal angle)		leg traction > popliteal angle	leg traction = popliteal angle	leg traction < popliteal angle; difference ≤1 column	leg traction < popliteal angle; difference > 1 column
HEAD CONTROL (sitting)		neck extension < neck flexion	neck extension = Neck flexion	neck extension > neck flexion; difference ≤ 1 column	neck extension > neck flexion; difference > 1 column
NECK AND AXIAL TONE (horizontal)		ventral suspension < head lag	ventral suspension = head lag	ventral suspension > head lag; difference ≤1 column	ventral suspension > head lag; difference > 1 column

1	.5	2	.5	3	.5	4	.5	5	
0	0	45	0	27	<1	27	0	1	**25-27w**
0	0	40	<1	40	0	20	<1	0	**28-29w**
0	0	34	<1	47	<1	18	0	1	**30-31w**
0	0	38	<1	36	<1	24	<1	2	**32-34w**
0	0	25	3	53	0	18	0	<1	**Full term**

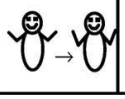
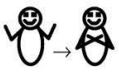
0	0	0	0	99	<1	0	0	1	**25-27w**
0	0	0	0	96	<1	3	0	1	**28-29w**
0	0	0	0	96	<1	2	0	2	**30-31w**
0	0	0	0	94	<1	2	0	4	**32-34w**
0	0	0	0	99	0	<1	0	<1	**Full term**

0	0	43	<1	34	0	21	<1	1	**25-27w**
0	0	41	0	39	<1	19	0	1	**28-29w**
0	0	38	0	36	<1	22	<1	4	**30-31w**
0	0	19	<1	50	<1	29	<1	2	**32-34w**
0	0	4	0	57	0	35	0	1	**Full term**

0	0	25	0	64	0	9	0	2	**25-27w**
0	0	17	0	70	0	13	0	0	**28-29w**
0	0	18	0	76	0	6	0	0	**30-31w**
0	0	23	0	64	0	13	0	0	**32-34w**
0	0	3	0	94	0	3	0	<1	**Full term**

0	0	20	0	39	0	35	0	6	**25-27w**
0	0	31	0	42	0	26	0	1	**28-29w**
0	0	24	0	49	0	26	0	1	**30-31w**
0	0	17	0	51	0	28	0	4	**32-34w**
0	0	24	0	58	0	18	0	<1	**Full term**

REFLEX ITEMS

TENDON REFLEX	absent	felt, not seen	seen	'exaggerated'	clonus
SUCK/GAG	no gag / no suck	weak irregular suck only: no stripping	weak regular suck some stripping	strong suck: (a) irregular (b) regular good stripping	no suck but strong clenching
PALMAR GRASP	no response **R L**	short, weak flexion of fingers **R L**	strong flexion of fingers **R L**	strong finger flexion, shoulder ↑ **R L**	very strong grasp; infant can be lifted off couch **R L**
PLANTAR GRASP	no response **R L**	partial plantar flexion of toes **R L**	toes curve around the examiner's finger **R L**		
PLACING	no response **R L**	dorsi - flexion of ankle onl y **R L**	full placing response with flexion of hip, knee & placing sole on surface **R L**		
MORO REFLEX	no response or opening of hands only	full abduction at shoulder and extension of the arms; no adduction 	full abduction but only delayed or partial adduction 	partial abduction at shoulder and extension of arms followed by smooth adduction 	• no abduction or adduction; • only forward extension of arms from the shoulders • marked adduction only  or

1	.5	2	.5	3	.5	4	.5	5	
0	0	9	0	55	7	13	3	13	**25-27w**
0	0	12	0	50	7	22	4	5	**28-29w**
0	0	24	1	52	1	13	0	9	**30-31w**
0	0	18	0	57	0	17	4	4	**32-34w**
<1	0	21	0	78	0	<1	0	<1	**Full term**

0	0	1	0	3	3	93	0	0	**25-27w**
0	0	3	0	7	0	90	0	0	**28-29w**
0	0	0	0	6	2	92	0	0	**30-31w**
0	0	4	0	10	0	86	0	0	**32-34w**
0	0	1	0	5	0	92	0	2	**Full term**

0	0	5	0	47	7	30	1	10	**25-27w**
0	0	3	1	40	8	43	1	4	**28-29w**
0	0	1	0	51	3	35	0	10	**30-31w**
0	0	7	0	53	3	30	0	7	**32-34w**
<1	0	6	0	84	0	9	0	<1	**Full term**

0	0	4	1	95	0	0	0	0	**25-27w**
0	1	5	2	92	0	0	0	0	**28-29w**
0	0	2	1	97	0	0	0	0	**30-31w**
0	0	2	2	96	0	0	0	0	**32-34w**
<1	0	2	0	98	0	0	0	0	**Full term**

5	2	12	3	78	0	0	0	0	**25-27w**
0	2	12	6	80	0	0	0	0	**28-29w**
1	0	8	8	83	0	0	0	0	**30-31w**
0	0	4	0	96	0	0	0	0	**32-34w**
1	0	18	0	81	0	0	0	0	**Full term**

0	0	13	1	61	4	20	0	1	**25-27w**
0	0	12	1	64	6	15	1	1	**28-29w**
0	0	12	1	51	3	28	0	5	**30-31w**
0	0	23	0	46	2	27	0	2	**32-34w**
0	0	1	0	20	0	79	0	0	**Full term**

MOVEMENTS AND ABNORMAL SIGNS

a

SPONTANEOUS MOVEMENT (quantity)	no movement	sporadic and short isolated movements	frequent isolated movements	frequent generalized movements	continuous exaggerated movements
SPONTANEOUS MOVEMENT (quality)	only stretches	stretches and random abrupt movements Some smooth movements	fluent movements but mono-tonous	fluent alternating movements of arms + legs; good variability	cramped synchr-onous mouthing jerky or other abnormal movement
HEAD RAISING	no movement	infant rolls head over, chin not raised	infant raises chin, rolls head over	infant brings head and chin up	infant brings head up and keeps it up

1	.5	2	.5	3	.5	4	.5	5	
0	0	15	3	28	3	51	0	0	25-27w
0	0	17	3	26	11	43	0	0	28-29w
0	0	13	0	31	8	48	0	0	30-31w
0	0	20	0	27	0	51	0	2	32-34w
<1	0	3	0	5	0	92	0	<1	Full term

0	0	16	4	42	11	23	1	3	25-27w
0	0	22	5	35	1	23	2	2	28-29w
0	0	20	6	34	2	36	0	2	30-31w
0	0	21	0	15	0	60	0	4	32-34w
2	0	5	0	<1	0	93	0	<1	Full term

0	0	36	6	34	6	14	1	3	25-27w
1	1	35	4	34	9	14	1	1	28-29w
1	1	40	5	28	1	21	1	2	30-31w
0	0	40	0	30	4	22	2	2	32-34w
<1	0	10	0	50	0	40	0	<1	Full term

b

ABN. HAND OR TOE POSTURES		hands open, toes straight most of the time	intermittent fisting or thumb adduction	continuous fisting or thumb adduction; index finger flexion, thumb opposition	continuous big toe extension or flexion of all toes
TREMOR		no trem or or trem or only when cry ing	tremor only after Moro or occasionally when awake	frequent tremors when awake	continuous tremors
STARTLE	no startle even to sudden noise	no spontan -eous startle but reacts to sudden noise	2-3 spontaneous startles	more than 3 spontaneous startles	continuous startles

1	.5	2	.5	3	.5	4	.5	5	
0	0	57	4	37	0	2	0	0	25-27w
0	0	64	6	28	0	2	0	0	28-29w
0	0	67	1	30	1	1	0	0	30-31w
0	0	75	2	21	0	2	0	0	32-34w
0	0	85	0	12	0	3	0	<1	Full term

0	0	43	1	29	8	16	0	3	25-27w
0	0	43	0	27	9	19	2	0	28-29w
0	0	54	0	24	3	19	0	0	30-31w
0	0	62	0	30	0	4	0	4	32-34w
0	0	88	0	12	0	<1	0	<1	Full term

22	0	40	7	20	1	10	0	0	25-27w
23	1	35	7	30	2	2	0	0	28-29w
37	1	32	1	25	1	3	0	0	30-31w
50	0	35	0	9	0	6	0	0	32-34w
<1	0	94	0	6	0	<1	0	<1	Full term

BEHAVIORAL SIGNS, VISION, HEARING

EYE APPEARANCE	**does not open eyes**		**full conjugated eye mov**	**transient nystagmus strabismus roving eye movemens sunsetting sign**	**persistent nystagmus strabismus roving eye movements dow nward deviation**
AUDITORY ORIENTATION	**no reaction**	**auditory startle; Brightens and stills; no true orientati - on**	**shifting of eyes, head might turn towards source**	**prolonged head turn to stimulus; search with eyes; smooth**	turns head and eyes towards noise every time; jerky abrupt
VISUAL ORIENTATION	**does not follow or focus on stimuli**	**stills, focuses follows briefly to the side but loses stimuli**	**follows horizontal -ly and vertically; no head turn**	**follows horizon -tally and vertically; turns head**	follows in a circle
ALERTNESS	**will not respond to stimuli**	**when awake, looks only briefly**	**when awake, looks at stimuli but loses them**	**keeps interest in stimuli**	does not tire (hyper-reactive)
IRRITABILITY	**quiet all the time, not irritable to any stimuli**	**awakes, cries some -times when handled**	**cries often when handled**	**cries always when handled**	cries even when not handled
CONSOLABILITY	**not crying consoling not needed**	**cries briefly; consol -ing not needed**	**cries; becomes quiet when talked to**	**cries; needs picking up to console**	cries cannot be consoled
CRY	**no cry at all**	**whimpe -ring cry only**	**cries to stimuli but normal pitch**		high pitched cry; often continuous

1	**.5**	**2**	**.5**	**3**	**.5**	**4**	**.5**	**5**	
6	0	0	0	74	4	16	0	0	25-27w
2	0	0	0	80	2	15	1	0	28-29w
5	0	0	0	80	2	13	0	0	30-31w
4	0	0	0	87	2	7	0	0	32-34w
7	0	0	0	92	0	1	0	<1	Full term

5	1	28	0	57	1	8	0	0	25-27w
2	0	23	10	50	6	9	0	0	28-29w
5	1	27	7	51	1	8	0	0	30-31w
3	0	14	0	73	3	7	0	0	32-34w
<1	0	30	0	50	0	20	0	<1	Full term

6	0	7	2	25	3	26	9	22	25-27w
0	0	7	1	33	7	21	15	16	28-29w
1	0	9	0	27	5	25	10	23	30-31w
0	0	10	0	42	10	38	0	0	32-34w
<1	0	7	0	41	0	51	0	1	Full term

6	0	22	1	48	3	20	0	0	25-27w
1	0	17	4	60	3	14	1	0	28-29w
0	0	21	1	43	2	33	0	0	30-31w
0	0	7	3	54	0	36	0	0	32-34w
1	0	2	0	48	0	49	0	<1	Full term

12	1	52	0	31	0	3	0	1	25-27w
16	2	47	2	27	1	5	0	0	28-29w
27	0	47	1	22	0	2	0	1	30-31w
23	0	49	0	23	0	5	0	0	32-34w
<1	0	93	0	5	0	2	0	<1	Full term

10	0	29	0	29	3	29	0	0	25-27w
17	1	19	2	29	7	22	1	2	28-29w
27	0	18	0	28	2	22	1	2	30-31w
23	0	9	0	32	2	28	0	6	32-34w
1	0	41	0	45	0	12	0	<1	Full term

11	0	11	0	78	0	0	0	0	25-27w
16	0	5	2	77	0	0	0	0	28-29w
26	1	3	1	69	0	0	0	0	30-31w
23	0	6	2	69	0	0	0	0	32-34w
<1	0	7	0	92	0	0	0	1	Full z

PROPOSED VITD CUTOFFS FOR VitDD, SUFFICIENCY AND INSUFFICIENCY:

According to the **Indian Academy of Paediatrics Revised (2021) Guidelines on Prevention and Treatment of VitDD and Rickets [103],**

VitD Deficiency	<12nanogram/millilitre
VitD Insufficiency	12-20nanogram/millilitre
VitD Sufficiency	>20nanagram/millilitre

CRITERIA FOR DISCHARGE FROM THE HOSPITAL:

- Baby maintaining euthermia in the cradle
- Mother confident enough to give KMC
- Direct breastfeeding well established or the mother confident enough to express and giving breast milk by paladai.
- Baby gaining weight >20gm/kg/day for 3 consecutive days or regained birth weight.
- Follow-up appointment at a high-risk clinic given

RESULTS

Table 1: Descriptive analysis of VitD Levels in the study group of preterm neonates (N=70):

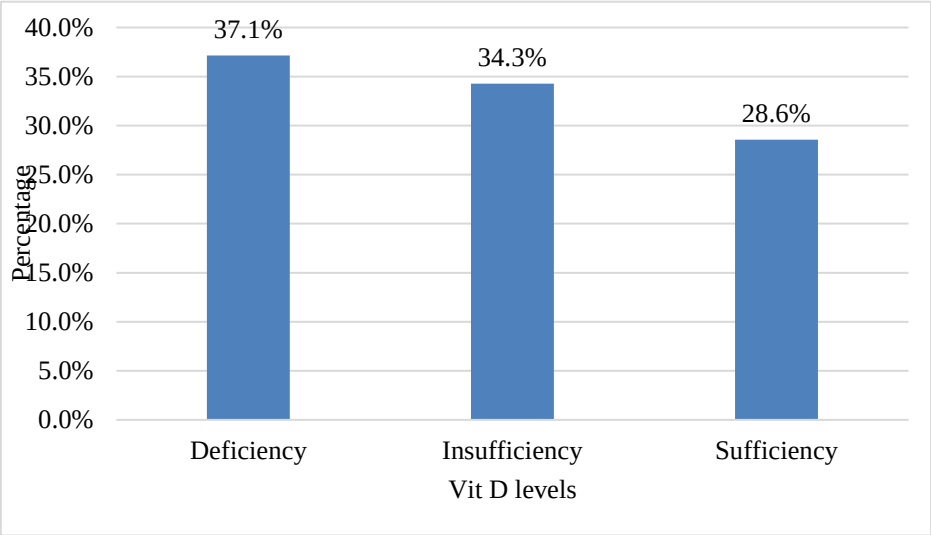
Parameters	Mean ± Standard deviation	Median	Minimum	Maximum
VitD Levels in ng/ml	20.27 ± 22.49	14.0	3.0	120.0

This descriptive analysis gives a comprehensive overview of the distribution of VitD levels in the preterm neonates. The mean value indicates the average level of VitD, the median represents the central value, indicating that half of the neonates has VitD levels below 14.0 ng/ml, and the other half has levels above. The minimum and maximum values provide insights into the range of VitD levels observed in the population, with a considerable variability from 3.0 nanogram/ml to 120.0 nanogram/ml.

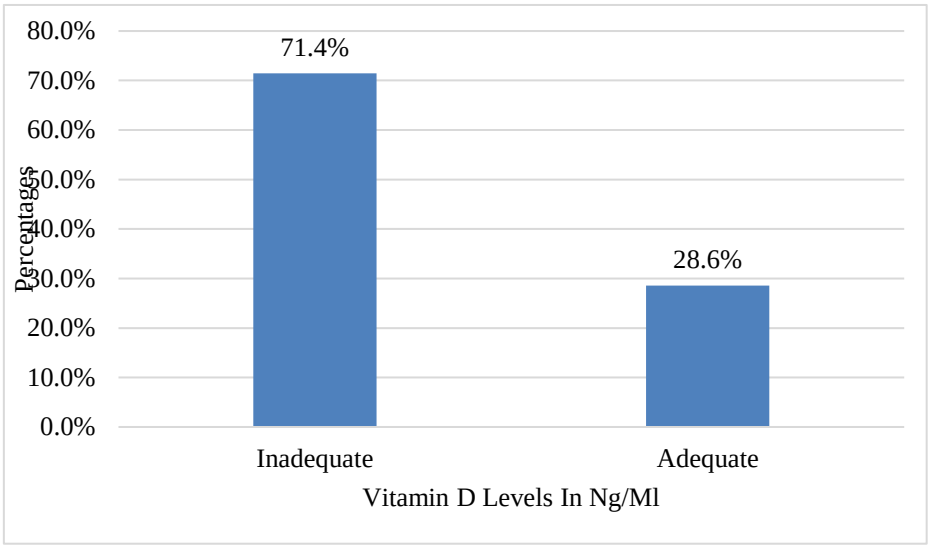
Table 2: Descriptive analysis of VitD levels in ng/ml and deficiency in the preterm neonates (N=70):

VitD	Frequency	Percentages
VitD Levels in ng/ml		
Deficiency	26	37.14%
Insufficiency	24	34.29%
Sufficiency	20	28.57%
VitDD		
Positive	50	71.43%
Negative	20	28.57%

Graph 1: Bar chart of VitD levels in ng/ml in the preterm neonates (N=70):



Graph 2: Bar chart of VitDD in the study group of preterm neonates (N=70):



The chart provides a descriptive analysis of VitD levels and deficiency status in preterm neonates. A significant proportion of preterm neonates, comprising 37.14%, exhibited VitDD (<12 ng/ml), while 34.29% had insufficient VitD levels (≤20 nanogram/ml) and 28.57% showed sufficiency (>20 nanogram/ml). A majority of the preterm neonates, accounting for 71.43%, tested positive for deficiency or insufficiency, while 28.57% tested negative, indicating sufficient VitD levels.

Table 3: Descriptive Analysis of Mother's age group (N=70):

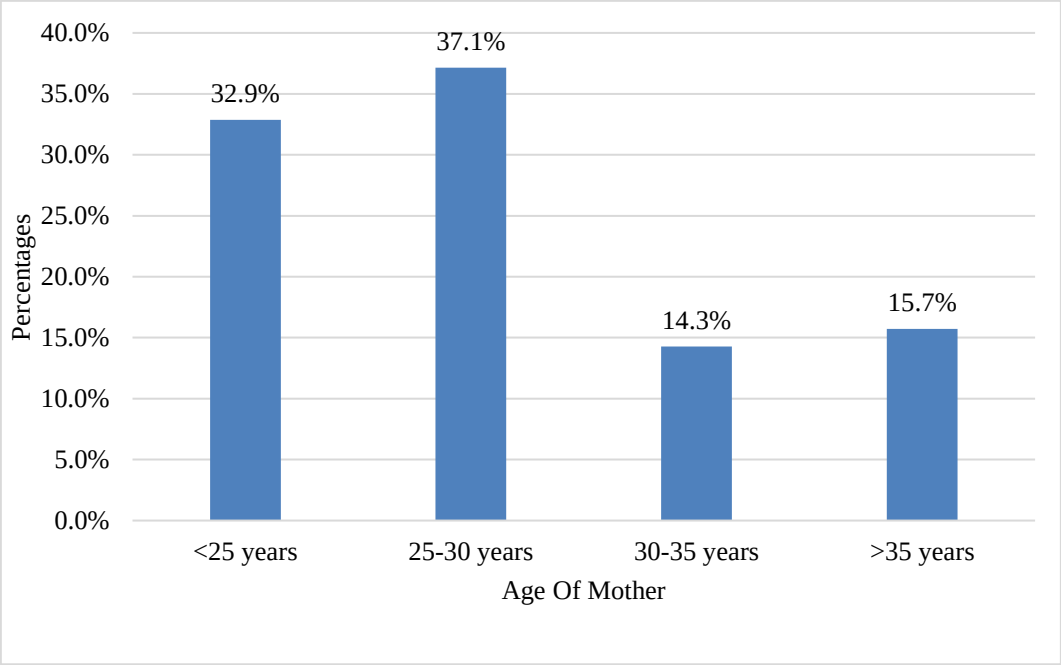
Parameter	Mean±SD	Median	Minimum	Maximum	95% C.I.	
					Lower	Upper
Age Of Mother	28.97 ± 6.3	28.0	20.0	48.0	27.5	30.5

The data show variability in the age distribution of mothers, with representation across different age ranges. The age distribution of the mothers was analysed. The mean mothers age was 28.97 years with a SD of 6.3 years, median age was 28.0 years, with ages ranging from 20.0 years to 48.0 years. The 95% confidence interval for the mean age was calculated to be between 27.5 and 30.5.

Table 4: Detailed Analysis of the mother's age group (N=70):

Age of Mother	Frequency	Percentages
<25 years	23	32.86%
25-30 years	26	37.14%
30-35 years	10	14.29%
>35 years	11	15.7%

Graph 3: Bar chart of mother's age group (N=70):

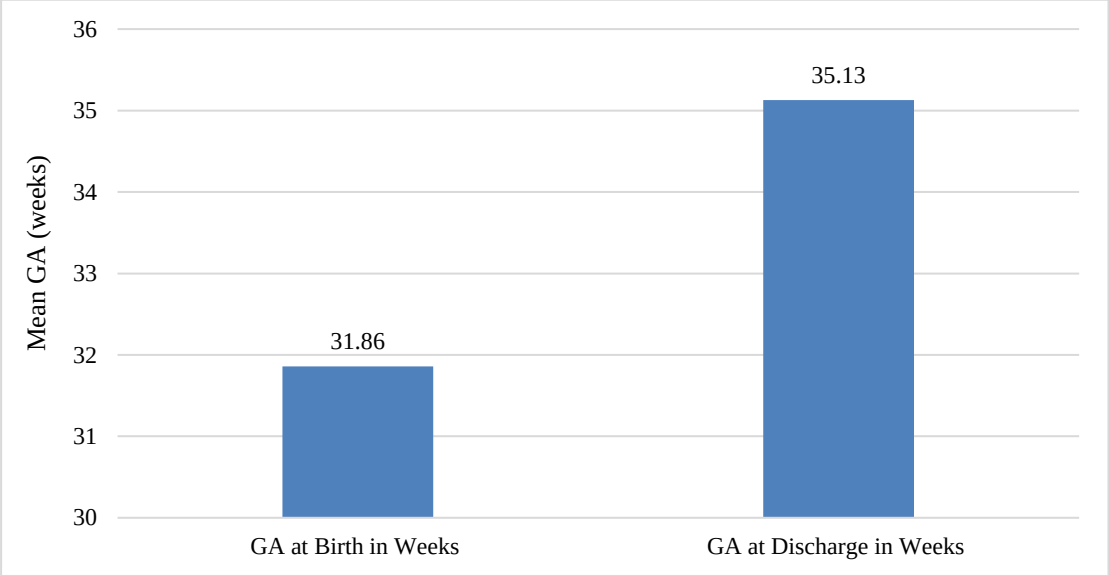


32.86% of mothers are below the age of 25. 37.14% of the mothers are between the age group of 25-30.14.29 % of the mothers are between the age group of 30-35.

Table 5: Descriptive Analysis of GA at birth and discharge (N=70):

	Mean ± SD.	Median.	Minimum.	Maximum.	95% C.I	
					Lower	Upper
GA at Birth in Weeks	31.86 ± 1.58	32.0	27.9	34.3	31.5	32.2
GA at Discharge in Weeks (N=67)	35.13 ± 1.27	35.3	31.4	37.7	34.8	35.4

Graph 4: Mean bar chart of GA at birth and discharge (N=70)

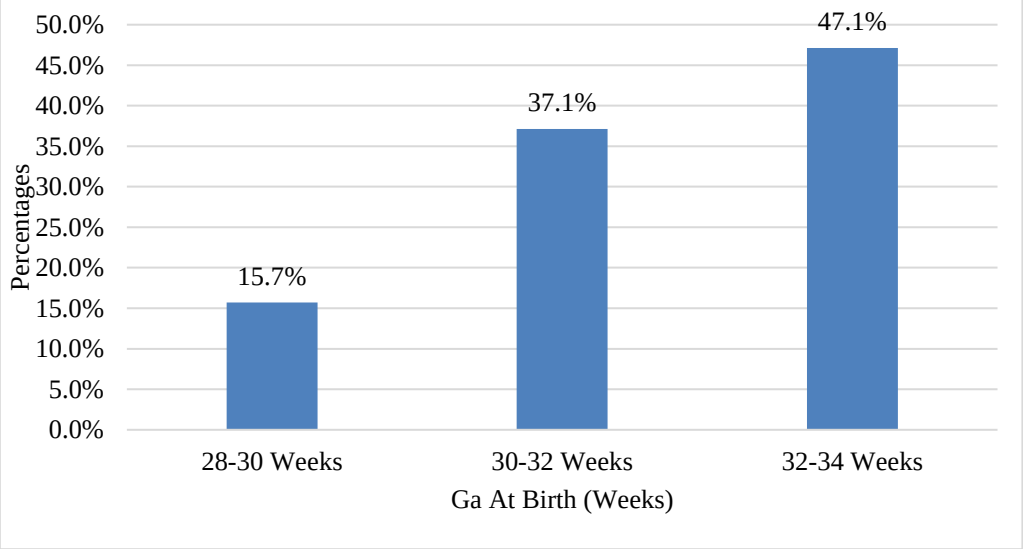


- The mean GA of the preterm neonates at birth was approximately 31.86 weeks, with a SD of 1.58 weeks. The median gestational age at birth is 32.0 weeks, indicating that half of the neonates were born before this gestational age and half were born after.
- Gestational ages at birth range from 27.9 weeks to 34.3 weeks
- The mean gestational age at discharge was approximately 35.13 weeks, with a SD of 1.27 weeks. The median gestational age at discharge is 35.3 weeks.
- Gestational ages at discharge range from 31.4 weeks to 37.7 weeks.

Table 6: Detailed analysis of GA of the preterm neonates (N=70)

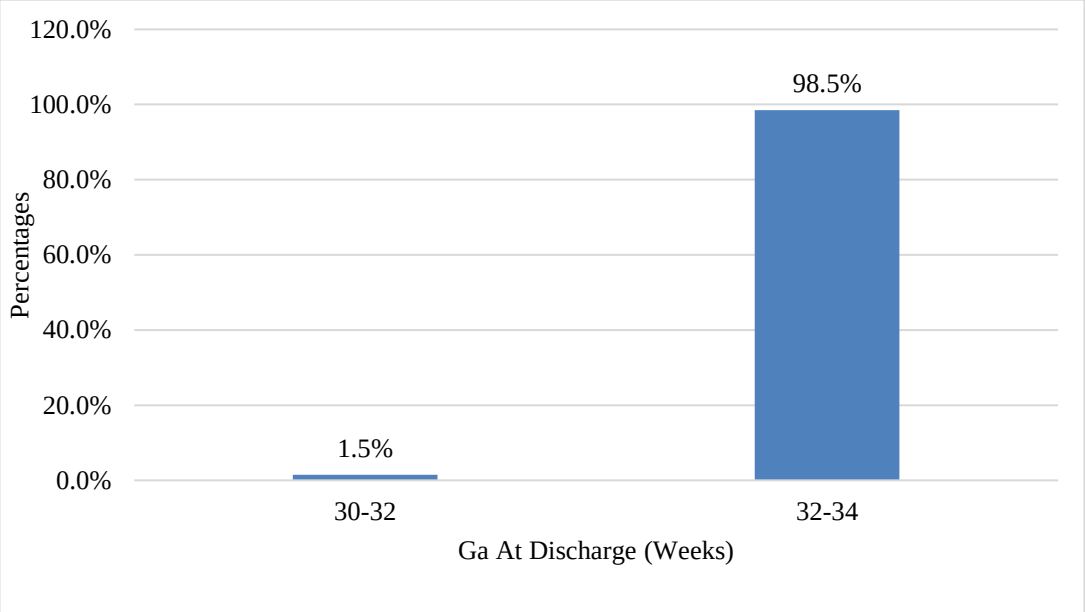
Parameter	Frequency	Percentages
GA At Birth (Weeks)		
28-30 Weeks	11	15.71%
30-32 Weeks	26	37.14%
32-34 Weeks	33	47.14%
GA At Discharge (Weeks) (N=67)		
30-32 Weeks	1	1.49%
32-34 Weeks	66	98.51%

Graph 5: Bar chart of GA at birth (weeks) in the study group of preterm neonates (N=70)



15.71% of the neonates were between 28 and 30 weeks of gestation at birth. This represents a smaller proportion of the sample. The majority of preterm neonates, comprising 37.14%, were between 30- and 32-weeks GA at birth. This age range represents a significant portion of the study group. 47.14% of preterm neonates were between 32-and 34weeks GA at birth. This age range also represents a substantial proportion of the sample.

Graph 6: Bar chart of Preterm neonate's GA at discharge (weeks) (N=67)



Only 1.49% of the neonates were discharged between 30-and 32-wks of gestation. This age range represents a very small proportion of the discharged neonates. The vast majority, comprising 98.51%, of the neonates were discharged between 32-and 34-wks of gestation. This age range represents the predominant group of discharged neonates.

Table 7: Comparison of mean GA of preterm neonates across VitD levels in ng/ml (N=70)

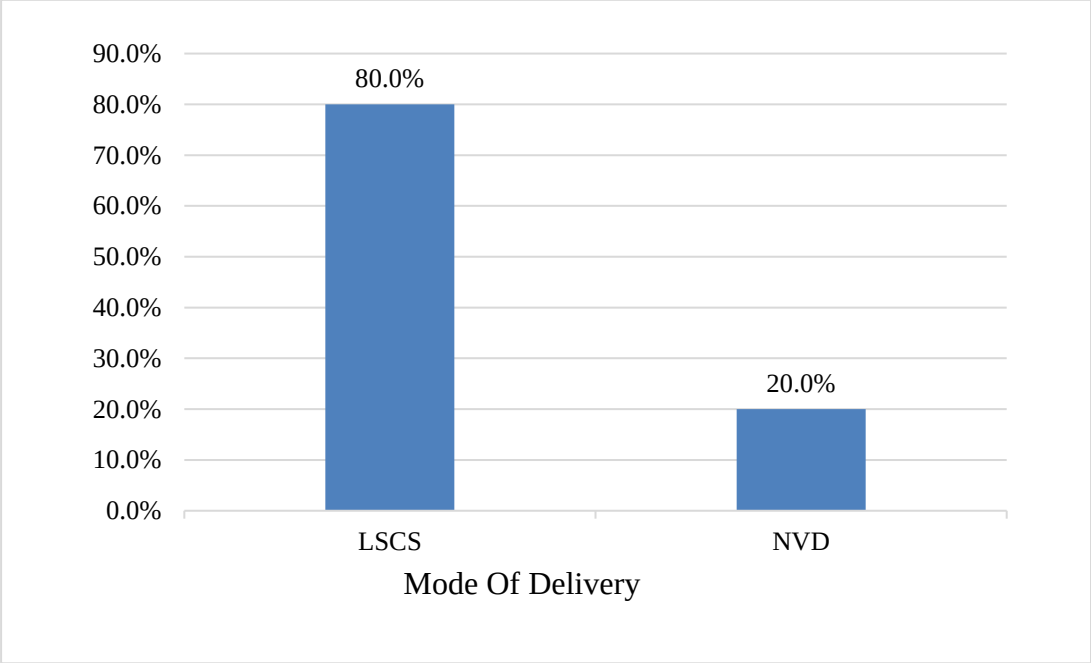
Parameter	VitD Levels in ng/ml			ANOVA P Value
	Deficiency (N=26)	**Insufficiency (N=24)**	**Sufficiency (N=20)**	
GA At Birth in Weeks	31.79 ± 1.6	32.01 ± 1.49	31.79 ± 1.72	0.864
GA At Discharge in Weeks (N=67)	35.07 ± 1.21	35.02 ± 1.45	35.35 ± 1.13	0.662
	Inadequate (N=50)		**Adequate (N=20)**	**Independent Sample t-test P value**
GA At Birth in Weeks	31.89 ± 1.53		31.79 ± 1.72	0.797
GA At Discharge in Weeks (N=67)	35.04 ± 1.32		35.35 ± 1.13	0.367

The provided data compares the mean GA at birth and at discharge across various levels of VitD in ng/ml using ANOVA and independent sample t-tests. GA at birth across VitD levels were 31.79 ±1.6, 32.01±1.49,31.79±1.72 for VitDD, VitD insufficiency, and VitD sufficiency respectively. GA at discharge were 35.07±1.21, 35.02±1.45, and 35.35±1.13 for VitDD, VitD insufficiency, and VitD sufficiency respectively. "The p value (0.864) indicates no significant correlation in the mean GA at birth across the three VitD level groups. The p value (0.662) suggests no significant correlation in the mean GA at discharge across the three VitD level groups."

Table 8: Descriptive Analysis of the mode of delivery of the preterm neonates (N=70)

Mode Of Delivery	Frequency	Percentages
LSCS	56	80.00 %
NVD	14	20.00 %

Graph 7: Bar Chart of the mode of delivery of tbe preterm neonates (N=70)

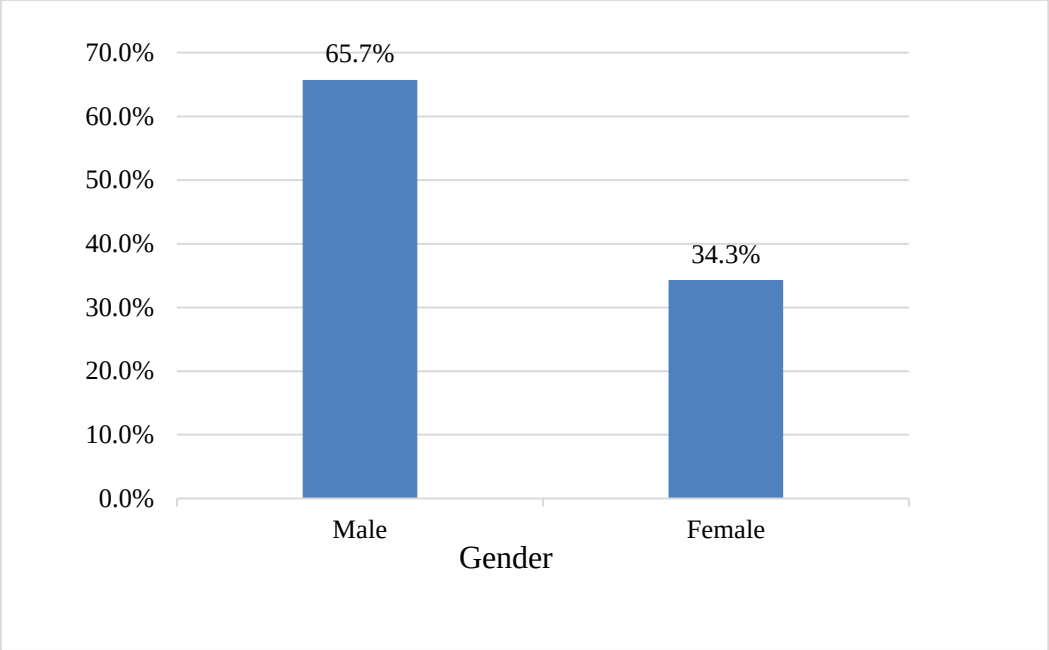


The majority of preterm neonates, accounting for 80.00%, were delivered via cesarean section. This high percentage suggests a tendency toward cesarean delivery in our study group. A minority of preterm neonates, comprising 20.00%, were delivered vaginally.

Table-9: Descriptive Analysis of gender difference of the preterm neonates (N=70)

Parameter	Frequency	Percentages
Male	46	65.71%
Female	24	34.29%

Graph-8: Bar chart of gender in the study group of preterm neonates (N=70)

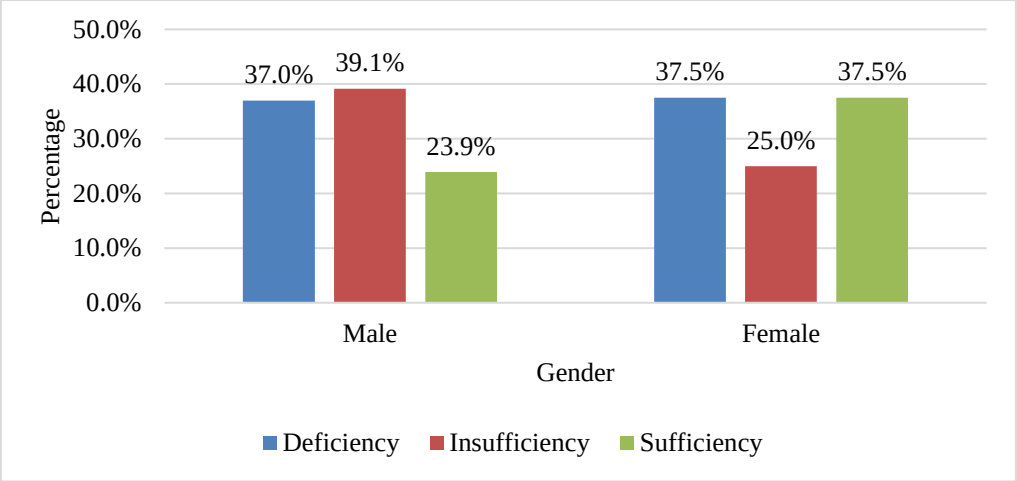


This descriptive analysis highlights the gender distribution within the study group. The majority of the subjects were male, with a frequency of 46, accounting for 65.71% of the total population. Female subjects comprised 24 of the study participants, representing 34.29% of the total population

Table 10: Comparison between VitD levels in ng/ml and gender (N=70)

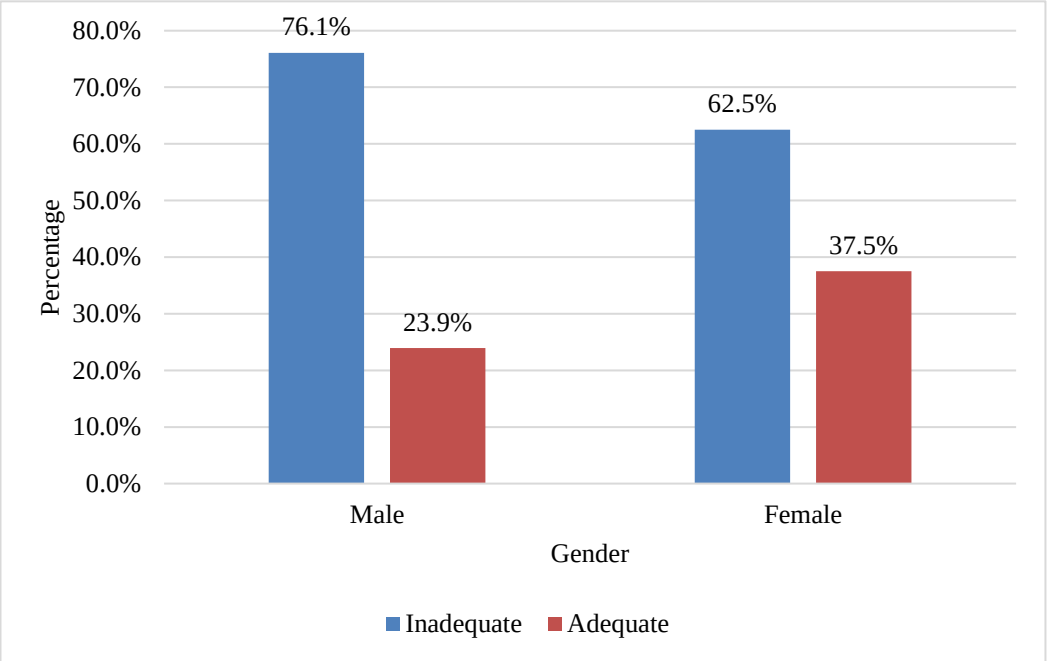
VitD Levels in ng/ml	Gender		Chi-square	P value
	Male (N=46)	Female (N=24)		
Deficiency	17 (36.96%)	9 (37.5%)	1.939	0.379
Insufficiency	18 (39.13%)	6 (25%)		
Sufficiency	11 (23.91%)	9 (37.5%)		
Inadequate	35 (76.09%)	15 (62.5%)	1.427	0.232
Adequate	11 (23.91%)	9 (37.5%)		

Graph-**9: Cluster bar chart of comparison between VitD levels in ng/ml with the gender of preterm neonates (N=70)**



The study assessed VitD levels in 70 participants, divided into 46 males and 24 females. The levels were classified as deficient, insufficient, or sufficient. Among males, 36.96% had a deficiency, 39.13% had insufficiency, and 23.91% had sufficient levels. For females, 37.5% had a deficiency, 25% had insufficiency, and 37.5% had sufficient levels. The chi-square analysis revealed no significant correlation between gender and VitD status ($\chi^2 = 1.939$, $p = 0.379$)

Graph 10: Cluster bar chart of comparison of VitD levels in ng/ml with gender (N=70)



Combining deficiency and insufficiency into an "inadequate" category, 76.09% of males and 62.5% of females had inadequate levels, while 23.91% of males and 37.5% of females had adequate levels. This comparison also showed no significant gender association ($\chi^2 = 1.427$, $p = 0.232$).

Table 11: Descriptive analysis of birth and discharge weight in the study group of preterm neonates (N=70)

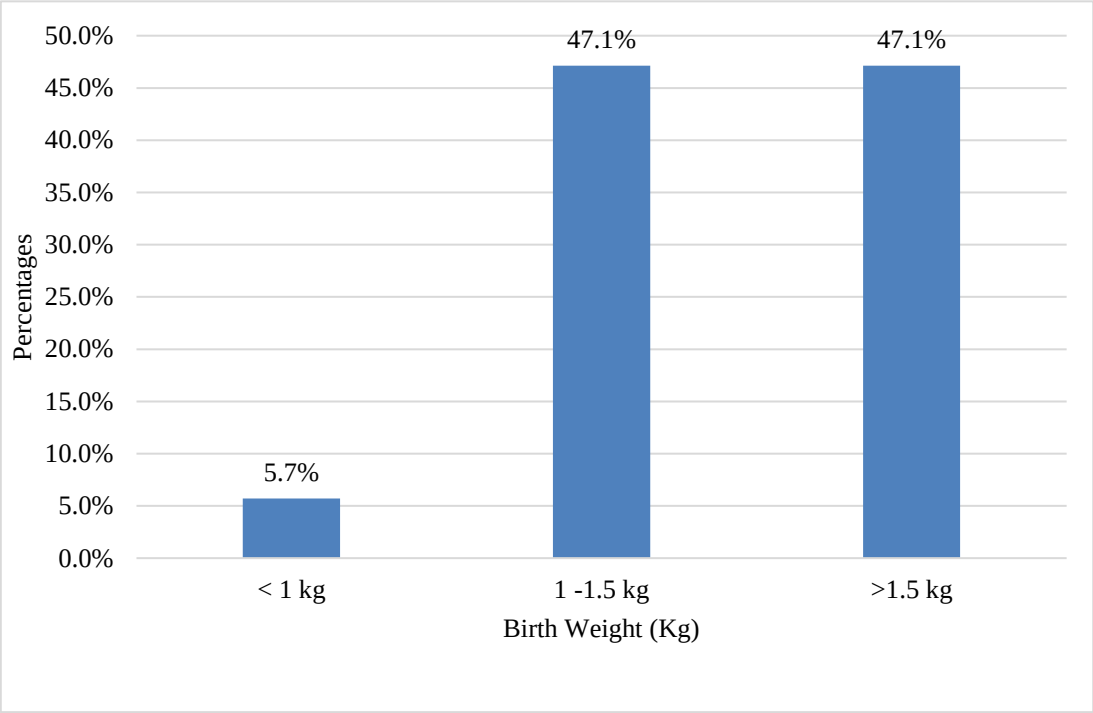
	Mean ± Standard deviation.	**Median.**	**Minimum.**	**Maximum.**	**95% C.I**	
					Lower	**Upper**
Birth Weight in Grams (N=70)	1540.17 ± 383.01	1500.0	725.0	2830.0	1448.9	1631.5
Discharge Weight in grams (N=67)	1568.22 ± 340.08	1560.0	931.0	2850.0	1485.3	1651.2

- The mean birth weight of the neonates is 1540.17 grams, with a standard deviation of 383.01 grams. The median birth weight is 1500.0 grams, suggesting that half of the neonates weighed ≤ 1500 g at birth, and the other half weighed more. The 95% CI for the average birth weight is between 1448.9 and 1631.5 grams, indicating that the true mean birth weight of the population likely falls within this range.
- The mean discharge weight of the neonates is 1568.22 grams, with a standard deviation of 340.08 grams, indicating variability similar to the birth weights. The median discharge weight is 1560.0 grams, suggesting that half of the neonates weighed less than or equal to 1560 grams at discharge, and the other half weighed more. The discharge weights ranged from a least value of 931.0 grams to a highest value of 2850.0 grams. The 95% CI for the mean discharge weight is between 1485.3 and 1651.2 grams, indicating that the true mean discharge weight of the population likely falls within this range.

Table-12: Descriptive Analysis of birth weight (kg) of the preterm neonates (N=70)

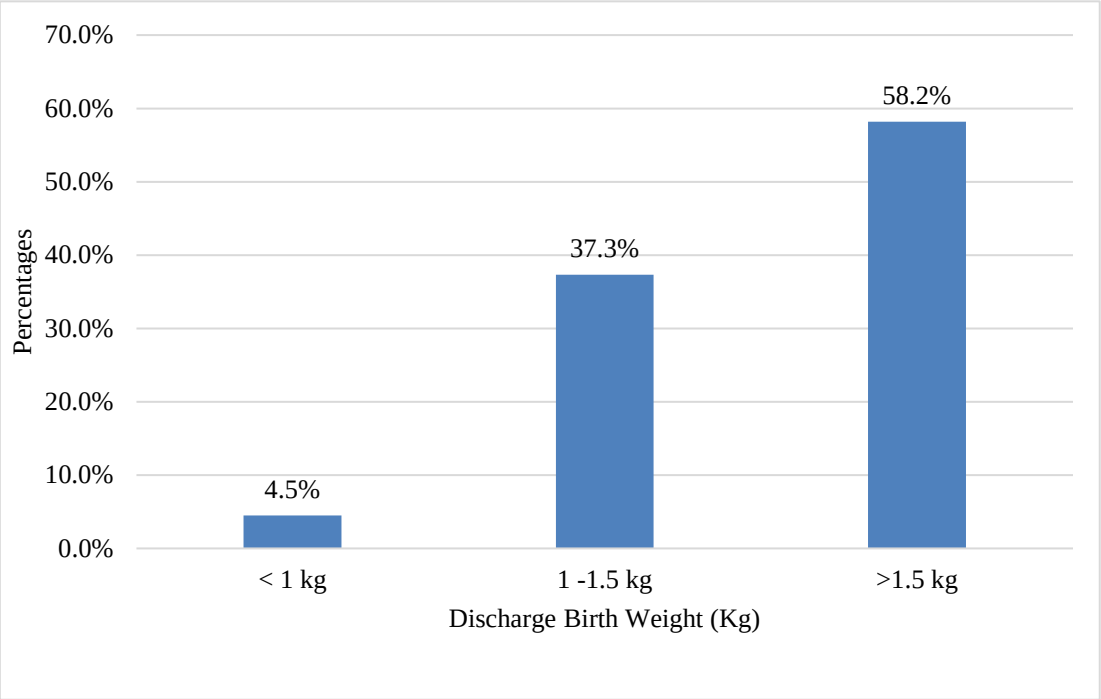
Parameter	Frequency	Percentages
Birth Weight (Kg)		
< 1 kg	4	5.71%
1 -1.5 kg	33	47.14%
>1.5 kg	33	47.14%
Discharge Birth Weight (Kg)		
< 1 kg	3	4.48%
1 -1.5 kg	25	37.31%
>1.5 kg	39	58.21%

Graph 11: Bar chart of birth weight (kg) in the study group of preterm neonates (N=70)



This indicates that 4 out of the 70 preterm neonates had a birth weight of less than 1 kg, constituting 5.71% of the study group of preterm neonates. 33 preterm neonates had a birth weight between 1 and 1.5 kg, making up 47.14% of the population. Similarly, another 33 neonates had a birth weight of more than 1.5 kg, also representing 47.14% of the study group of preterm neonates.

Graph 12: Bar chart of discharge birth weight (kg) in the study group of preterm neonates (N=67)



This indicates that 3 preterm neonates had a discharge weight of less than 1 kg, constituting 4.48% of the 67 neonates for whom discharge weight data is available. 25 preterm neonates had a discharge weight between 1 and 1.5 kg, making up 37.31% of the population. The majority, 39 neonates, had a discharge weight of more than 1.5 kg, representing 58.21% of the study group of preterm neonates.

Table 13: Comparison of mean Birth Weight across VitD levels in ng/ml (N=70)

Parameter	VitD Levels in ng/ml			ANOVA P Value
	Deficiency (N=26)	**Insufficiency (N=24)**	**Sufficiency (N=20)**	
Birth Weight in Grams	1611.81 ± 450.31	1516.67 ± 271.83	1475.25 ± 405.37	0.461
Discharge Weight (N=67)	1619.74 ± 441.6	1503.21 ± 227.93	1587 ± 322.69	0.487
	Inadequate (N=50)		**Adequate (N=20)**	**Independent Sample t-test P value**
Birth Weight in Grams	1566.14 ± 374.76		1475.25 ± 405.37	0.374
Discharge Weight Grams (N=67)	1560.23 ± 350.3		1587 ± 322.69	0.771

This table provides a comparison of mean birth weight across different levels of VitD and between inadequate and adequate VitD levels.

- The ANOVA test compares the means of birth weight across three levels of VitD: The p-value obtained (0.461) suggests that there is no significant correlation in birth weight among these three groups.
- Similarly, for discharge weight, the p-value obtained (0.487) also suggests no significant association across the three levels of VitD. For birth weight, the p-value obtained (0.374) suggests that there is no significant association in birth weight among those with inadequate and adequate levels of VitD. For discharge weight, the p-value obtained (0.771) also indicates no significant association between the 2 groups. There is no correlation found between VitD levels and birth weight or discharge weight.

Table-14: Descriptive Analysis of APGAR score of the preterm neonates at birth (N=70)

	Mean ± SD	Median	Minimum	Maximum	95% C.I	
					Lower	Upper
Apgar at 1 Min	6.27 ± 1.82	7.0	1.0	9.0	5.8	6.7
Apgar at 5 Min	8.16 ± 1.22	8.0	5.0	10.0	7.9	8.5

This descriptive analysis provides insights into the "APGAR scores at 1 minute and 5 minutes after birth in the study group of preterm neonates."

The average "APGAR score at 1 minute" is approximately 6.27, with a standard deviation of 1.82. APGAR scores at 1 minute range from least value of 1.0 to a maximum value of 9.0. The 95% confidence interval indicates that the true population mean falls between 5.8 and 6.7

The average "APGAR score at 5 minutes" in the study group of preterm neonates of preterm neonates is approximately 8.16, with a standard deviation of 1.22. APGAR scores at 5 minutes range from a "minimum of 5.0 to a maximum of 10.0". The 95% confidence interval suggests that the true population mean of APGAR score at 05 minutes falls between 7.9 and 8.5

Table 15: Comparison of mean APGAR score across VitD levels in nanograms/ml (N=70)

Parameter	"VitD Levels in ng/ml"			ANOVA P Value
	Deficiency (N=26)	Insufficiency (N=24)	Sufficiency (N=20)	
Apgar At 1 Min	5.92 ± 2.19	6.71 ± 1.33	6.2 ± 1.77	0.310
Apgar At 5 Min	7.81 ± 1.39	8.42 ± 1.06	8.3 ± 1.13	0.177
	Inadequate (N=50)		Adequate (N=20)	Independent Sample t-test P value
Apgar At 1 Min	6.3 ± 1.85		6.2 ± 1.77	0.837
Apgar At 5 Min	8.1 ± 1.27		8.3 ± 1.13	0.541

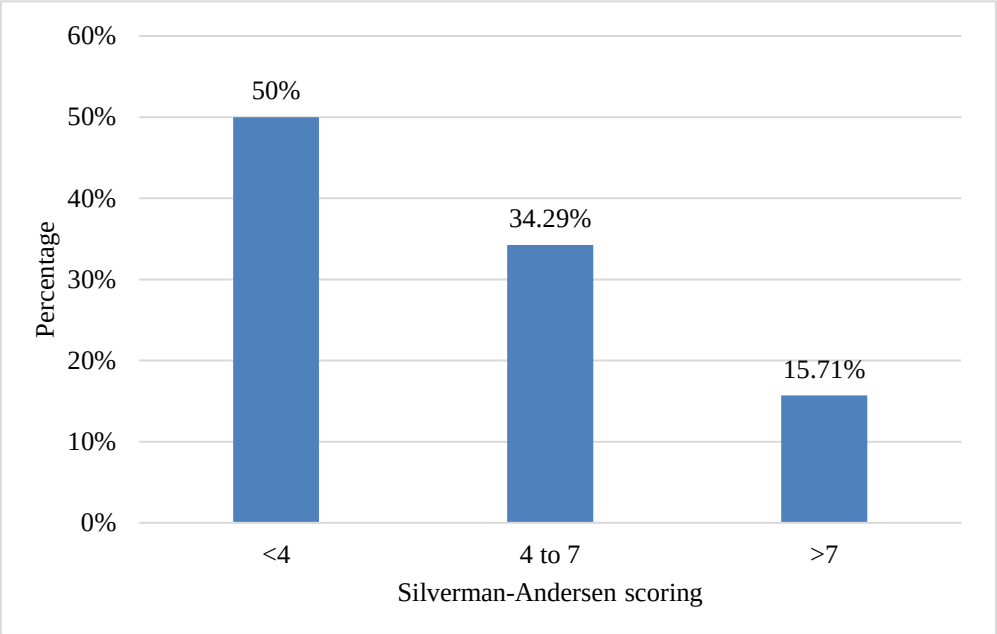
The study analysed the relationship between VitD levels and "Apgar scores at 1 and 5 minutes" in newborns. VitD levels were categorized as deficient, insufficient, or sufficient, and the differences in Apgar scores were assessed using ANOVA. For the deficiency group, the average "Apgar scores at 1 and 5 minutes" were 5.92 ± 2.19 and 7.81 ± 1.39, respectively. For the insufficiency group, the scores were 6.71 ± 1.33 and 8.42 ± 1.06, and for the sufficiency group, they were 6.2 ± 1.77 and 8.3 ± 1.13. Nil significant differences were noted in "Apgar scores at 1 minute (P = 0.310) or 5 minutes (P = 0.177)" across these categories. Further analysis grouped VitD levels into inadequate (deficiency and insufficiency) and adequate categories. Independent sample t-tests showed nil significant differences in "Apgar scores at 1 minute" (6.3 ± 1.85 for inadequate vs. 6.2 ± 1.77 for adequate, P = 0.837) or 5 minutes (8.1 ± 1.27 for inadequate vs. 8.3 ± 1.13 for adequate, P = 0.541)amongst these 2 groups. These findings suggest "no significant association between VitD levels and Apgar scores in the study group of preterm neonates."

Table-16: Analysis of Silverman Anderson scoring in the study group of preterm neonates (N=70)

Parameters	Mean ± SD	Median	Minimum	Maximum	95% CI	
					Lower	Upper
Silverman Anderson Scoring	3.49 ± 3.28	3.00	0.00	10.00	2.70	4.27

Silverman Andersen score	Frequency	Percentage
<4	35	50%
4-7	24	34.29%
>7	11	15.71%

Graph 13: Bar chart of Silverman Anderson scoring in the study group of preterm neonates (N=70)



The average Silverman-Anderson score in the study group of preterm neonates is approximately 3.49, with a standard deviation of 3.28. The median Silverman-Anderson score is 3.00, indicating the middle value of the score distribution. Silverman-Anderson scores range from "least value of 0.00 to a maximum of 10.00." 95% confidence interval suggests that the true population mean of the Silverman-Anderson score falls between 2.70 and 4.27

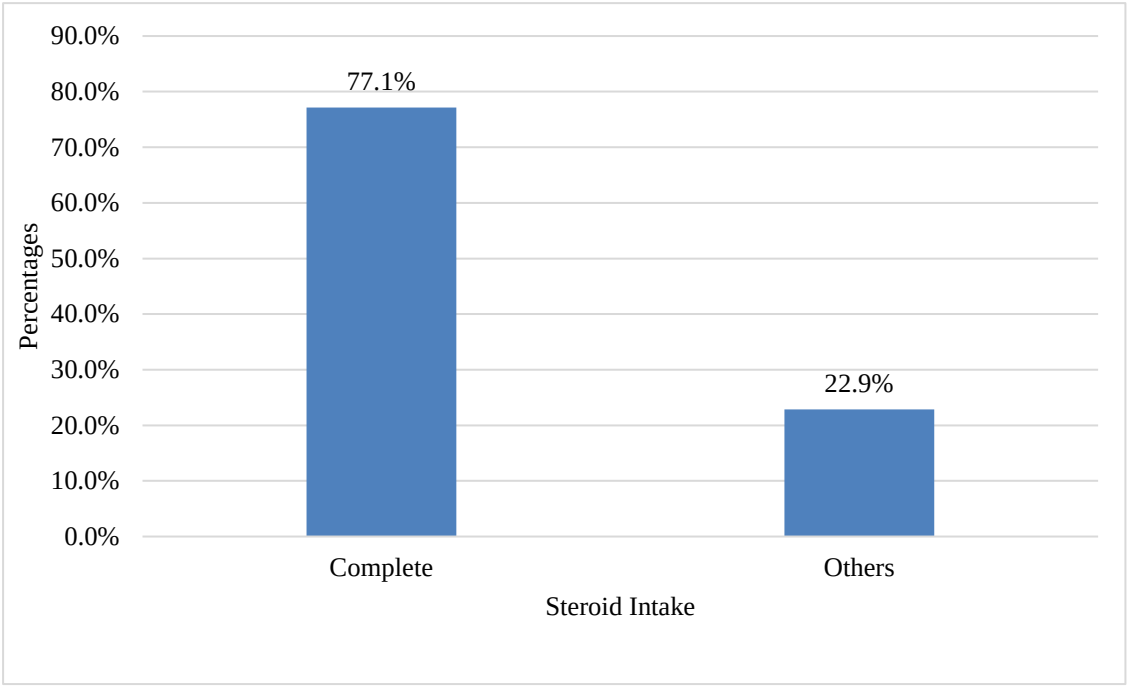
The table provides a descriptive analysis of the Silverman-Anderson scoring in the preterm neonates. "50% of the preterm neonates have Silverman-Anderson scores below 4." A score below 4 generally indicates Mild respiratory distress. 34.29% of the neonates have Silverman-Anderson scores between 4 and 7. Scores in this range suggest moderate respiratory distress. 15.71% of the neonates have Silverman-Anderson scores above 7. Scores above 7 indicate severe respiratory distress.

Table 17: Descriptive analysis of steroid intake in the study group of preterm neonates (N=70)

Steroid Intake	Frequency	Percentages
Complete	54	77.14%
Others	16	22.86%

Others include 13 incomplete and 3 not taken.

Graph 14: Bar chart of steroid intake in the study group of preterm neonates (N=70)

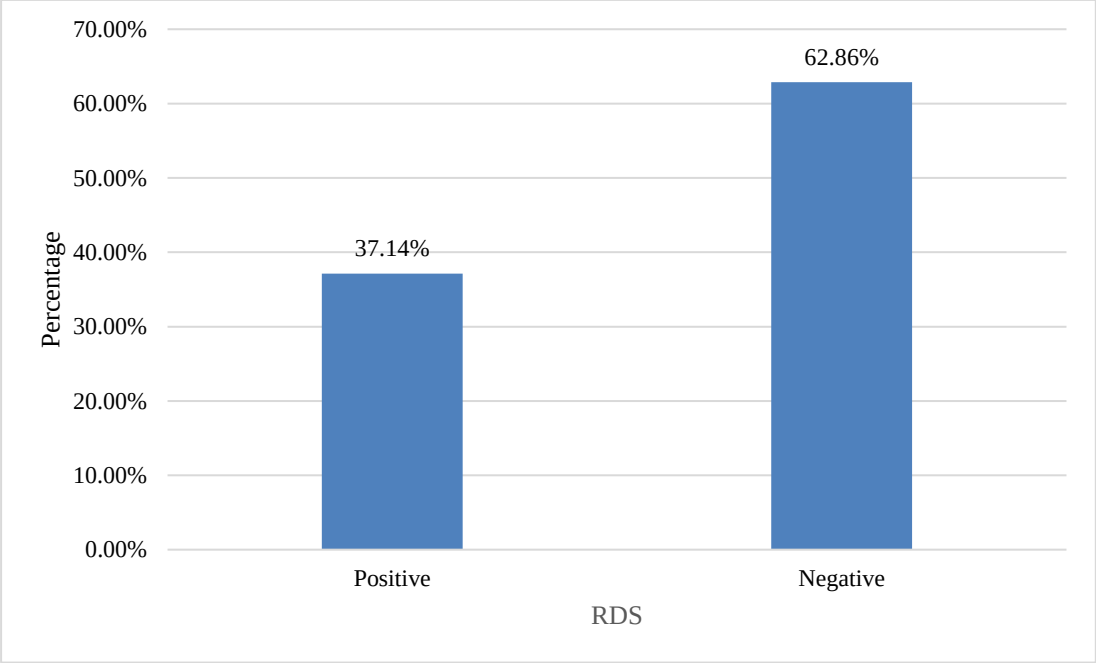


In the study group comprising 70 preterm neonates, 77.14% of the mothers received complete steroid intake, whereas 22.86% contributed to mothers who didn't receive/received an incomplete course of antenatal steroids.

Table 18: Descriptive analysis of RDS in the study group of preterm neonates (N=70)

RDS	Frequency	Percentages
Positive	26	37.14%
Negative	44	62.86%

Graph 15: Bar chart of RDS in the study group of preterm neonates (N=70)

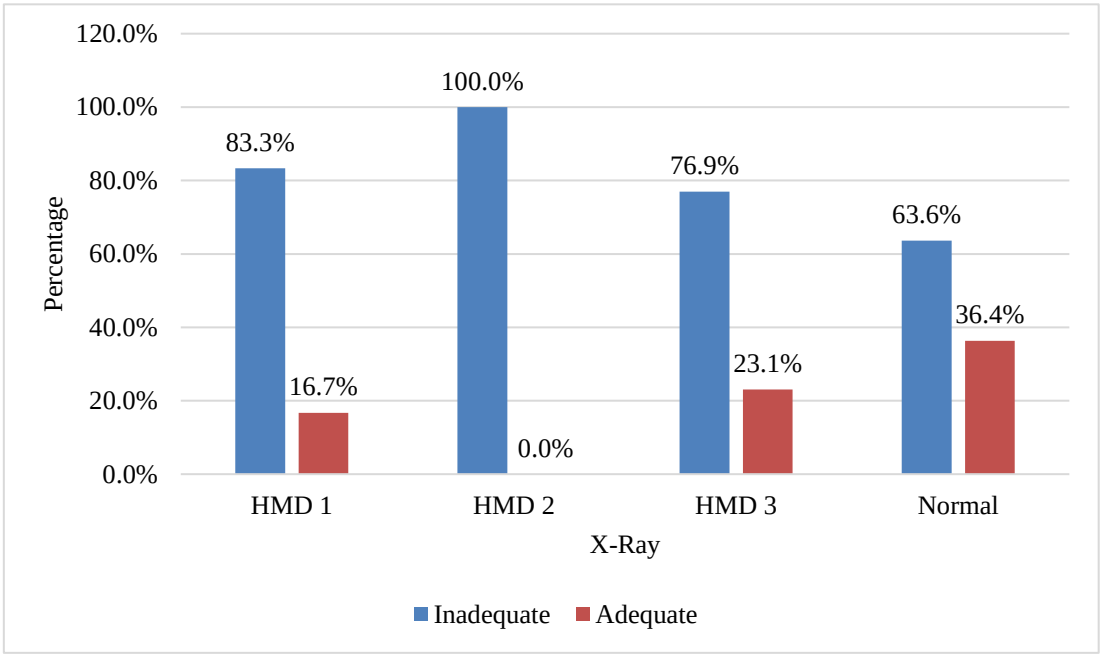


This table and chart provides information regarding distribution of Respiratory distress syndrome (RDS) statuses in population of 70 neonates. 26 out of the 70 individuals were diagnosed as RDS. This group constitutes slightly over one-third (37.14%) of the total population, suggesting a significant presence of the condition within the group. The majority of the participants, 44 individuals, did not have RDS, making up 62.86% of the study group of preterm neonates. This indicates that nearly two-thirds of the population was not affected by RDS.

Table 19: Comparison of Silverman Anderson scoring across RDS (N=70)

Silverman Anderson Scoring	RDS			Kappa Value	Chi-square	P value
	Positive (N=26)	Negative (N=42)	Expired (N=2)			
<4	0 (0%)	35 (83.33%)	0 (0%)	0.479	52.42	<0.001
4-7	15 (57.69%)	7 (16.67%)	2 (100%)			
>7	11 (42.31%)	0 (0%)	0 (0%)			

Graph 16: Cluster bar chart of comparison of Silverman Anderson scoring with RDS (N=70)

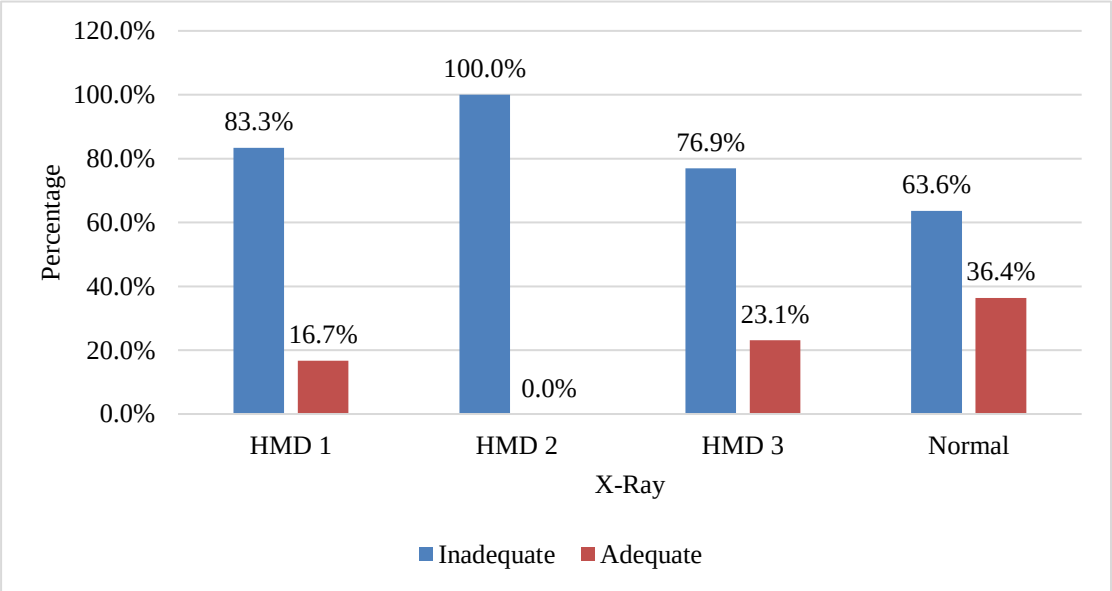


This table and bar chart show the relation between Silverman-Andersen scoring and the presence of RDS.83.33% of the cases without RDS had an SAS of <4. When SAS is >7, all the neonates had RDS. A p value of <0.001 indicates that Silverman Andersen scoring appears to correlate well with the severity of RDS.

Table 20: Comparison of VitD levels in ng/ml across Silverman Anderson scoring (N=70)

VitD Levels In ng/ml	Silverman Anderson scoring			Kappa Value	Chi-square	P value
	<4 (N=35)	4-7 (N=24)	>7 (N=11)			
Deficiency	11 (31.43%)	11 (45.83%)	4 (36.36%)	-0.008	5.302	0.258
Insufficiency	10 (28.57%)	10 (41.67%)	4 (36.36%)			
Sufficiency	14 (40%)	3 (12.5%)	3 (27.27%)			
Inadequate	21 (60%)	21 (87.5%)	8 (72.73%)	-0.206	5.872	0.071
Adequate	14 (40%)	3 (12.5%)	3 (27.27%)			

Graph 17: Cluster bar chart of comparison of VitD levels in ng/ml across Silverman Anderson scoring (N=70)

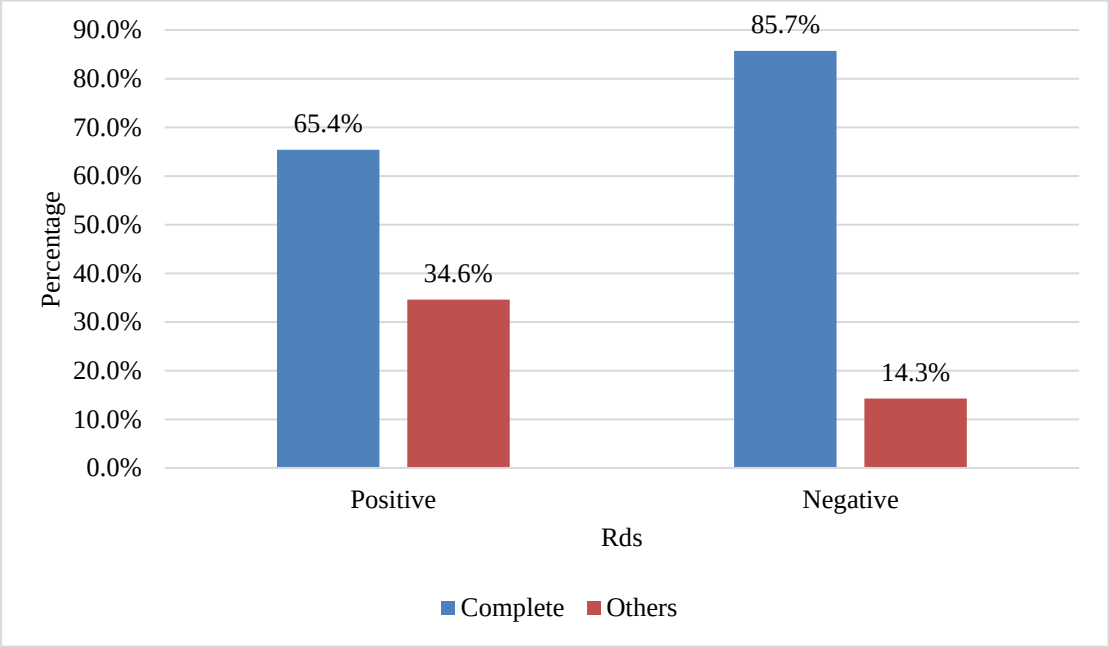


The table presents the comparison of VitD levels in ng/ml across Silverman Anderson scoring categories among a sample of 70 preterm neonates. There is no significant relationship when SAS is compared across VitD

Table 21: Comparison between steroid intake and RDS (N=68)

Steroid Intake	RDS		Chi-square	P value
	Positive (N=26)	Negative (N=42)		
Complete	17 (65.38%)	36 (85.71%)	3.860	0.049
Others	9 (34.62%)	6 (14.29%)		

Graph 18: Cluster bar chart of comparison of steroid intake with RDS (N=68)

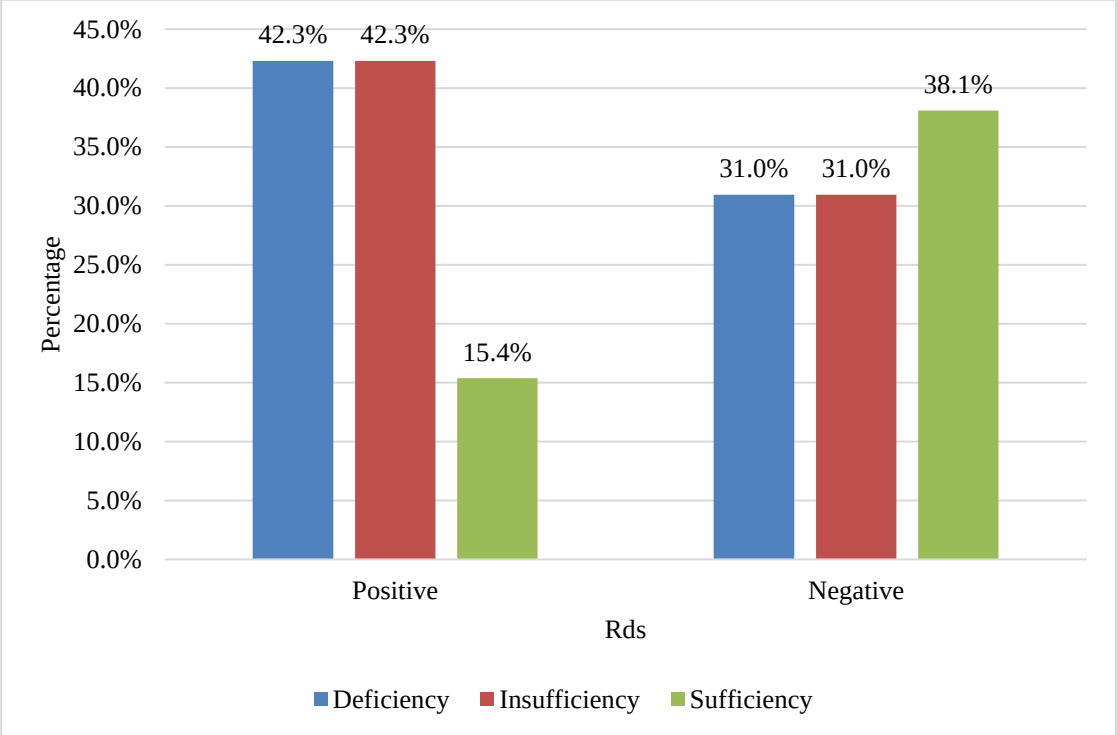


Among those with RDS, 17 out of 26 (65.38%) received complete steroid intake, while among those without RDS, 36 out of 42 (85.71%) received complete steroid intake. The chi-square value obtained is 3.860 with a corresponding "**P-value of 0. 049**." There is a significant correlation amongst steroid intake and RDS neonates.

Table 22: Comparison between VitD levels in ng/ml and RDS (N=68)

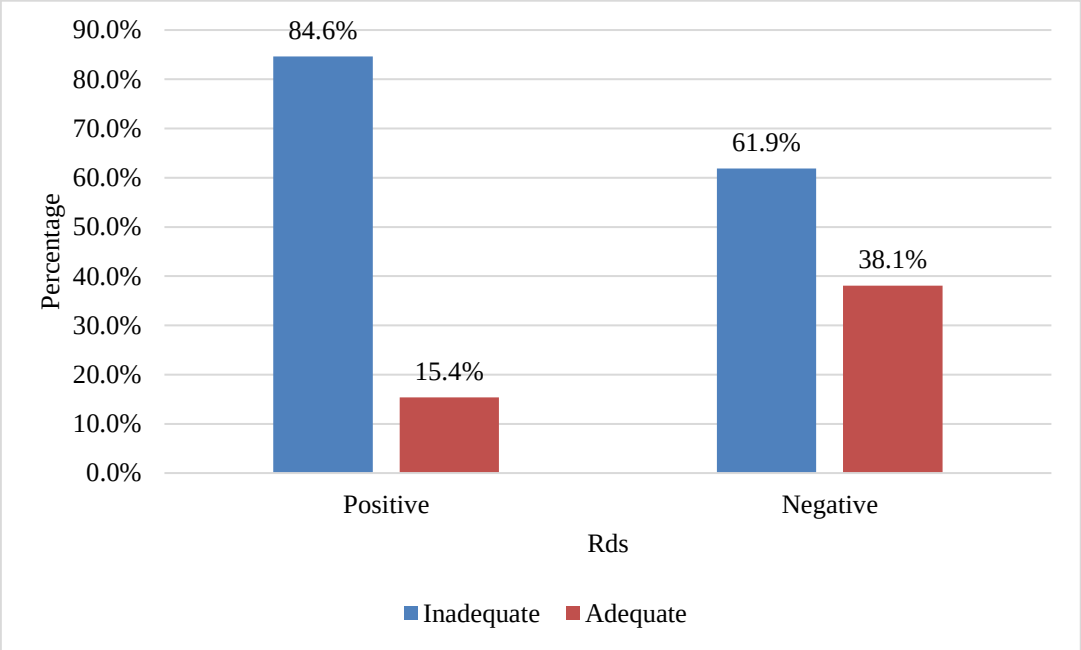
VitD Levels In ng/ml	RDS		Kappa Value	Chi-square	P value
	Positive (N=26)	Negative (N=42)			
Deficiency	11 (42.31%)	13 (30.95%)	-0.001	3.989	0.136
Insufficiency	11 (42.31%)	13 (30.95%)			
Sufficiency	4 (15.38%)	16 (38.1%)			
Inadequate	22 (84.62%)	26 (61.9%)	0.188	3.989	0.046
Adequate	4 (15.38%)	16 (38.1%)			

Graph:19 Cluster bar chart of comparison VitD levels in ng/ml with RDS (N=68)



The relationship with VitD levels and RDS was assessed using Kappa statistics and chi-square analysis. Among individuals who tested positive for RDS, 42.31% were deficient in VitD, 42.31% had VitD insufficiency and 15.38% had sufficient VitD levels. However, the Kappa value of -0.001 suggests no agreement between VitDD and RDS diagnosis. “The chi-square test showed a non-significant P value of 0.136, indicating no significant correlation between VitDD and RDS diagnosis.”

Graph 20: Cluster bar chart of comparison of VitD levels in ng/ml with RDS (N=68)

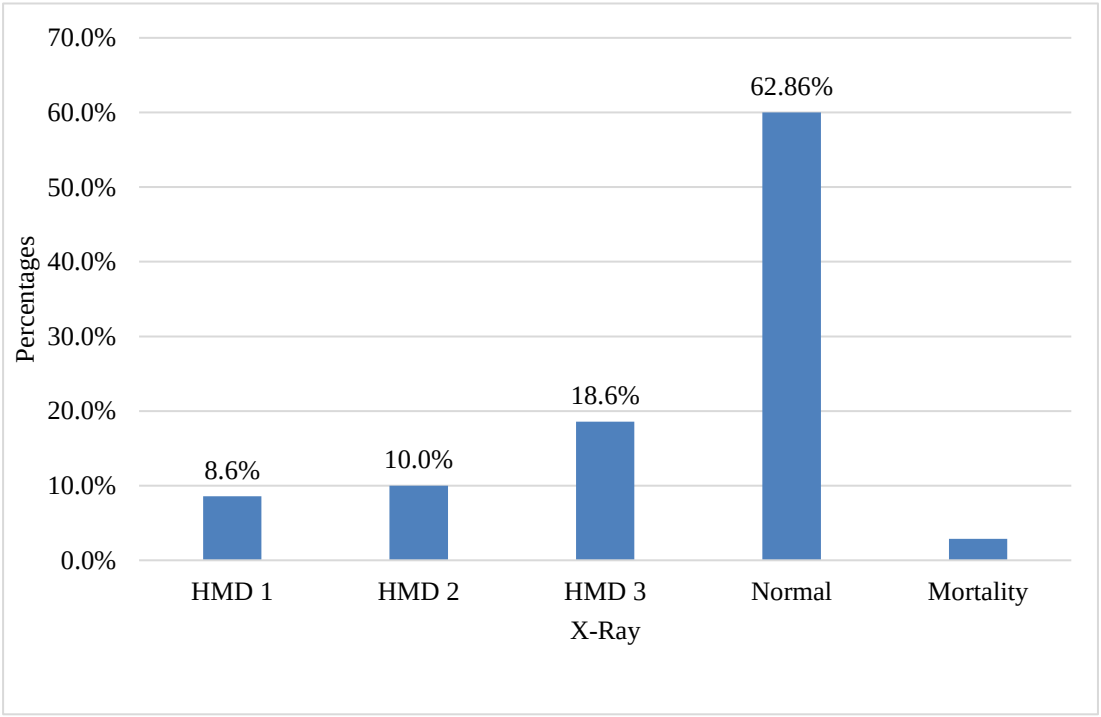


Furthermore, when considering inadequate (deficiency and insufficiency combined) versus adequate VitD levels, 84.62% of individuals with inadequate levels exhibited RDS, compared to 61.9% of those with adequate levels. “The chi-square test showed a statistically significant association between inadequate VitD levels and RDS ($p = 0.046$). These findings suggest a potential link between inadequate VitD levels and an increased risk of RDS, warranting further investigation.”

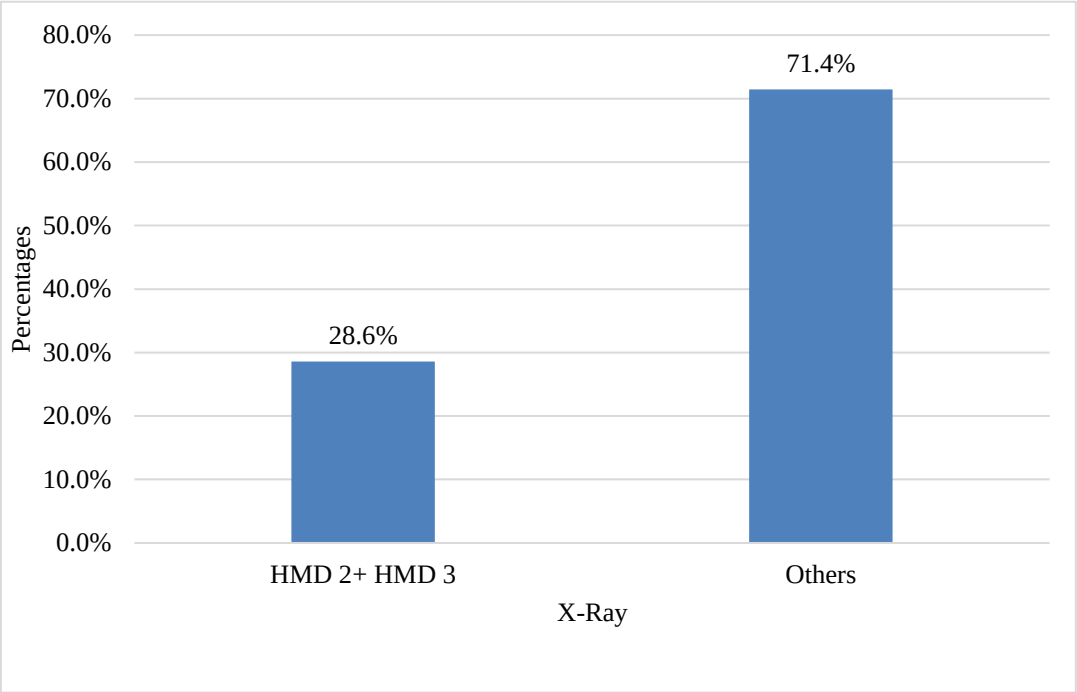
Table 23: Descriptive analysis of X-ray in the study group of preterm neonates (N=70)

X-Ray	Frequency	Percentages
HMD Grade 1	6	8.57%
HMD Grade 2	7	10.00%
HMD Grade 3	13	18.57%
Normal	44	62.86%
X-Ray		
HMD Grade 2+ HMD Grade 3	20	28.57%
Others	50	71.43%

Graph 21: Bar chart of X-ray in the study group of preterm neonates (N=70)



Graph 22: Bar chart of x-ray in the study group of preterm neonates (N=70)

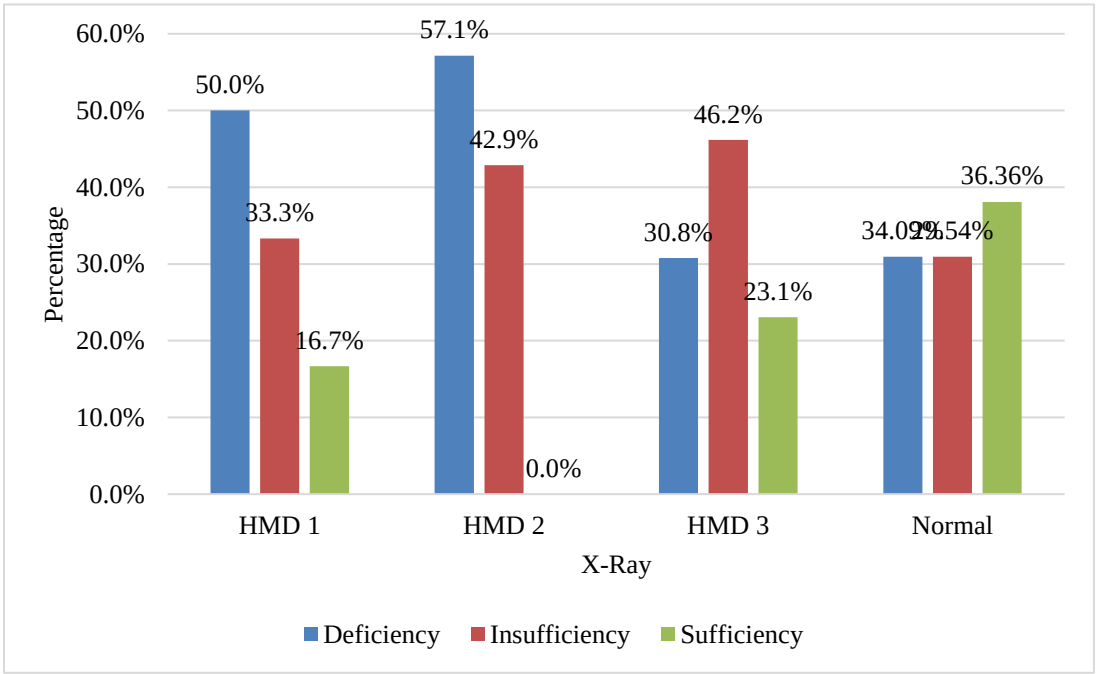


The table provides a detailed overview of the X-ray findings in a population of preterm neonates, highlighting the prevalence of HMD and the proportion of neonates with normal findings. This indicates that 6 out of the 70 preterm neonates had Stage 1 HMD, representing 8.57% of the population. 7 neonates were found to have Stage 2 HMD, making up 10% of the preterm babies. The largest group within the HMD categories, 13 neonates, had Stage 3 HMD, accounting for 18.57% of the population. The majority of the neonates, 44 out of 70, had normal X-ray findings, constituting 62.9% of the population. Combining the frequencies of HMD Stage 2 and Stage 3, 20 neonates were affected, which is 28.57% of the population. The "Others" category includes neonates with normal X-ray findings and those with HMD Stage 1, totalling 50 neonates or 71.43% of the population.

Table 24: Comparison of VitD levels in ng/ml across x-ray (N=68)

VitD Levels In ng/ml	X-Ray				Kappa Value	Chi-square	P value
	HMD 1 (N=6)	HMD 2 (N=7)	HMD 3 (N=13)	Normal (N=44)			
Deficiency	3 (50%)	4 (57.14%)	4 (30.77%)	15 (34.09%)	0.011	5.617	0.467
Insufficiency	2 (33.33%)	3 (42.86%)	6 (46.15%)	13 (29.55%)			
Sufficiency	1 (16.67%)	0 (0%)	3 (23.08%)	16 (36.36%)			
Inadequate	5 (83.33%)	7 (100%)	10 (76.92%)	28 (63.64%)	-0.020	4.718	0.194
Adequate	1 (16.67%)	0 (0%)	3 (23.08%)	16 (36.36%)			

Graph 23: Cluster bar chart of comparison of VitD levels in ng/ml across x-ray findings (N=68)



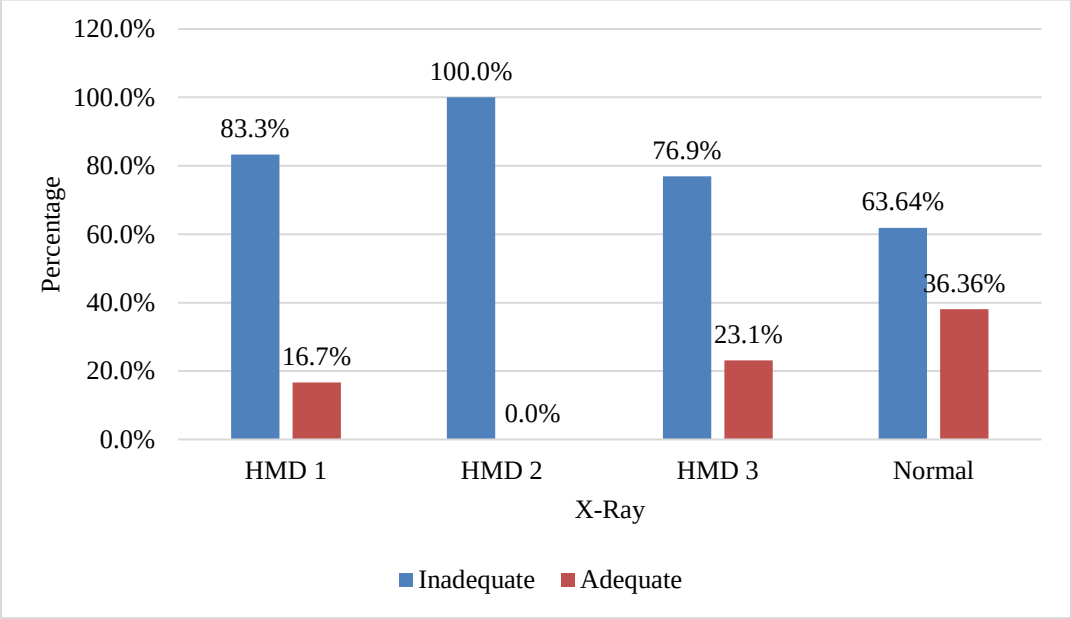
The table provides a "**comparative analysis of VitD levels (in ng/ml) across different X-ray findings"** in a population of preterm neonates (N=68). The categories for VitD levels are – "deficiency, insufficiency, and sufficiency, and they are analysed concerning different X-ray diagnoses: HMD 1, HMD 2, HMD 3, and normal."

- Neonates with HMD 1 and HMD 2 have higher proportions of VitDD compared to those with normal X-rays. HMD 2 has the highest deficiency rate at 57.14%, while normal X-rays have the lowest at 30.95%.
- HMD 3 has the highest proportion of neonates with VitD insufficiency (46.15%), while normal X-rays have the lowest (30.95%)
- The normal group has the highest proportion of neonates with sufficient VitD levels (38.1%), while HMD 2 has none with sufficient levels.
- The HMD 2 group has the largest proportion of neonates with inadequate VitD levels (100%), followed by HMD 1 (83.33%) and HMD 3 (76.92%). The normal group has the lowest proportion of inadequate levels (61.9%).
- The normal group has the highest proportion of neonates with adequate VitD levels (38.1%), while the HMD 2 group has none.
- "The Chi-square values for both comparisons suggest some association between VitD levels and X-ray findings, but the associations are not statistically significant at the conventional 0.05 level"
- "Among the 20 neonates with moderate to severe respiratory respiratory distress, 17 neonates had Inadequate VitD levels, but P-values indicate that the differences in VitD levels across X-ray findings are not statistically significant."

Table:25 Comparison of VitD levels in ng/ml across x-ray findings (N=70)

VitD levels in ng/ml	Xray			Chi-square	P value
	HMD 1	HMD 2 AND 3	Normal		
Inadequate	5	17	28	3.99	0.135
Adequate	1	3	16		

Graph 24: Cluster bar chart of comparison of VitD levels in ng/ml across x-ray (N=68)



- The proportions of neonates with inadequate VitD levels are highest in the HMD 1 (83.33%) and HMD 2+3 (85%) groups compared to the normal group (63.64%). This analysis shows that neonates with HMD are at more risk of having inadequate VitD levels compared to those with normal X-rays. But statistically, “the Chi-square value (3.99) and the P value (0.135) indicate that the differences in VitD adequacy across the different X-ray findings are not statistically significant.”

Table 26: Descriptive analysis of Respiratory Support received in the study population (N=70)

VENTILATOR	FREQUENCY	PERCENTAGE
Yes	15	21.43%
No	55	78.57%
CPAP		
Yes	38	54.29%
No	32	45.71%
Oxygen Follow-up		
Yes	27	38.57%
No	43	61.43%
Respiratory Support		
Yes	45	64.29%
No	25	35.71%

Table 27: Comparison of VitD levels in ng/ml with preterm neonates on ventilator support (N=70)

VitD Levels In ng/ml	Ventilator		Chi-square	P value
	Yes (N=15)	No (N=55)		
Deficiency	7 (46.67%)	19 (34.55%)	0.814	0.666
Insufficiency	4 (26.67%)	20 (36.36%)		
Sufficiency	4 (26.67%)	16 (29.09%)		
Inadequate	11 (73.33%)	39 (70.91%)	0.034	0.854
Adequate	4 (26.67%)	16 (29.09%)		

Table 28: Comparison of VitD levels in ng/ml with preterm neonates on CPAP (N=70)

VitD Levels In ng/ml	CPAP		Chi-square	P value
	Yes (N=38)	No (N=32)		
Deficiency	15 (39.47%)	11 (34.38%)	0.975	0.614
Insufficiency	14 (36.84%)	10 (31.25%)		
Sufficiency	9 (23.68%)	11 (34.38%)		
Inadequate	29 (76.32%)	21 (65.63%)	0.973	0.324
Adequate	9 (23.68%)	11 (34.38%)		

Table 29: Comparison of VitD levels in ng/ml with preterm neonates who received Oxygen by nasal prongs (N=70)

VitD Levels In ng/ml	Oxygen requirement by nasal prongs		Chi-square	P value
	Yes (N=27)	No (N=43)		
Deficiency	12 (44.44%)	14 (32.56%)	1.016	0.602
Insufficiency	8 (29.63%)	16 (37.21%)		
Sufficiency	7 (25.93%)	13 (30.23%)		
Inadequate	20 (74.07%)	30 (69.77%)	0.151	0.698
Adequate	7 (25.93%)	13 (30.23%)		

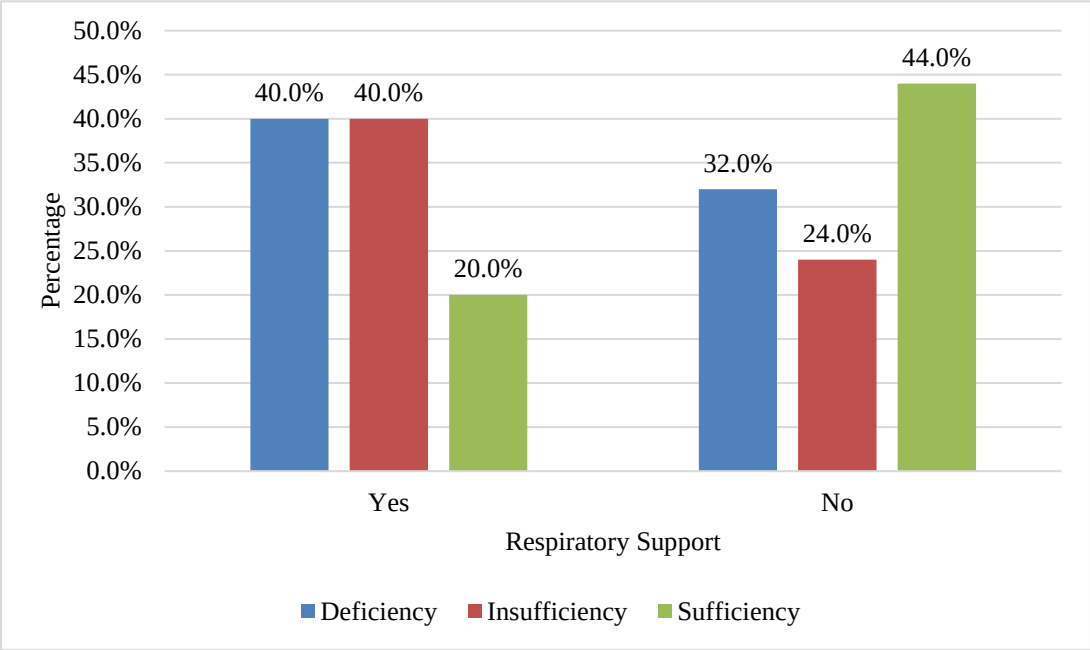
In the above tables, the requirement of Mechanical ventilation, CPAP, and oxygen support by nasal prongs were compared across VitD levels and there was no statistical significance between them.

Table 30: Comparison between VitD levels in ng/ml and Requirement of at least one form of respiratory Support (N=70)

VitD Levels In ng/ml	Respiratory Support		Chi-square	P value
	Yes (N=45)	No (N=25)		
Deficiency	18 (40%)	8 (32%)	4.717	0.095
Insufficiency	18 (40%)	6 (24%)		
Sufficiency	9 (20%)	11 (44%)		
Inadequate	36 (80%)	14 (56%)	4.536	0.033
Adequate	9 (20%)	11 (44%)		

The above table with a p-value <0.05 signifies that preterm neonates with inadequate VitD levels required at least one form of respiratory support during the hospital stay.

Graph 25: Cluster bar chart of comparison between VitD levels in ng/ml and mode of received Respiratory Support (N=70)



Graph 26: Cluster bar chart of comparison of VitD levels in ng/ml with mode of received Respiratory Support (N=70)

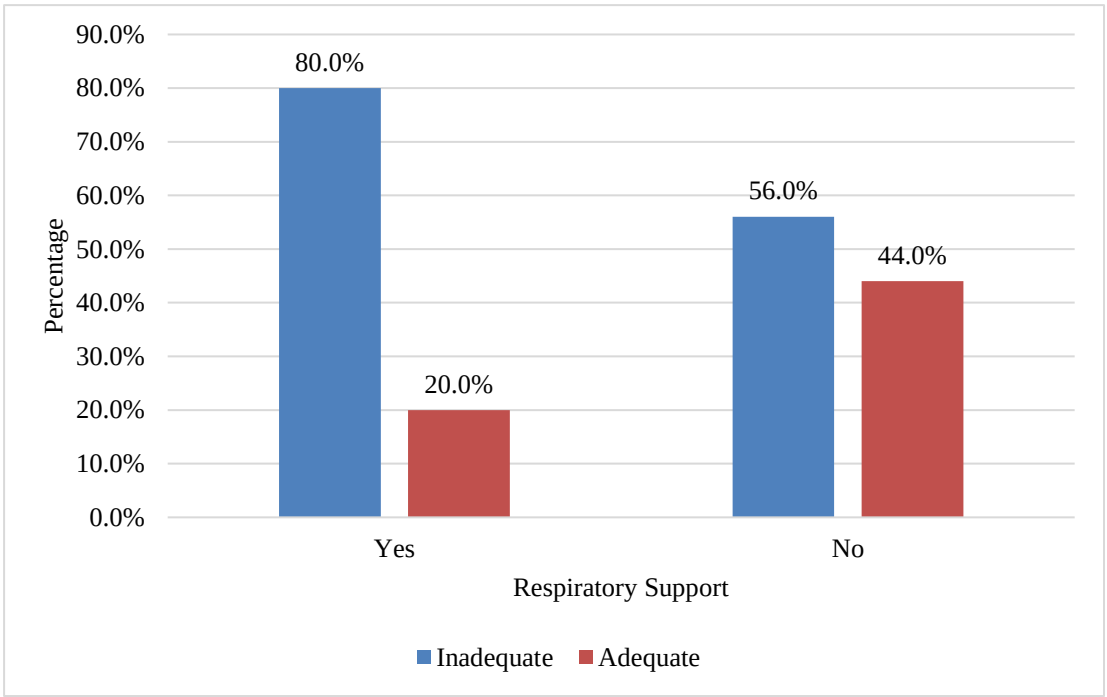


Table 31: Descriptive analysis of ventilator (days), CPAP (days), and oxygen by nasal prongs (days) in the study group of preterm neonates

	Mean ± SD	Median	Minimum	Maximum	95% C I	
					Lower	Upper
Ventilator (Days) (N=15)	2.5 ± 1.9	2.0	1.0	7.0	1.5	3.6
CPAP (Days) (N=37)	1.91 ± 1.09	2.0	0.5	5.0	1.6	2.3
Oxygen by Nasal prongs (Days) (N=27)	1.12 ± 0.72	1.0	0.2	3.0	0.8	1.4

The table provides a descriptive analysis of three respiratory support parameters—ventilator days, CPAP (Continuous Positive Airway Pressure) days, and oxygen by nasal prongs days—in a study group of preterm neonates.

On average, preterm neonates in this group required ventilator support for 2.5 days, with a standard deviation of 1.9 days, indicating some variability in the duration of ventilator use. The median duration is 2.0 days, suggesting that half of the neonates needed ventilator support for 2 days or less. The duration of ventilator support varied from 1 to 7 days, showing that some neonates required significantly longer support. The 95% confidence interval suggests that the average duration of ventilator support in the population is likely between 1.5 and 3.6 days.

The mean duration of CPAP support was 1.91 days, with a standard deviation of 1.09 days, indicating moderate variability. The median is 2.0 days, suggesting that half of the neonates needed CPAP for 2 days or less. CPAP support duration varied from 0.5 to 5.0 days. The 95% confidence interval suggests that the average duration of CPAP support is likely between 1.6 and 2.3 days.

On average, neonates required oxygen by nasal prongs for 1.12 days, with a standard deviation of 0.72 days, indicating relatively low variability. The median is 1.0 days, indicating that half of the neonates required this form of support for a day or less. The duration ranged from 0.2 to 3.0 days. The 95% confidence interval suggests that the average duration of oxygen support by nasal prongs is likely between 0.8 and 1.4 days.

Table 32: Comparison of mean ventilator, CPAP, and oxygen by nasal prongs days of across VitD levels in ng/ml (N=70)

Parameter	VitD Levels in ng/ml			ANOVA P Value
	Deficiency (N=26)	Insufficiency (N=24)	Sufficiency (N=20)	
Ventilator (Days) (N=15)	2.07 ± 1.88	2.25 ± 1.5	3.5 ± 2.38	0.498
CPAP (Days) (N=37)	1.94 ± 1.04	2.21 ± 1.24	1.39 ± 0.78	0.209
Oxygen Nasal prongs (Days) (N=27)	1.26 ± 0.73	1.08 ± 0.84	0.93 ± 0.59	0.623
	Inadequate (N=50)		Adequate (N=20)	Independent Sample t-test P value
Ventilator (Days) (N=15)	2.14 ± 1.67		3.5 ± 2.38	0.232
CPAP (Days) (N=37)	2.08 ± 1.13		1.39 ± 0.78	0.100
Oxygen by nasal prongs (Days) (N=27)	1.19 ± 0.76		0.93 ± 0.59	0.413

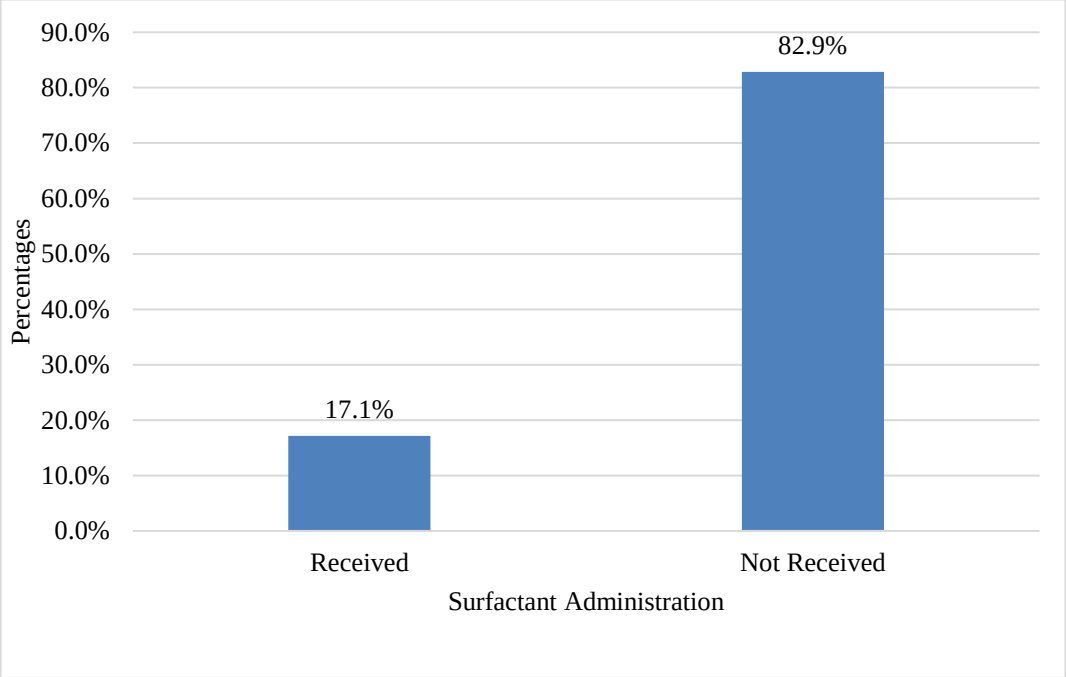
The provided data compares the mean number of days patients spent on ventilators, CPAP (Continuous Positive Airway Pressure), and oxygen by nasal prongs across different levels of VitD (in ng/ml) among a sample size of 70 individuals.

- "There is a trend towards increased ventilator days in the sufficient VitD group compared to the deficient and insufficient groups, but this difference is not statistically significant (ANOVA P value = 0.498, t-test P value = 0.232)."
- While the sufficient VitD group shows fewer CPAP days compared with the deficient and insufficient groups, this difference does not show any statistical significance (ANOVA P value = 0.209, t-test P value = 0.100).
- "The average days of oxygen use by nasal prongs are slightly lower in the sufficient group and is not statistically significant (ANOVA P value = 0.623, t-test P value = 0.413)."

Table 33: Descriptive analysis of surfactant administration in the study group of preterm neonates (N=70)

Surfactant Administration	Frequency	Percentages
Received	12	17.14%
Not Received	58	82.86%

Graph 27: Bar chart of surfactant administration in the study group of preterm neonates (N=70)

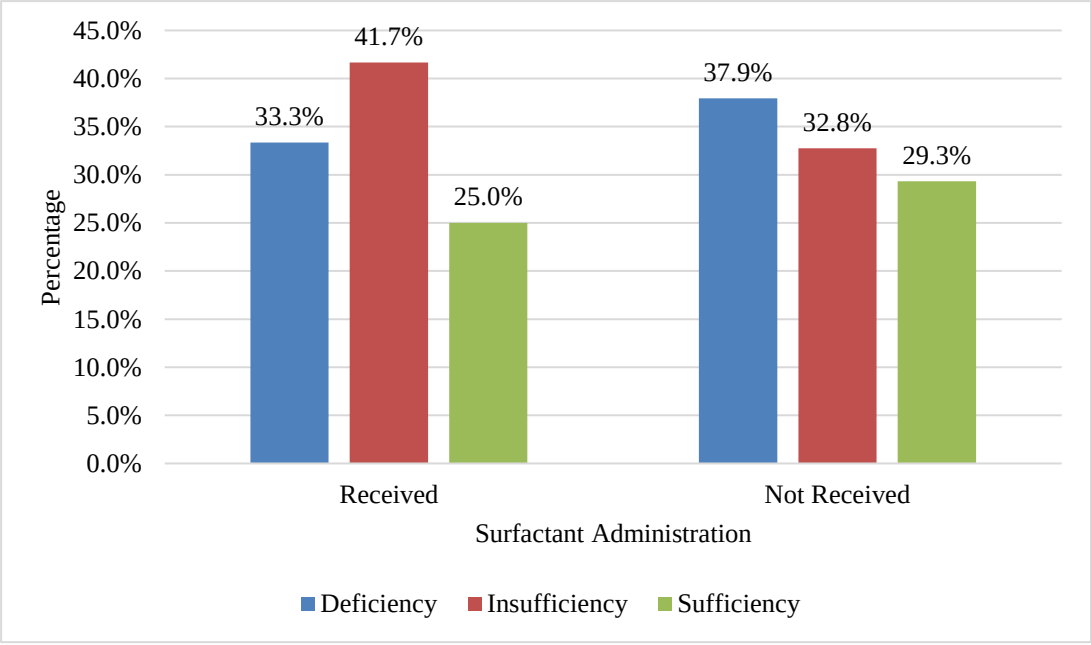


This indicates that 12 out of the 70 preterm neonates received surfactant therapy. This group constitutes 17.14% of the total study group of preterm neonates. The majority of the neonates, 58 out of 70, did not receive surfactant therapy, making up 82.86% of the study group of preterm neonates

Table 34: Comparison of VitD levels in ng/ml with surfactant requirement (N=70)

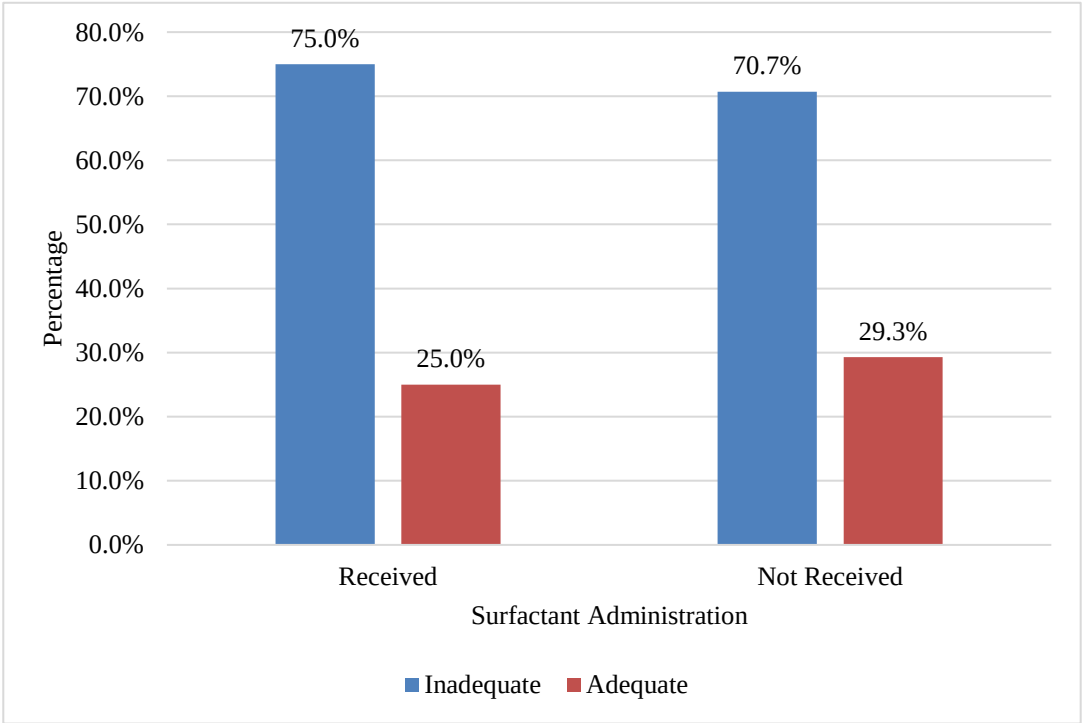
VitD Levels in ng/ml	Surfactant Administration Dose		Chi-square	P value
	Received (N=12)	Not Received (N=58)		
Deficiency	4 (33.33%)	22 (37.93%)	0.351	0.839
Insufficiency	5 (41.67%)	19 (32.76%)		
Sufficiency	3 (25%)	17 (29.31%)		
Inadequate	9 (75%)	41 (70.69%)	0.091	0.764
Adequate	3 (25%)	17 (29.31%)		

Graph 28: Cluster bar chart of comparison of VitD levels in ng/ml with surfactant requirement (N=70)



- The table provides a comparative analysis of VitD levels (in ng/ml) between preterm neonates who received surfactant administration (N=12) and those who did'nt receive surfactant (N=58). The categories for VitD levels are deficiency, insufficiency, and sufficiency.
- Among the babies who received surfactant(N=12), 33.33%,41.67%, and 25% had deficiency, insufficiency, and sufficiency respectively. Among babies who were needless of surfactant requirement(N=58), 37.93%,32.76%, and 23.31% had VitDD, insufficiency, and sufficiency respectively. This indicates that a similar proportion of neonates in both groups had VitDD. A major population of neonates who received surfactant had VitD insufficiency compared to those who haven't receive surfactant.

Graph 29: Cluster bar chart of comparison of VitD levels in ng/ml with surfactant requirement (N=70)

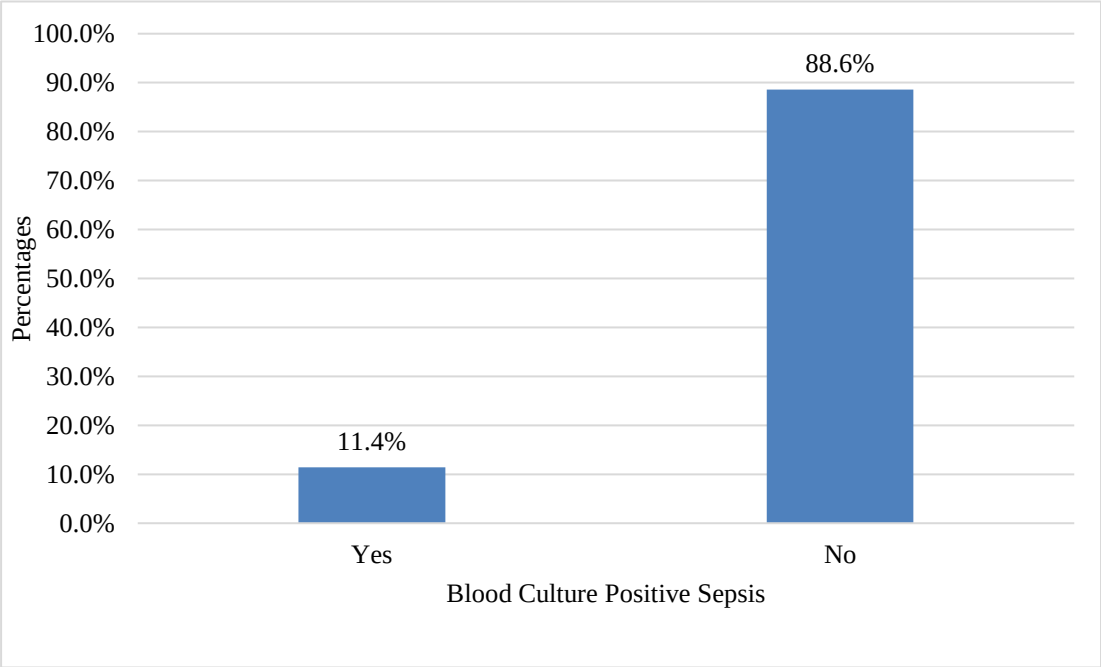


- Combining deficiency and insufficiency into an "inadequate" category, the most neonates in both groups had inadequate VitD levels (75% in neonates who received surfactant and 70.69% who had not received surfactant), with a slightly higher proportion in the surfactant administration group. A similar number of neonates in both groups had adequate VitD levels.
- "The Chi-square values for both comparisons are relatively low, suggesting nil significant association between surfactant administration and VitD levels. (χ^2 = 0.091, P = 0.764)."
- The P-values are far from significant, indicating that the differences in VitD levels between neonates who had received surfactant and those who had not are not statistically significant.

Table 35: Descriptive analysis of blood culture-positive sepsis in the study group of preterm neonates (N=70)

Blood Culture Positive Sepsis	Frequency	Percentages
Yes	8	11.43%
No	62	88.57%

Graph 30: Bar chart of blood culture-positive sepsis in the study group of preterm neonates (N=70)

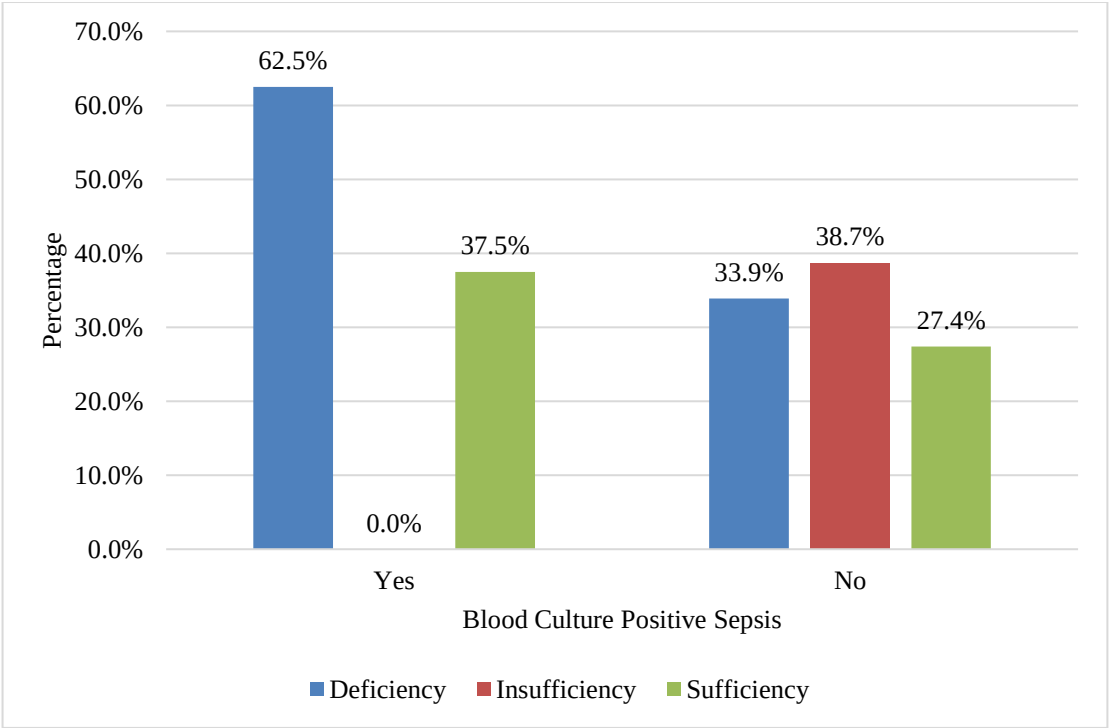


The table provides information on the occurrence of sepsis confirmed by blood culture among a population of preterm neonates. This indicates that 8 out of the 70 preterm neonates had sepsis confirmed by a positive blood culture, constituting 11.43% of the preterm neonates. The majority of the neonates, 62 out of 70, did not have sepsis confirmed by a positive blood culture, making up 88.57% of the study group of preterm neonates.

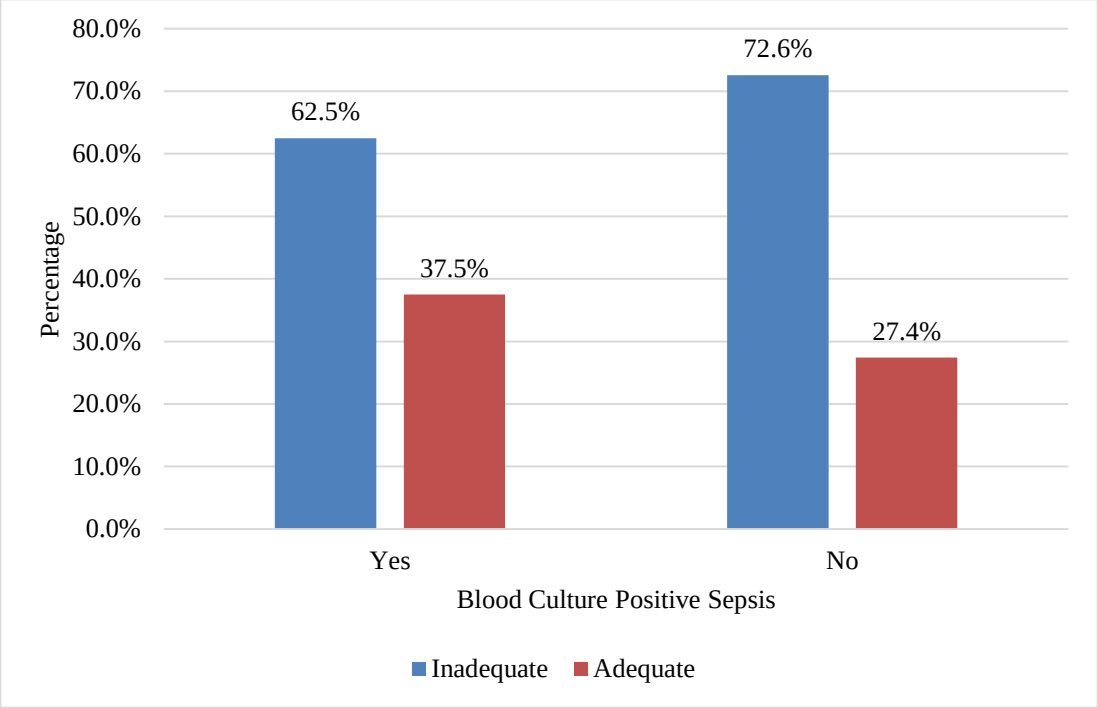
Table 36: Comparison between VitD levels in ng/ml with Blood Culture Positive Sepsis (N=70)

VitD Levels in ng/ml	Blood Culture Positive Sepsis		Kappa value	Chi-square	P value
	Yes (N=8)	No (N=62)			
Deficiency	5 (62.5%)	21 (33.87%)	0.104	4.91	0.086
Insufficiency	0 (0%)	24 (38.71%)			
Sufficiency	3 (37.5%)	17 (27.42%)			
Inadequate	5 (62.5%)	45 (72.58%)	-0.031	0.353	0.680
Adequate	3 (37.5%)	17 (27.42%)			

Graph 31: Cluster bar chart of comparison of VitD levels in ng/ml with blood culture positive sepsis (N=70)



Graph 32: Cluster bar chart of comparison of VitD levels in ng/ml between blood culture positive sepsis (N=70)



The table and bar chart provide a comparative analysis of VitD levels (in ng/ml) between preterm neonates with blood culture-positive sepsis (N=8) and those without (N=62).

The table denotes that a higher proportion of neonates with sepsis had VitDD compared to those without sepsis, whereas a significant proportion of those without sepsis had insufficient VitD levels. None of the neonates with sepsis had VitD insufficiency. The majority of neonates in both groups had inadequate VitD levels, though the proportion is slightly lower in the sepsis group, also “A higher proportion of neonates with sepsis had adequate VitD levels.”

The Kappa values are very low, indicating poor agreement between the presence of sepsis and VitD levels. The Chi-square value for the VitD categories

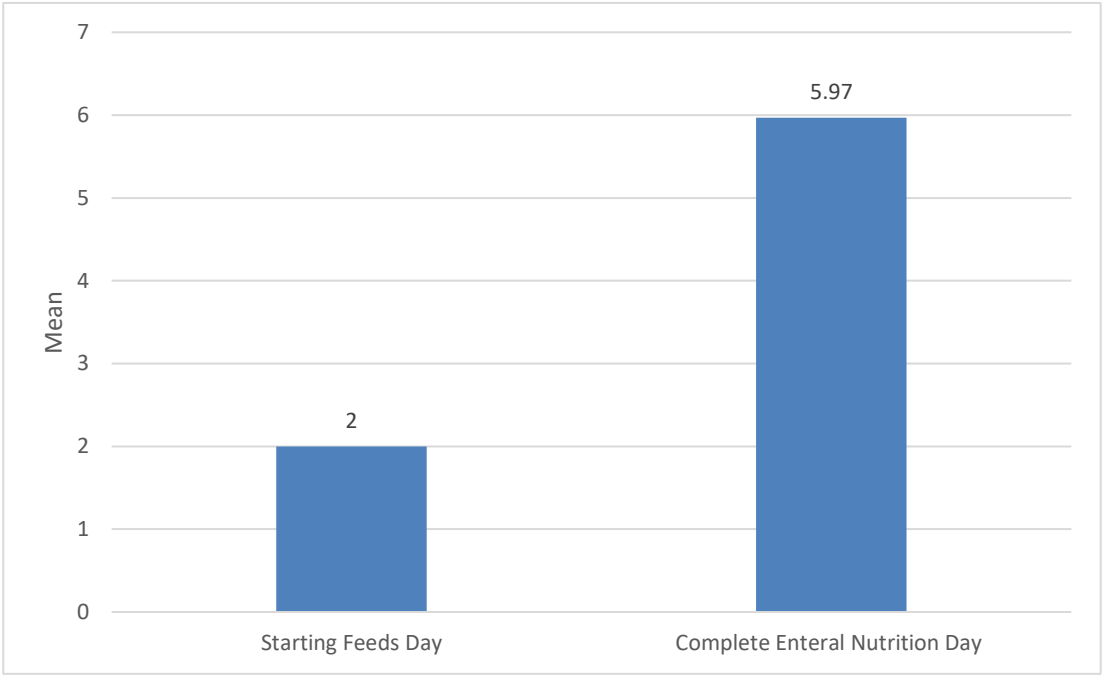
suggests some association, but it is not statistically significant at the conventional 0.05 level.

"The P-values indicate that the differences in VitD levels between neonates with and without sepsis are not statistically significant. Specifically, the P-value of 0.086 suggests a trend towards significance for the VitD categories but does not meet the threshold of 0.05. The P-value of 0.680 for adequacy is not significant."

Table 37: Descriptive analysis of day of starting feeds and the day of achieving complete Enteral Nutrition within the study group of preterm neonates (N=70)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C I	
					Lower	Upper
Starting Feeds Day (N=68)	2 ± 1.75	1.5	1.0	10.0	1.6	2.4
Complete Enteral Nutrition Day (N=67)	5.97 ± 3.87	4.0	2.0	20.0	5.0	6.9

Graph 33: Mean bar chart of starting feeds day and Complete Enteral Nutrition Day within the study group of preterm neonates (N=70)



On average, feeds were started on the 2nd day of life, with a standard deviation of 1.75 days, indicating some variability around this mean. The median day for starting feeds is 1.5 days, suggesting that half of the neonates started feeds within the first day and a half after birth on average, complete enteral nutrition was achieved by the 5.97th day, with a standard deviation of 3.87 days, “indicating significant variability in the time taken to reach full enteral feeding.” The median day for achieving complete enteral nutrition is 4.0 days, suggesting that half of the neonates reached full enteral feeding by the 4th day. The time to achieve complete enteral nutrition ranged from 2.0 days to 20.0 days, reflecting a wide range of clinical scenarios and individual neonate conditions. The 95% confidence interval indicates that the true mean day for achieving complete enteral nutrition in the population is between 5.0 and 6.9 days.

Table 38: Comparison between VitD levels in ng/ml and the mean day of starting feeds (N=68)

Parameter	VitD Levels in ng/ml			ANOVA P Value
	Deficiency (N=26)	**Insufficiency (N=24)**	**Sufficiency (N=20)**	
Starting Feeds Day (N=68)	2.21 ± 2.08	1.92 ± 1.1	1.85 ± 2.01	0.769
	Inadequate (N=48)		**Adequate (N=20)**	**Independent Sample t-test P value**
Starting Feeds Day (N=68)	2.06 ± 1.66		1.85 ± 2.01	0.652

The table compares the mean Starting Feeds Day across different levels of VitD among 68 participants. The levels of VitD are categorized into three groups: "Deficiency, Insufficiency, and Sufficiency." The analysis is further divided by considering "Inadequate (combining Deficiency and Insufficiency) and Adequate VitD levels." The mean duration of starting feeds in the deficiency, insufficiency, and sufficiency group were 2.21±2.08, 1.92±1.1, and 1.85±2.01 respectively. The data represented does not have statistical significance according to the p-value(p=0.769)

Table 39: Comparison between VitD levels and the mean duration of achieving complete enteral nutrition (N=68)

Parameter	VitD Levels in ng/ml			ANOVA P Value
	Deficiency (N=23)	**Insufficiency (N=24)**	**Sufficiency (N=20)**	
Complete Enteral Nutrition Day (N=67)	6.83 ± 4.77	5.04 ± 2.76	6.1 ± 3.78	0.285
	Inadequate (N=47)		**Adequate (N=20)**	**Independent Sample t-test P value**
Complete Enteral Nutrition Day (N=67)	5.91 ± 3.94		6.1 ± 3.78	0.859

The ANOVA test suggests that the differences in mean complete enteral nutrition day across these VitD levels are not statistically significant, with a Pvalue of 0.285. This indicates that there's no strong evidence to suggest that the mean complete enteral nutrition day varies significantly between different levels of VitD.

Table 40: Descriptive analysis of Duration of Hospital Stay in Days within the study group of preterm neonates (N=70)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Duration Of Hospital Stay in Days (N=67)	20.46 ± 8.04	21.0	5.0	40.0	18.5	22.4

The average stay for preterm neonates in hospital is 20.46 days, with a standard deviation of 8.04 days. The median duration of hospital stay is 21.0 days, suggesting that half of the neonates stayed for less than or equal to 21 days and the other half stayed longer. This median value being close to the mean indicates a relatively symmetrical distribution of hospital stay durations. The average duration of hospital stays ranges from a minimum of 5.0 days to a maximum of 40.0 days. "The 95% confidence interval for the average duration of hospital stay ranges from 18.5 to 22.4 days. This means we can be 95% confident that the true mean duration of hospital stay for the entire population of preterm neonates falls within this interval."

Table 41: Comparison of mean Duration of Hospital Stay in Days across VitD levels in ng/ml (N=67)

Parameter	VitD Levels in ng/ml			ANOVA P Value
	Deficiency (N=23)	Insufficiency (N=24)	Sufficiency (N=20)	
Duration of Hospital Stay in Days (N=67)	19.61 ± 8.94	19.38 ± 6.83	22.75 ± 8.24	0.319
	Inadequate (N=47)		Adequate (N=20)	Independent Sample t-test P value
Duration of Hospital Stay in Days (N=67)	19.49 ± 7.85		22.75 ± 8.24	0.130

This table compares the mean duration of hospital stay in days across different VitD levels among 67 individuals. The data is presented in two ways: “across three VitD categories (deficiency, insufficiency, and sufficiency)” and as a comparison between inadequate (deficient + insufficient) and adequate VitD levels.This difference, however, there is no statistical significance (t-test P value = 0.130). “The mean duration of hospital stay is slightly higher in the sufficient VitD group compared to the deficient and insufficient groups. However, this difference is not statistically significant (ANOVA P value = 0.319).”

Table 42: Descriptive analysis of HNNE score in the study group of preterm neonates (N=70)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C I	
					Lower	Upper
HNNE Score At 40 Weeks (N=67)	31.16 ± 2.48	32.0	16.0	34.0	30.6	31.8

The mean HNNE score at 40 weeks for the preterm neonates is 31.16, with a standard deviation of 2.48. This indicates that, on average, the neonates scored just over 31 on the neurological examination, with some variability around this mean. The median HNNE score is 32.0, suggesting that half of the neonates scored 32 or below, and the other half scored above 32. This median being close to the mean indicates a relatively symmetrical distribution of scores. The 95% confidence interval for the mean HNNE score ranges from 30.6 to 31.8. This means we can be 95% confident that the true mean HNNE score for the entire population of preterm neonates at 40 weeks lies within this interval.

Table 43: Comparison of mean HNNE Score across VitD levels in ng/ml (N=67)

Parameter	VitD Levels in ng/ml			ANOVA P Value
	Deficiency (N=26)	**Insufficiency (N=24)**	**Sufficiency (N=20)**	
HNNE Score At 40 Weeks (N=67)	31.35 ± 1.27	30.88 ± 3.48	31.3 ± 2.18	0.780
	Inadequate (N=47)		**Adequate (N=20)**	**Independent Sample t-test P value**
HNNE Score At 40 Weeks (N=67	31.11 ± 2.62		31.3 ± 2.18	0.773

The ANOVA test compares the means of HNNE scores across three levels of VitD: “deficiency, insufficiency, and sufficiency”. The p-value obtained (0.780) suggests that there is no significant difference in HNNE scores among these three groups. The p-value obtained (0.773) indicates that there is no significant difference in HNNE scores between those with inadequate and adequate levels of VitD.

Table 44: Correlation between HNNE Score and VitD levels in ng/ml (N=68)

Pair	Pearson Correlation (r)	P-value
HNNE Score At 40 Weeks Vs VitD levels	-0.030	0.809

"The correlation between HNNE Score at 40 weeks and VitD levels was assessed using Pearson correlation analysis. The correlation coefficient (r) was found to be -0.030, indicating a very weak negative correlation between the two variables. However, this correlation was not statistically significant (p = 0.809), suggesting that there is no meaningful relationship between HNNE Score at 40 weeks and VitD levels in the study group of preterm neonates."

DISCUSSION

"A Hospital based prospective observational study was done in the Neonatal Intensive Care Unit of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre among preterm neonates born between 28 and 34 weeks of gestation from April 2023 to February 2024 with the primary objective of estimating the prevalence of VitD deficiency among respiratory distress syndrome neonates.

The mean age of the mothers was 28.97 ± 6.3 years. The largest proportion of mothers falls within the age range of 25 to 30 years, comprising 37.14% of the study population. This suggests that a considerable number of mothers in the sample are in their mid to late twenties. A smaller proportion of mothers, accounting for 14.29%, are between 30 and 35. While less common, this age group still contributes to the overall age distribution of mothers in our study. Maternal age can influence various health outcomes for both the mother and the infant. In a study done in a neonatal unit in northern India by Natarajan et al [105], the mean gestational age group of mothers in their study with 2 groups were 28.1± 5.4 and 28.3 ± 4.7. In a prospective cohort study conducted by Chike Onwuneme et al[111], the mean age maternal group in their study group was. 27.43± 5.27.

Preterm neonates in the study population were born on average around 31.86 weeks of gestation with a standard deviation of 1.58 weeks and were discharged on average around 35.13 weeks of gestation with a standard deviation of 1.27 weeks. 50% of the study group were born < 32 weeks of gestation and the other half were above 32 weeks of gestation. Gestational ages at birth range from 27.9 weeks to 34.3 weeks. The variability in gestational ages at birth and discharge underscores the

diverse needs and challenges associated with caring for preterm infants. The mean gestational age at birth in the study by Natarajan et al [105] , was 32.4± 1.9 and 32.5 ± 1.8. The mean gestational age at birth in the study by Onwuneme et al [106] was 28.8± 2.09.

Most preterm neonates were born between 30 and 34 weeks of gestation, with the highest proportion between 32 and 34 weeks of gestation. A study done in South India in the NICU of a tertiary care hospital by Tergestina et al [107] among 111 preterm neonates had most of their mothers in the study group between 30 and 32 weeks of gestation. At discharge, the majority of preterm neonates were within the 32-34 weeks gestational age range, indicating the duration of hospital stay and the readiness for discharge in this population. The discharge of the newborn from the NICU is in turn dependent on a variety of factors like weight gain, euthermia, hemodynamic stability, achievement of complete enteral nutrition etc.

In our study, we enrolled 70 preterm newborns ≤ 34 weeks of gestation. The male: female ratio was 1.92:1, with 65.71% of the study group being male gender and 34.29% were of female gender. Similar to our study, in the study by Tergestina et al [107], males contributed to a majority of the study group comprising 55.8%. In a study done in India among preterm neonates to estimate the prevalence of VitD deficiency by Suresh Kumar Tripathy et Al [15], the female gender contributed to the majority of the study group. 57.5% of the study group were of female gender with a male-to-female ratio of 0.74:1. In a study by Jafari et Al [96], done to find the association between VitD insufficiency and respiratory problems in preterm neonates, females contributed to 77% of the study group with a male: female ratio of 0.30:1.

The majority of preterm neonates, accounting for 80.00%, were delivered via cesarean section. This high percentage suggests a tendency toward cesarean delivery for preterm births in the study group. A minority of preterm neonates, comprising 20.00%, were delivered vaginally

The significant difference in percentages between cesarean section and vaginal delivery for preterm neonates underscores the prevailing clinical indication for cesarean delivery in this specific population.The high proportion of cesarean deliveries highlights the importance of ensuring optimal neonatal care, particularly for preterm infants who may require specialized medical attention and support immediately after birth.

Exploring the factors influencing the choice of delivery mode for preterm neonates, such as gestational age, maternal health status, and fetal conditions, can provide valuable insights into clinical decision-making and perinatal care practices.

Many studies carried out in preterm neonates around the world had Cesarean section as their mode of delivery predominantly. In a similar study by Fettah et Al [98], 84% of the newborns were born by cesarean section.

The median APGAR score at 1 minute is 7.0, suggesting that half of the neonates had an APGAR score of 7 or higher at 1 minute after birth.

The median APGAR score at 5 minutes is 8.0, indicating that half of the neonates had an APGAR score of 8 or higher at 5 minutes after birth

APGAR score was developed in 1950, by Dr.Virginia Apgar to assess the physical condition of the newborn and the need for active resuscitation. A nationwide study was done by Cnattingius S et Al [108], to assess the outcome of the neonate in

terms of survival efficacy with APGAR score at 5 and 10 minutes of birth. The risk of neonatal deaths was predicted substantially across preterm neonates born at varied gestational age groups by APGAR scores at 5 and 10 minutes of life. With decreasing APGAR scores the risk of neonatal death was consistently increased

VitD Deficiency Status: When considering deficiency status, a majority of the preterm neonates, accounting for 71.43%, tested positive for deficiency or insufficiency (VitD levels ≤20ng/ml), while 28.57% tested negative, indicating sufficient VitD levels. This highlights the high prevalence of VitD inadequacy among the preterm neonates in this study. In our study, male neonates comprised the majority of the population and 76.09% of the male population had inadequate VitD levels. Among the 34.29% of the female neonates in the population, 62.5% had inadequate VitD levels.

Studies conducted in other centres estimated a similar prevalence among preterm neonates. A study conducted in India by Natarajan et Al [105], found the prevalence of VitD deficiency in preterm neonates to be 83% (VitD levels <20ng/ml).

A study done by Chike Onwuneme et Al [106] to estimate the VitD status of Preterm NICU inmates at birth found the prevalence of VitD deficiency <20ng/ml to be 92% and levels less than 12ng/ml to be 64%. These studies had similar results of having a large proportion of preterm neonates with VitD deficiency.

The average birth weight of around 1540 grams indicates that the study group includes a significant number of low birth weight (LBW) and very low birth weight (VLBW) neonates, which is typical for preterm populations.

Variability: The wide range in birth weights (725 to 2830 grams) reflects the diversity in gestational ages and growth restrictions commonly seen in preterm births. The range in discharge weights (931 to 2850 grams) shows that while some neonates had significant weight gain, others may have had slower growth or additional medical issues affecting their weight.

The mean birth weight of 1540.17 grams and the mean discharge weight of 1568.22 grams, along with the respective ranges and variability, highlight the challenges and progress in the growth and development of preterm neonates. These metrics are crucial for assessing the effectiveness of neonatal care practices and interventions aimed at promoting healthy growth and development in this vulnerable population.

At discharge, the distribution shifts, with a higher percentage of neonates (58.21%) weighing more than 1.5 kg, compared to their birth weights. This indicates the weight gain of these neonates during the hospital stay.

- **Weight Gain**: The percentage of neonates weighing less than 1 kg at discharge slightly decreased from 5.71% at birth to 4.48%, indicating that some of the smallest neonates gained sufficient weight.
- 1 **- 1.5 kg Category**: The percentage of neonates in the 1 - 1.5 kg category decreased from 47.14% at birth to 37.31% at discharge, reflecting weight gain and movement into the higher weight category (>1.5 kg).

The shift in weight distribution from birth to discharge, with more neonates moving into higher weight categories, reflects successful medical and nutritional interventions during their hospital stay. When a comparison was made between birth

and discharge weight categories with VitD levels, we found no statistical significance between VitD levels and birth or discharge weight.

Comparing with the study done by Natarajan et Al [105], the mean discharge weight in the 2 study groups was 1655±411 grams and 1694 ± 513 grams. In the study by Onwuneme et al [106], the mean birth weight in the study group with VitD deficiency (taken as <30nmol/l) and VitD levels ≥30nmol/l was 1171 ± 363 grams and 1229 ± 398 grams respectively.

The majority of the study population, comprising 50%, had a Silverman Andersen score of less than 4, indicating minimal to no respiratory distress. Meanwhile, 34.29% fell within the score range of 4-7, suggesting mild to moderate respiratory distress. A significant proportion, 15.71%, had scores exceeding 7, implying severe respiratory distress in this subset of the population.

SAS was used to assess the severity of respiratory distress in the preterm neonates. SAS has a direct relationship with the severity of respiratory distress. The lower the score, the lesser the distress. In this study scores 4 to 7 and more than 7 were started on CPAP and Mechanical ventilation respectively. A Prospective cohort study was done by Anna Bruett Hedstrom et Al [109] to determine if SAS did within 1 hour of life is useful in predicting the Carbon dioxide level (PCO2) and if it is a valuable score for assessing the need for increased respiratory support. The study concluded that the Silverman- Andersen respiratory severity score may be valuable for predicting the need for escalation of respiratory support and facilitating decision making for transfer in low-resource settings. Patients with respiratory scores ≥5 had their respiratory support increased within 24 hours more often than those with scores <5 (79% vs. 28%, p < 0.001). Similar to this study in our study, we found a

significant relationship between SAS and RDS outcomes, with scores more than 7 have a 100% chance of developing RDS.

The data shows that RDS is present in over one-third of the study population (37.14%). This indicates a relatively high prevalence of the condition within the group being studied. A larger portion, 62.86%, did not have RDS, suggesting that the majority of the study population was not affected by this condition. The high prevalence of RDS (37.14%) suggests that there is a substantial portion of the population that may require medical intervention and continuous monitoring for RDS-related complications.

This study reveals that a substantial majority, 77.14%, of the study population underwent complete steroid intake. 22.86% of the population constituted the group which either received incomplete doses/not received steroids. This breakdown provides insight into the prevalence of different steroid intake patterns, which could be crucial for understanding treatment trends and outcomes related to steroid therapy. 85.71% of the cases without RDS received complete steroid intake and 65.38% of the neonates with RDS received complete doses of steroids.

The chi-square value of 3.860 and the corresponding p-value of 0.049 indicate that there is a statistically significant difference in the distribution of steroid intake between the positive and negative groups.

The analysis shows a significant association between the status of steroid intake (positive vs. negative) and the type of steroid intake (complete vs. others) in the RDS population. Specifically, individuals with negative steroid intake are more likely to have RDS compared to those with positive steroid intake.

Among neonates tested positive for RDS, 42.31% were deficient in VitD, 42.31% had VitD insufficiency and 15.38% had sufficient VitD levels. Both deficiency and insufficiency in VitD levels are more prevalent in the Positive RDS group compared to the Negative RDS group, but the difference is not statistically significant (P value = 0.136). A higher percentage of individuals without RDS have sufficient VitD levels compared to those with RDS. **The combined inadequate levels (deficiency + insufficiency) are significantly more common in the Positive RDS group (84.62%) compared to the Negative RDS group (61.9%), with a P value of 0.046 indicating statistical significance**.

- There is a notable difference in the distribution of VitD levels between those with and without RDS.
- Individuals with RDS tend to have a higher prevalence of inadequate VitD levels (deficiency or insufficiency).
- **The difference in the prevalence of inadequate VitD levels between the groups is statistically significant, suggesting a potential link between VitD inadequacy and the presence of RDS**. Further research may be needed to explore this relationship in more depth.

Similarly, in a study done in India by Suresh Kumar Tripathy et al [15] to assess the prevalence of VitD deficiency and its associated co-morbidities, RDS was found in a higher proportion of VitD deficient Very low birth weight neonates.

A prospective study was done in a tertiary care centre by Dogan et al [95], to study the effect of VitD deficiency on respiratory distress syndrome. Of the 72 patients included in the study, 49 had RDS, while 23 did not have RDS.

Preterm neonates with RDS had significantly decreased mean 25 (OH)D levels (p=0.04).

Patients with greater 25(OH)D levels may be preventive for developing RDS, according to multivariate analysis (odds ratio 0.89; 95% confidence interval 0.8–0.99; p=0.04).

Similarly, a study was done by Fettah et al [98] to investigate the relationship between cord blood 25-hydroxyVitD (25(OH)D) levels and respiratory distress syndrome (RDS) development in preterm infants. Higher levels of 25 (OH)D have been shown through multivariate analysis to be protective against the dev elopment of RDS (odds ratio, 0.6; 95% confidence interval (0.5–0.8); p ¼ 0.001). Lower levels of 25(OH)D in cord blood may be linked to a higher risk of RDS in pre mature children born with extremely low birth weights.

Similarly, a case control study was done by Hegazy et al [97], to find the association between VitD levels at birth and the presence of RDS. The study concluded that neonates with RDS had significantly low VitD levels compared to those without RDS.

According to the chest X-ray findings in our study, HMD grade 1, HMD grade 2, and HMD grade 3 were 8.57%, 10%, and 18.57% respectively. 28.57% of the preterm neonates had HMD 2 and 3. 62.86% of the preterm neonates had normal X-ray findings. The Chi-square and P-values indicate that the differences in VitD levels across different X-ray findings are not statistically significant. The analysis suggests variability in VitD levels among preterm neonates with different X-ray findings, with a notable deficiency in the HMD 2 group and better VitD status in those with normal X-rays. However, these differences are not statistically significant.

The preterm neonates on average required ventilator support for a period of 2.5 days with an SD of 1.9 days with the duration of ventilatory support required ranging from 1 to 7 days. Half of the neonates needed CPAP for 2 days or less. CPAP support duration varied from 0.5 to 5.0 days. Neonates with minimal respiratory distress required oxygen (by nasal prongs) for 1.12 days with a SD of 0.72 days. Half of the neonates received oxygen for a day or less.

Though the neonates with sufficient VitD levels were on fewer days on CPAP, the difference was not statistically significant (ANOVA P value = 0.209, t-test P value = 0.100). The mean duration of oxygen received through nasal prongs was also lower in the group with sufficient VitD levels although showing no statistical significance (ANOVA P value = 0.623, t-test P value = 0.413). However, the duration of days on the Ventilator were more in the group with sufficient VitD levels.

There are observed differences in the number of days on ventilator, CPAP, and oxygen by nasal prongs across different VitD levels, with the sufficient group generally showing fewer days for CPAP and oxygen by nasal prongs. However, none of these differences reach statistical significance, indicating that VitD levels might not have a significant impact on the duration of these respiratory supports in our study.

In a study by Jafari et al [96], of the 113 preterm infants, 65 (58%) had low VitD levels and 48 (42%) had normal levels; these individuals were categorised into groups I and II, respectively. 20 cases (41.7%) in group II and 40 cases (61.5%) in group I had respiratory distress syndrome (RDS) and required surfactant therapy (P = 0.036). Furthermore, non-invasive ventilation (NIV) was required for 46 babies (70.8%) in the first group and 22 babies (45.8%) in the second group (P = 0.007). VitD status and RDS were significantly correlated, according to multiple logistic

regression (OR, 95% CI = 2.840 (1.083–7.447), P = 0.034), indicating the need for (OR, 95% CI = 2.840 (1.083–7.447), P = 0.034) and **need for NIV** (OR, 95% CI = 3.929 (1.526–10.113), P = 0.005). The study found no significant relation between VitD levels and the need for Mechanical ventilation. No association was found between the need for supplemental oxygen.

The study by Onwuneme et al [106], also found no significant relationship between VitD levels and the need for mechanical ventilation. But, in our study, when preterm neonates with inadequate VitD levels were compared with the requirement of respiratory support, a significant p-value was found.

The relatively low percentage (17.14%) of surfactant administration may reflect the clinical criteria used to determine which neonates require this treatment. Surfactant therapy is typically administered to neonates with significant respiratory distress syndrome (RDS) or other conditions where it is deemed necessary to improve lung function. Comparing the neonates with received/not received surfactant across VitD levels, a slightly lower proportion of neonates who received surfactant had sufficient VitD levels compared to those who did not receive surfactant. The Chi-square values for both comparisons are relatively low. ($\chi^2 = 0.091$, $p = 0.764$). Both groups exhibit similar proportions of deficiency, insufficiency, and sufficiency. The study by Onwuneme et al [106] also found no significant association between VitD levels and surfactant administration.

The majority of neonates in both groups had inadequate VitD levels, with no significant difference between the two groups. Similarly, the proportion of neonates with adequate VitD levels did not differ significantly between the groups. The Chi-

square values and P-values indicate that there is no statistically significant association between surfactant administration and VitD levels among preterm neonates.

The majority of the neonates, 62 out of 70, did not have sepsis confirmed by a positive blood culture, making up 88.57% of the study population. With 11.43% of the neonates affected, it highlights that sepsis is a significant concern in this population, though the majority (88.57%) did not experience this complication.

A larger proportion of neonates with sepsis had VitD deficiency compared to those without sepsis (62.5% vs. 33.87%). This could suggest a potential link between low VitD levels and susceptibility to sepsis, but further research would be needed due to a lack of statistical significance.

When combining deficiency and insufficiency into an "inadequate" category, the majority of neonates in both groups fall into this category. However, the sepsis group had a slightly lower proportion of inadequate VitD levels compared to the non-sepsis group (62.5% vs. 72.58%).

The lack of statistically significant P-values suggests that while there are observable differences in VitD levels between the sepsis and non-sepsis groups, these differences are not strong enough to rule out the possibility that they are due to chance.

The analysis suggests some differences in VitD levels between preterm neonates with and without blood culture positive sepsis, particularly with a higher prevalence of deficiency in the sepsis group. However, the lack of statistical significance means that these findings should be interpreted with caution. Further research with larger sample sizes and more rigorous study designs would be necessary

to clarify the potential role of VitD in the susceptibility to sepsis among preterm neonates.

However, a study in Southern India by Srinivasan N et al [99] found that low VitD levels can be a risk factor for the development of sepsis. Another Indian study by Suresh Kumar Tripathy et al [15] also found that sufficient VitD levels can be protective for neonatal sepsis. Another Indian study by Behere et al[17] also found a significant correlation between VitD levels and the development of Neonatal Sepsis.

Early Feeding: The early initiation of feeds (mean of 2 days and median of 1.5 days) reflects current clinical practices that support early enteral feeding to promote gut health and development in preterm neonates.

Variability: The variability (range from 1 to 10 days) indicates that some neonates may have had clinical conditions or complications that delayed the start of feeding.

Achievement of Full Nutrition: Achieving complete enteral nutrition by an average of approximately 6 days, with a median of 4 days, suggests that many preterm neonates can transition to full enteral feeds relatively quickly.

Clinical Variability: The wide range (2 to 20 days) underscores the diversity of health conditions and responses to feeding protocols among preterm neonates. This variability necessitates individualized feeding plans and close monitoring.

Early initiation of feeds (mean of 2 days) and relatively quick achievement of complete enteral nutrition (mean of approximately 6 days) highlight effective feeding strategies. However, the variability in both parameters indicates that individualized care is essential to accommodate the different clinical needs of preterm neonates.

In our study, we found no statistical significance when neonates with inadequate VitD levels were compared with the mean duration of starting and achieving complete enteral nutrition. In the study by Natarajan et al [105], no significant

relation was found between VitD levels and feeding intolerance. Onvuneme et al [106] also found no correlation between VitD levels and the time taken to achieve complete enteral nutrition.

The wide range (5 to 40 days) highlights the variability in the health conditions and recovery times among preterm neonates. Some neonates may have less severe conditions and recover quickly, while others with more severe health issues or complications may need prolonged hospital care. With an average stay of approximately 20.5 days and a range from 5 to 40 days, the data highlights the significant variability in hospitalization needs, with no significant association found in the duration of hospital stay in the groups with adequate and inadequate VitD levels. Similarly, in the study by Onvuneme et al [106], no significant correlation was found between VitD levels and the duration of hospital stay.

The HNNE score is a measure of neurological function, and the average score of 31.16 suggests that, on the whole, the preterm neonates in this study population have relatively good neurological outcomes by 40 weeks. However, the wide range of scores indicates considerable variability in neurological function. The median score is close to the mean suggests a relatively normal distribution of HNNE scores, with no extreme skewing in the data. The HNNE scores ranged from a minimum of 16.0 to a maximum of 34.0, showing a wide range. In this study, no statistical significance was found between HNNE scores done at 40 weeks postmenstrual age across VitD levels. Pearson correlation also showed nil significant association. To our knowledge, there are no studies with correlate VitD levels and neurological outcomes assessed by HNNE scores.

CONCLUSION

1. VitDD was found in 37.14% of the study group and VitD insufficiency was found in 34.29% of the study group of preterm neonates. About 71.43percentage of the neonates in this study group had inadequate (VitDD and insufficiency) VitD levels.
2. "A significant association was found between VitD levels and the development of RDS where inadequate VitD levels had a significant correlation with the development of RDS."
3. All neonates with HMD grade 2 and 77.92% of neonates with HMD grade 3 had inadequate VitD levels.
4. "A significant association was found between Inadequate VitD levels and preterm neonates requiring at least one form of respiratory support during the hospital stay."
5. Silverman Andersen scoring can be used as a clinical criterion to diagnose RDS.
6. "Steroid intake was found to be preventive for the development of RDS."
7. "Neonates with inadequate VitD levels had increased incidence of neonatal sepsis."
8. "No significant correlation was found between VitD levels and HNNE score at 40 weeks PMA."
9. The effect of optimal VitD levels on neonatal clinical outcomes requires further evaluation.

SUMMARY

"A hospital-based prospective observational study was carried out at KLES Dr.Prabhakar Kore Hospital and MRC" from April 2023 to February 24 , & primary objective of finding "the association between VitDD and RDS among Preterm neonates between ≤34 weeks of gestation."

Out of 70 preterm neonates, 26 (37.14%) had VitDD, and 24 (34.29%) had VitD insufficiency. "The prevalence of VitDD + insufficiency in the population was found to be 71.43%"

"The mean average age of the mothers in this study group was 28.97± 6.3 years. The majority of the mothers were between the age group of 25-30 years. The mean gestational age at birth was 31.86± 1.58 weeks with the majority of the study group born between 32-34 weeks. 80% of the preterm neonates were born by Lower segment Cesarean section and 46 out of 70(65.71%) were of male gender. The mean GA (Gestational age) at discharge was 35.13±1.27 weeks. No significant relationship was found when GA (gestational age) at birth and discharge were compared across VitD levels."

The average mean birth weight of our study group was 1540.17± 383.01 grams. >90% of the preterm neonates were more than 1kg. The mean discharge weight was 1568.22±340.08 grams. No significant relation was found when mean birth and discharge weight were compared across VitD levels.

The mean APGAR at 1 and 5 minutes of birth was 6.27±1.82 and 8.16±1.22 respectively. Nil significant association was found between mean APGAR scores and VitD levels.

The mean Silverman Andersen scoring in the study group was 3.49±3.28. 26 out of 70 (37.14%) preterm neonates had respiratory distress syndrome. When SAS was compared with RDS, SAS directly correlated with RDS with SAS >7 had a 100 % chance of developing RDS. "No significant association was found between SAS and VitD levels."

"Preterm neonates who had received a complete course of antenatal steroids had less chance of developing RDS."

A significant association was found when RDS was compared across VitD levels where preterm neonates with inadequate (deficiency + insufficiency) VitD levels had an increased chance of developing RDS. When the severity of RDS was compared with VitD levels, no association was found. Preterm neonates with Inadequate VitD levels require at least one form of respiratory support (Mechanical ventilation, CPAP, or Oxygen by nasal prongs).12(17.14%) out of 70 preterm neonates received surfactant. "No significant association was found between surfactant requirement and VitD levels."

8 out of 70 (11.43%) neonates had developed neonatal sepsis. 5 out of 8 neonates who developed sepsis had VitDD. However, the results were not significant statistically.

The mean day of life on which enteral nutrition was started was 2 ± 1.75 SD. The mean Day of life on which complete enteral nutrition was achieved was 5.97 ± 3.87 SD. Nil association was found when compared across VitD levels.

The average mean duration of hospital stay of the preterm neonates was 20.46 ± 8.04 SD. No association was found when compared across VitD levels.

When HNNE scores at 40 weeks PMA were compared across VitD levels, no significant association was found.

LIMITATIONS OF THE STUDY

- Neonatal VitD levels were not correlated with Maternal VitD levels.
- Though we found a clinical difference in the neonatal health outcomes with VitDD, the results were not statistically significant due warranting further studies with greater sample size.
- There are no universal criteria for the diagnosis of VitDD.We found variations with the criteria defined for VitDD in studies conducted in India and across the world which made comparison difficult with other studies.

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ANNEXURE I – CONSENT FORM

STATEMENT OF CONSENT

Patient no:

Project title: Prevalence of VitD deficiency and its association among preterm neonates with respiratory distress syndrome

Name of principal investigator: _____

Contact address:

The content of the provided information sheet, has been carefully read by me/explained to me in detail, in a language that I comprehend and have understood its content. I confirm that I have had the opportunity to ask questions.

The nature and purpose of study and its potential risks/benefits and expected duration of study, and relevant details of study have been explained to me in detail. I understand that the participation of my newborn in this study is voluntary and that I am free to withdraw at any time without giving reasons, without my medical care or legal rights being affected.

I understand that information collected about me from my participation in this research and section of any of my medical notes, may be looked at by responsible individuals from regulatory authorities where relevant. I give permission for these individuals to have access to my records.

Signature of the subject/left thumb impression & date: _____

Place: _____

Name of the participant: _____

Name of the parent/guardian: _____

Signature of Parent /Guardian: _____

This is to certify that above consent has been obtained in my presence.

ANNEXURE II – PROFORMA

Name: **IP Number:**

1.Age of mother: …… **LMP:** **EDD:**

2.Antenatal risk factors: Diabetes /Hypertension/Thyroid/PROM

3.Mode of delivery: Normal vaginal delivery/LSCS/Assisted: Ventouse/Forceps

Indication:

4.Liquor: Clear/ Meconium Stained

5.Antenatal steroid intake: Complete/Incomplete/Not taken

6.Infant's details:

Ip number:

DOB:

Sex: Male/Female/Ambiguous

Gestational age:

Birth weight: .....

7.Resuscitation Details (at delivery room):

Cried at birth: Yes/No

Bag and mask ventilation: Yes/No

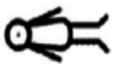
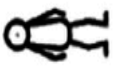
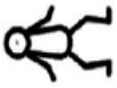
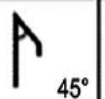
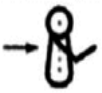
CPAP: Yes/No

Intubated: Yes/No

APGAR: /10 (1min) /10 (5min) /10 (10min)

Apgar score	0	1	2
Heart rate	Absent	<100	>100
Respiration	Absent	Weak cry	Good cry
Muscle tone	Limp	Some flexion	Active motion
Reflex irritability	No response	Grimace	Cry/Active withdrawal
Skin colour	Blue or pale	Acrocyanotic	Completely pink

Neuromuscular Maturity

Score	-1	0	1	2	3	4	5
Posture							
Square window (wrist)	 >90°	 90°	 60°	 45°	 30°	 0°	
Arm recoil		 180°	 140°–180°	 110°–140°	 90°–110°	 <90°	
Popliteal angle	 180°	 160°	 140°	 120°	 100°	 90°	 <90°
Scarf sign							
Heel to ear							

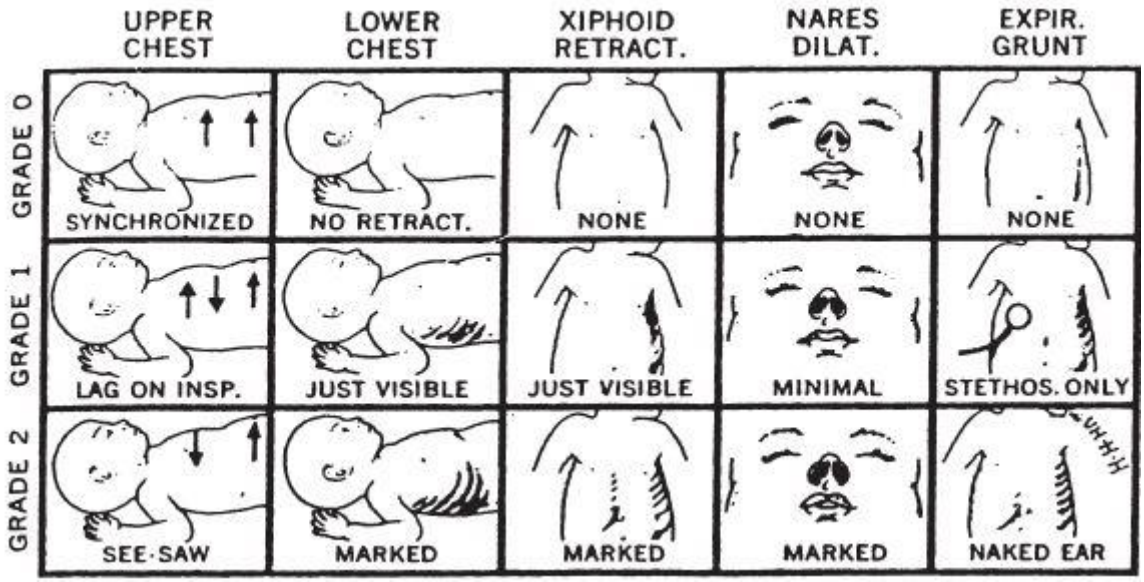
Physical Maturity

Skin	Sticky, friable, transparent	Gelatinous, red, translucent	Smooth, pink; visible veins	Superficial peeling and/or rash; few veins	Cracking, pale areas; rare veins	Parchment, deep cracking; no vessels	Leathery, cracked wrinkled
Lanugo	None	Sparse	Abundant	Thinning	Bald areas	Mostly bald	
Plantar surface	Heel-toe 40-50 mm: –1 <40 mm: –2	>50 mm, no crease	Faint red marks	Anterior transverse crease only	Creases anterior ⅔	Creases over entire sole	
Breast	Imperceptible	Barely perceptible	Flat areola, no bud	Stippled areola, 1–2 mm bud	Raised areola, 3–4 mm bud	Full areola, 5–10 mm bud	
Eye/Ear	Lids fused loosely: –1 tightly: –2	Lids open; pinna flat; stays folded	Slightly curved pinna; soft; slow recoil	Well curved pinna; soft but ready recoil	Formed and firm, instant recoil	Thick cartilage, ear stiff	
Genitals (male)	Scrotum flat, smooth	Scrotum empty, faint rugae	Testes in upper canal, rare rugae	Testes descending, few rugae	Testes down, good rugae	Testes pendulous, deep rugae	
Genitals (female)	Clitoris prominent, labia flat	Clitoris prominent, small labia minora	Clitoris prominent, enlarging minora	Majora and minora equally prominent	Majora large, minora small	Majora cover clitoris and minora	

Maturity Rating

Score	Weeks
-10	20
-5	22
0	24
5	26
10	28
15	30
20	32
25	34
30	36
35	38
40	40
45	42
50	44

8. Silverman Andersen <u>scoring system</u>(On admission):



Total Score: .....

9. Serum 25(OH) vitamin D level:

10.Other investigations:

11.Culture positive sepsis: Yes/No

12.NICU admission:

	Yes	No	Duration(in days)
SIMV			
CPAP			
O2			

13. Hypotension: Yes/No

14.Ionotopes: Yes/No Duration:

15.Enteral nutrition:

Day of starting: Started with:

16.Day of starting full enteral nutrition:

17. Treatment given (tick the box if any):

IV fluids: ☐

Antibiotics: ☐

Surfactant administration: ☐ Doses: _____

Others:

18.Status @ discharge:

Head circumference:

Weight:

Length:

HNNE:

19. Date of admission:

Date of discharge:

Duration of hospital stay:

HAMMERSMITH NEONATAL NEUROLOGICAL EXAMINATION

POSTURE	arms & legs extended or very slightly flexed	legs slightly flexed	leg well-flexed but not adducted	leg well flexed & adducted near to abdomen	abnormal posture: opisthotonus a) arms flexed, b) legs extended
ARM RECOIL	arms do not flex	arms flex slowly not always; not completely	arms flex slowly; more complete	arms flex quickly and completely	arms difficult to extend; snap back forcefully
ARM TRACTION	arms remain straight; no resistance	arms flex slightly or some resistance felt	arms flex well till shoulder lifts, then straighten	arms flex to approx. 100° & maintained as shoulder lifts	flexion of arms <100°; maintained when body lifts up
LEG RECOIL	No flexion	incomplete or variable flexion	complete but slow flexion	complete fast flexion	legs difficult to extend; snap back forcefully
LEG TRACTION	legs straight - no resistance	legs flex slightly or some resistance felt	legs flex well till bottom lifts up	knee flexes & remains flexed when bottom up	flexion stays when back & bottom up
POPLITEAL ANGLE	180°	≈ 150°	≈110°	≈90°	<90°
HEAD CONTROL (1)	no attempt to raise head	infant tries: effort better felt than seen	raises head but drops forward or back	raises head: remains vertical	
HEAD CONTROL (2)	no attempt to raise head	infant tries: effort better felt than seen	raises head but drops forward or back	raises head: remains vertical; it may wobble	head upright or extended; cannot be passively flexed
HEAD LAG	head drops & stays back	tries to lift head but it drops back	able to lift head slightly	lifts head in line with body	head in front of body
VENTRAL SUSPENSION	back curved, head & limbs hang straight	back curved, head ↓, limbs slightly flexed	back slightly curved, limbs flexed	back straight, head in line, limbs flexed	back straight, limbs above body

1	.5	2	.5	3	.5	4	.5	5	
3	0	9	6	60	9	12	0	1	25-27w
1	0	6	2	61	16	12	1	1	28-29w
2	0	4	2	65	17	8	0	2	30-31w
0	0	0	2	81	4	9	0	4	32-34w
0	0	0	0	6	3	90	1	0	Full term

3	1	9	9	44	9	23	2	0	25-27w
1	1	3	4	42	15	33	0	1	28-29w
1	0	8	3	42	10	36	0	0	30-31w
0	0	2	2	54	15	25	0	2	32-34w
0	0	5	2	22	3	67	1	0	Full term

3	0	17	5	51	10	14	0	0	25-27w
7	1	14	7	45	8	18	0	0	28-29w
7	2	15	4	51	7	14	0	0	30-31w
6	2	25	0	59	4	4	0	0	32-34w
0	0	1	0	22	8	69	0	0	Full term

3	0	14	4	18	5	52	0	4	25-27w
0	0	5	2	24	5	62	0	2	28-29w
0	0	10	2	34	2	50	0	2	30-31w
0	0	9	0	38	2	49	0	2	32-34w
0	0	3	1	4	1	91	0	0	Full term

3	1	17	6	35	6	27	1	4	25-27w
1	1	17	2	36	6	35	1	1	28-29w
2	0	21	8	38	5	25	0	1	30-31w
0	4	29	10	43	2	10	0	2	32-34w
0	0	0	1	12	12	72	0	3	Full term

3	0	22	8	46	6	14	0	0	25-27w
5	1	16	5	48	7	17	1	0	28-29w
2	0	15	10	53	5	15	0	0	30-31w
2	0	26	4	49	4	13	0	2	32-34w
0	0	5	5	19	20	51	0	0	Full term

3	0	17	4	46	9	21	0	0	25-27w
0	0	13	5	46	12	24	0	0	28-29w
3	0	14	2	48	13	20	0	0	30-31w
4	0	15	4	55	4	18	0	0	32-34w
0	0	0	6	26	12	56	0	0	Full term

3	0	3	5	57	11	21	0	0	25-27w
1	2	6	4	50	13	24	0	0	28-29w
1	0	2	2	63	11	21	0	0	30-31w
0	0	4	2	77	2	15	0	0	32-34w
0	0	0	4	29	15	52	0	0	Full term

3	3	27	13	36	3	15	0	0	25-27w
3	3	18	7	40	14	15	0	0	28-29w
7	3	16	5	46	7	16	0	0	30-31w
4	0	21	4	56	0	15	0	0	32-34w
0	0	9	4	44	12	31	0	0	Full term

0	0	21	11	38	11	15	4	0	25-27w
3	0	25	8	44	8	10	0	2	28-29w
3	0	22	8	47	5	14	1	0	30-31w
2	0	17	2	56	2	19	0	2	32-34w
0	0	4	5	47	16	28	0	0	Full term

TONE PATTERN ITEMS

FLEXOR TONE (compare arm and leg traction)		arm flexion < leg flexion	arm flexion = leg flexion	arm flexion > leg flexion; difference ≤1 column	arm flexion > leg flexion; difference > 1 column
FLEXOR TONE (resting posture)			arms and legs generally flexed	strong arm flexion with strong leg extension *intermittent*	strong arm flexion with strong leg extension *continuous*
LEG TONE (leg traction and popliteal angle)		leg traction > popliteal angle	leg traction = popliteal angle	leg traction < popliteal angle; difference ≤1 column	leg traction < popliteal angle; difference > 1 column
HEAD CONTROL (sitting)		neck extension < neck flexion	neck extension = Neck flexion	neck extension > neck flexion; difference ≤ 1 column	neck extension > neck flexion; difference > 1 column
NECK AND AXIAL TONE (horizontal)		ventral suspension < head lag	ventral suspension = head lag	ventral suspension > head lag; difference ≤1 column	ventral suspension > head lag; difference > 1 column

1	**.5**	**2**	**.5**	**3**	**.5**	**4**	**.5**	**5**	
0	0	45	0	27	<1	27	0	1	**25-27w**
0	0	40	<1	40	0	20	<1	0	**28-29w**
0	0	34	<1	47	<1	18	0	1	**30-31w**
0	0	38	<1	36	<1	24	<1	2	**32-34w**
0	0	25	3	53	0	18	0	<1	**Full term**

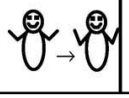
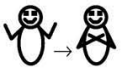
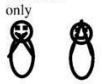
0	0	0	0	99	<1	0	0	1	**25-27w**
0	0	0	0	96	<1	3	0	1	**28-29w**
0	0	0	0	96	<1	2	0	2	**30-31w**
0	0	0	0	94	<1	2	0	4	**32-34w**
0	0	0	0	99	0	<1	0	<1	**Full term**

0	0	43	<1	34	0	21	<1	1	**25-27w**
0	0	41	0	39	<1	19	0	1	**28-29w**
0	0	38	0	36	<1	22	<1	4	**30-31w**
0	0	19	<1	50	<1	29	<1	2	**32-34w**
0	0	4	0	57	0	35	0	1	**Full term**

0	0	25	0	64	0	9	0	2	**25-27w**
0	0	17	0	70	0	13	0	0	**28-29w**
0	0	18	0	76	0	6	0	0	**30-31w**
0	0	23	0	64	0	13	0	0	**32-34w**
0	0	3	0	94	0	3	0	<1	**Full term**

0	0	20	0	39	0	35	0	6	**25-27w**
0	0	31	0	42	0	26	0	1	**28-29w**
0	0	24	0	49	0	26	0	1	**30-31w**
0	0	17	0	51	0	28	0	4	**32-34w**
0	0	24	0	58	0	18	0	<1	**Full term**

REFLEX ITEMS

TENDON REFLEX	absent	felt, not seen	seen	'exaggerated'	clonus
SUCK/GAG	no gag / no suck	weak irregular suck only: no stripping	weak regular suck some stripping	strong suck: (a) irregular (b) regular good stripping	no suck but strong clenching
PALMAR GRASP	no response R L	short, weak flexion of fingers R L	strong flexion of fingers R L	strong finger flexion, shoulder ↑ R L	very strong grasp; infant can be lifted off couch R L
PLANTAR GRASP	no response R L	partial plantar flexion of toes R L	toes curve around the examiner's finger R L		
PLACING	no response R L	dorsi - flexion of ankle onl y R L	full placing response with flexion of hip, knee & placing sole on surface R L		
MORO REFLEX	no response or opening of hands only	full abduction at shoulder and extension of the arms; no adduction 	full abduction but only delayed or partial adduction 	partial abduction at shoulder and extension of arms followed by smooth adduction 	• no abduction or adduction; • only forward extension of arms from the shoulders • marked adduction only  or

1	.5	2	.5	3	.5	4	.5	5	
0	0	9	0	55	7	13	3	13	**25-27w**
0	0	12	0	50	7	22	4	5	**28-29w**
0	0	24	1	52	1	13	0	9	**30-31w**
0	0	18	0	57	0	17	4	4	**32-34w**
<1	0	21	0	78	0	<1	0	<1	**Full term**

0	0	1	0	3	3	93	0	0	**25-27w**
0	0	3	0	7	0	90	0	0	**28-29w**
0	0	0	0	6	2	92	0	0	**30-31w**
0	0	4	0	10	0	86	0	0	**32-34w**
0	0	1	0	5	0	92	0	2	**Full term**

0	0	5	0	47	7	30	1	10	**25-27w**
0	0	3	1	40	8	43	1	4	**28-29w**
0	0	1	0	51	3	35	0	10	**30-31w**
0	0	7	0	53	3	30	0	7	**32-34w**
<1	0	6	0	84	0	9	0	<1	**Full term**

0	0	4	1	95	0	0	0	0	**25-27w**
0	1	5	2	92	0	0	0	0	**28-29w**
0	0	2	1	97	0	0	0	0	**30-31w**
0	0	2	2	96	0	0	0	0	**32-34w**
<1	0	2	0	98	0	0	0	0	**Full term**

5	2	12	3	78	0	0	0	0	**25-27w**
0	2	12	6	80	0	0	0	0	**28-29w**
1	0	8	8	83	0	0	0	0	**30-31w**
0	0	4	0	96	0	0	0	0	**32-34w**
1	0	18	0	81	0	0	0	0	**Full term**

0	0	13	1	61	4	20	0	1	**25-27w**
0	0	12	1	64	6	15	1	1	**28-29w**
0	0	12	1	51	3	28	0	5	**30-31w**
0	0	23	0	46	2	27	0	2	**32-34w**
0	0	1	0	20	0	79	0	0	**Full term**

MOVEMENTS AND ABNORMAL SIGNS

a

SPONTANEOUS MOVEMENT (quantity)	no movement	sporadic and short isolated movements	frequent isolated movements	frequent generalized movements	continuous exaggerated movements
SPONTANEOUS MOVEMENT (quality)	only stretches	stretches and random abrupt movements Some smooth movements	fluent movements but mono-tonous	fluent alternating movements of arms + legs; good variability	cramped synchr-onous mouthing jerky or other abnormal movement
HEAD RAISING	no movement	infant rolls head over, chin not raised	infant raises chin, rolls head over	infant brings head and chin up	infant brings head up and keeps it up

1	.5	2	.5	3	.5	4	.5	5	
0	0	15	3	28	3	51	0	0	25-27w
0	0	17	3	26	11	43	0	0	28-29w
0	0	13	0	31	8	48	0	0	30-31w
0	0	20	0	27	0	51	0	2	32-34w
<1	0	3	0	5	0	92	0	<1	Full term

0	0	16	4	42	11	23	1	3	25-27w
0	0	22	5	35	1	23	2	2	28-29w
0	0	20	6	34	2	36	0	2	30-31w
0	0	21	0	15	0	60	0	4	32-34w
2	0	5	0	<1	0	93	0	<1	Full term

0	0	36	6	34	6	14	1	3	25-27w
1	1	35	4	34	9	14	1	1	28-29w
1	1	40	5	28	1	21	1	2	30-31w
0	0	40	0	30	4	22	2	2	32-34w
<1	0	10	0	50	0	40	0	<1	Full term

b

ABN. HAND OR TOE POSTURES		hands open, toes straight most of the time	intermittent fisting or thumb adduction	continuous fisting or thumb adduction; index finger flexion, thumb opposition	continuous big toe extension or flexion of all toes
TREMOR		no trem or or trem or onl y when cry ing	tremor only after Moro or occasionally when awake	frequent tremors when awake	continuous tremors
STARTLE	no startle even to sudden noise	no spontan -eous startle but reacts to sudden noise	2-3 spontaneous startles	more than 3 spontaneous startles	continuous startles

1	.5	2	.5	3	.5	4	.5	5	
0	0	57	4	37	0	2	0	0	25-27w
0	0	64	6	28	0	2	0	0	28-29w
0	0	67	1	30	1	1	0	0	30-31w
0	0	75	2	21	0	2	0	0	32-34w
0	0	85	0	12	0	3	0	<1	Full term

0	0	43	1	29	8	16	0	3	25-27w
0	0	43	0	27	9	19	2	0	28-29w
0	0	54	0	24	3	19	0	0	30-31w
0	0	62	0	30	0	4	0	4	32-34w
0	0	88	0	12	0	<1	0	<1	Full term

22	0	40	7	20	1	10	0	0	25-27w
23	1	35	7	30	2	2	0	0	28-29w
37	1	32	1	25	1	3	0	0	30-31w
50	0	35	0	9	0	6	0	0	32-34w
<1	0	94	0	6	0	<1	0	<1	Full term

BEHAVIORAL SIGNS, VISION, HEARING

EYE APPEARANCE	**does not open eyes**		**full conjugated eye mov**	**transient nystagmus strabismus roving eye movemens sunsetting sign**	**persistent nystagmus strabismus roving eye movements dow nward deviation**
AUDITORY ORIENTATION	**no reaction**	**auditory startle; Brightens and stills; no true orientati - on**	**shifting of eyes, head might turn towards source**	**prolonged head turn to stimulus; search with eyes; smooth**	turns head and eyes towards noise every time; jerky abrupt
VISUAL ORIENTATION	**does not follow or focus on stimuli**	**stills, focuses follows briefly to the side but loses stimuli**	**follows horizontal -ly and vertically; no head turn**	**follows horizon -tally and vertically; turns head**	follows in a circle
ALERTNESS	**will not respond to stimuli**	**when awake, looks only briefly**	**when awake, looks at stimuli but loses them**	**keeps interest in stimuli**	does not tire (hyper-reactive)
IRRITABILITY	**quiet all the time, not irritable to any stimuli**	**awakes, cries some -times when handled**	**cries often when handled**	**cries always when handled**	cries even when not handled
CONSOLABILITY	**not crying consoling not needed**	**cries briefly; consol -ing not needed**	**cries; becomes quiet when talked to**	**cries; needs picking up to console**	cries cannot be consoled
CRY	**no cry at all**	**whimpe -ring cry only**	**cries to stimuli but normal pitch**		high pitched cry; often continuous

1	.5	2	.5	3	.5	4	.5	5	
6	0	0	0	74	4	16	0	0	25-27w
2	0	0	0	80	2	15	1	0	28-29w
5	0	0	0	80	2	13	0	0	30-31w
4	0	0	0	87	2	7	0	0	32-34w
7	0	0	0	92	0	1	0	<1	Full term

5	1	28	0	57	1	8	0	0	25-27w
2	0	23	10	50	6	9	0	0	28-29w
5	1	27	7	51	1	8	0	0	30-31w
3	0	14	0	73	3	7	0	0	32-34w
<1	0	30	0	50	0	20	0	<1	Full term

6	0	7	2	25	3	26	9	22	25-27w
0	0	7	1	33	7	21	15	16	28-29w
1	0	9	0	27	5	25	10	23	30-31w
0	0	10	0	42	10	38	0	0	32-34w
<1	0	7	0	41	0	51	0	1	Full term

6	0	22	1	48	3	20	0	0	25-27w
1	0	17	4	60	3	14	1	0	28-29w
0	0	21	1	43	2	33	0	0	30-31w
0	0	7	3	54	0	36	0	0	32-34w
1	0	2	0	48	0	49	0	<1	Full term

12	1	52	0	31	0	3	0	1	25-27w
16	2	47	2	27	1	5	0	0	28-29w
27	0	47	1	22	0	2	0	1	30-31w
23	0	49	0	23	0	5	0	0	32-34w
<1	0	93	0	5	0	2	0	<1	Full term

10	0	29	0	29	3	29	0	0	25-27w
17	1	19	2	29	7	22	1	2	28-29w
27	0	18	0	28	2	22	1	2	30-31w
23	0	9	0	32	2	28	0	6	32-34w
1	0	41	0	45	0	12	0	<1	Full term

11	0	11	0	78	0	0	0	0	25-27w
16	0	5	2	77	0	0	0	0	28-29w
26	1	3	1	69	0	0	0	0	30-31w
23	0	6	2	69	0	0	0	0	32-34w
<1	0	7	0	92	0	0	0	1	Full z

ANNEXURE III – MASTER CHART

sl no	NAME	GENDER	AGE OF MOTHER	APGAR AT 1MIN	APGAR AT 5 MIN	MODE OF DELIVERY	GA AT BIRTH IN WEEKS	BALLARD SCORING	GA AT DISCHARGE IN WEEKS	STEROID INTAKE	VITAMIN D LEVELS IN NG/ML	VITAMIN D DEFICIENCY	SILVERMAN ANDERSON SCORING	RDS	XRAY	VENTILATOR	CPAP	OXYGEN FU	SURFACTANT ADMINISTRATION	DURATION OF HOSPITAL STAY IN DAYS	BLOOD CULTURE POSITIVE SEPSIS	STARTING FEEDS DAY	FEEDS STARTED WITH	COMPLETE ENTERAL NUTRITION DAY	BIRTH WEIGHT IN GRAMS	DISCHARGE WEIGHT	HNNE SCORE AT 40 WEEKS
1	BABY OF PRASSANA KUMARI	MALE	27	6	8	NVD	33+4	27,34-36WEEKS	34+3	COMPLETE	17.5	+	0	-	-	-	-	YES (4HRS)	-	11	NO	1	SF	2	1526	1570	32
2	BABY OF SUMAYYA	MALE	26	8	9	NVD	33+2	25,34-36WEEKS	34+4	COMPLETE	16.2	+	1	-	-	-	-	-	-	11	NO	1	SF	2	2080	1910	28
3	BABY OF PRADNYA	FEMALE	30	6	7	LSCS	29+6	15,30WEEKS	35+4	COMPLETE	11.5	+	5	+	HMD 3	6 DAYS	3DAYS	1DAY	2DOSES ON D1 AND D2 OF LIFE	40	NO	8	RT	12	1000	1170	32
4	BABY OF GOURI	MALE	30	6	8	NVD	33+5	21,32-34WEEKS	36+2	INCOMPLETE	31	-	5	-	-	-	1DAY	1DAY	-	16	NO	2	RT	5	2260	2270	32
5	BABY OF TANUJA 1	FEMALE	25	7	8	LSCS	31+3	21,32-34WEEKS	35+3	COMPLETE	6.27	+	0	-	-	-	-	-	-	28	NO	1	RT	4	1500	1400	32
6	BABY OF TANUJA 2	MALE	25	6	8	LSCS	31+3	22,32-34WEEKS	35+3	COMPLETE	4.56	+	4	-	-	-	2DAYS	-	-	28	NO	1	RT	5	1200	1120	30
7	BABY OF SNEHA SUDDIR	MALE	28	7	9	LSCS	32+5	22,32-34WEEKS	35	COMPLETE	16.9	+	8	+	HMD 3	1DAY	5DAYS	1DAY	1 DOSE ON 24/11/23	16	NO	3	RT	9	1660	1560	16
8	BABY OF CHANDANA	FEMALE	21	6	9	LSCS	33+5	27,34-36WEEKS	35+2	COMPLETE	5.54	+	5	+	HMD 2	-	2DAYS	-	-	11	NO	1	RT	4	1464	1400	33
9	BABY OF RAJALAKSHMI	FEMALE	33	2	6	LSCS	30+3	17,30-32WEEKS	34+3	COMPLETE	12.36	+	10	+	HMD 3	1DAY	3DAYS	-	1 DOSE OF NEOSURF ON 12/12/23	28	NO	2	RT	7	1260	1500	33
10	BABY OF DEEPALI TUKARAM	FEMALE	31	5	8	LSCS	33+3	18,30-32WEEKS	35+6	COMPLETE	28.6	-	5	+	HMD 1	-	2	1	-	17	NO	2	RT	5	1580	1540	32
11	BABY OF KAVERI 1	FEMALE	22	6	7	NVD	32	26,34-36WEEKS	35+5	COMPLETE	18.1	+	6	+	HMD 2	-	2DAYS	-	-	26	NO	3	RT	4	1760	1580	34
12	BABY OF KAVERI 2	MALE	22	6	8	NVD	32	25,34WEEKS	35+5	COMPLETE	12.5	+	6	-	-	-	1DAY	-	-	26	NO	1	RT	3	1840	1870	33
13	BABY OF ZEBA	MALE	21	7	9	LSCS	30+6	18,30-32WEEKS	34+2	COMPLETE	14.3	+	0	-	-	-	-	-	-	24	NO	1	RT	3	1650	1720	31
14	BABY OF JYOTI 1	MALE	30	7	8	LSCS	31+5	20,32WEEKS	34+1	COMPLETE	13.2	+	6	-	-	-	1DAY	-	-	17	NO	2	RT	5	1480	1500	33
15	BABY OF VARSHA 1	MALE	31	9	10	LSCS	32+6	23,32-34WEEKS	34+5	COMPLETE	6.2	+	0	-	-	-	-	-	-	13	NO	1	RT	3	1800	1730	30
16	BABY OF VARSHA 2	MALE	31	9	10	LSCS	32+6	23,32-34WEEKS	34+5	COMPLETE	3	+	1	-	-	-	-	-	-	13	NO	1	RT	3	2160	1970	30
17	BABY OF DEEPA SOMAYYA ATTIGAD	MALE	30	7	8	NVD	32+2	21,32-34WEEKS	36	INCOMPLETE	8.6	+	0	-	-	-	-	-	-	18	NO	1	RT	3	1920	1860	31
18	BABY OF SHANTATAI	MALE	48	2	6	LSCS	29+2	13,28-30WEEKS	34+1	INCOMPLETE	40.9	-	10	+	HMD 3	2	1	2	1 DOSE OF NEOSURF	34	NO	1	RT	6	1100	1130	32
19	BABY OF TRUPTI 1	FEMALE	40	7	9	LSCS	34+2	25,34WEEKS	35+4	COMPLETE	49.6	-	3	-	-	-	1DAY	6HRS	-	10	NO	1	RT	4	1600	1520	26
20	BABY OF TRUPTI 2	MALE	40	7	9	LSCS	34+2	22,30-34WEEKS	35+4	COMPLETE	49.4	-	3	-	-	-	12HRS	6HRS	-	10	NO	1	RT	3	2500	2400	29
21	BABY OF DIPIKA	MALE	29	7	8	LSCS	31	15,30WEEKS	33+5	COMPLETE	12.38	+	5	-	-	-	1	-	-	17	NO	2	RT	6	1370	1420	32
22	BABY OF AMBIKA1	MALE	22	7	9	LSCS	30+5	15,30WEEKS	35+2	COMPLETE	105.1	-	0	-	-	-	-	-	-	32	NO	2	RT	4	1510	1830	31
23	BABY OF AMBIKA 2	FEMALE	22	7	9	LSCS	30+5	14,28-30WEEKS	35+2	COMPLETE	120	-	2	-	-	-	-	-	-	32	NO	2	RT	4	1150	1510	32
24	BABY OF SUPRIYA	FEMALE	24	7	9	LSCS	33+3	25,34WEEKS	37+3	COMPLETE	18.3	+	0	-	-	-	-	1	-	28	NO	3	SF	4	1170	1340	32
25	BABY OF KAVYA	FEMALE	22	2	5	LSCS	31+2	16,30-32WEEKS	34+4	INCOMPLETE	29.2	-	8	+	HMD 3	2DAYS	3DAYS	-	1 DOSE OF NEOSURF 6ML ON 8/9/23	21	NO	2	RT	5	1200	1320	25
26	BABY OF SHRIYA	FEMALE	20	7	8	LSCS	31+6	18,30-32WEEKS	35+3	COMPLETE	16.58	+	7	+	HMD 3	-	4DAYS	-	1 DOSE OF NEOSURF 3ML ON 8/9/23	25	NO	2	RT	4	987	1220	32
27	BABY OF NEETA PAWATE	FEMALE	34	7	9	LSCS	33+1	20,32WEEKS	34+6	COMPLETE	23.45	-	0	-	-	-	-	-	-	12	NO	1	SF	2	1900	1800	32
28	BABY OF KAVERI	MALE	28	7	9	LSCS	33	23,32-34WEEKS	35+5	INCOMPLETE	12.7	+	6	+	HMD 2	-	2.5DAYS	12HRS	-	10	NO	1	RT	6	1710	1680	31
29	BABY OF SHIFA	FEMALE	24	1	5	NVD	33+3	23,32-34WEEKS	34+3	COMPLETE	3	+	2	-	-	-	-	1DAY	-	8	NO	2	SF	3	1920	1870	33
30	BABY OF POONAM	FEMALE	28	7	9	LSCS	31+1	16,30-32WEEKS	34+5	COMPLETE	10.76	+	6	+	HMD 2	-	4DAYS	1DAY	-	24	NO	4	RT	12	1316	1300	31
31	BABY OF SHRUTI VEERESH	FEMALE	30	7	8	NVD	32+6	20,32WEEKS	35+5	COMPLETE	3.47	+	0	-	-	-	-	-	-	8	NO	1	SF	2	1880	1820	29
32	BABY OF UJJAWALA	MALE	35	7	9	NVD	32+5	21,32-34WEEKS	35+5	COMPLETE	20.2	-	0	-	-	-	-	-	-	22	YES	10	SF	12	1590	1680	33
33	BABY OF NEETA	MALE	22	5	7	NVD	32	18,30-32WEEKS	34+5	COMPLETE	8.1	+	6	-	-	-	1DAY	2DAYS	-	12	NO	2	RT	5	1860	1780	33
34	BABY OF MAHALAKSHMI	MALE	29	4	5	LSCS	27+6	8,26-28WEEKS	exp	COMPLETE	9.3	+	5	-	-	1DAY	11HRS	3DAYS	-	EXP	YES	2	RT	EXP	1020	-	-
35	BABY OF GEETA	FEMALE	38	5	7	NVD	33	-	EXP	NOT TAKEN	3	+	8	+	HMD2	1DAY	16HRS	-	-	EXP	NO	EXP	EXP	EXP	1540	-	-
36	BABY OF SHAMBAVI	FEMALE	24	7	9	LSCS	30+1	-	EXP	INCOMPLETE	7.2	+	5	-	-	EXP	EXP	EXP	-	EXP	NO	EXP	EXP	EXP	1450	-	-
37	BABY OF SUDHA	MALE	24	2	7	LSCS	32+5	17 , 30-32WEEKS	36+2	INCOMPLETE	6.44	+	4	+	HMD 2	3DAYS	-	1DAY	-	25	NO	2	RT	10	1490	1550	30
38	BABY OF AALIYA	MALE	28	8	9	LSCS	34+1	24,32-34WEEKS	36+3	NOT TAKEN	23.6	-	0	-	-	-	-	-	-	15	NO	1	RT	4	1670	1700	32

sl no	NAME	GENDER	AGE OF MOTHER	APGAR AT 1MIN	APGAR AT 5 MIN	MODE OF DELIVERY	GA AT BIRTH IN WEEKS	BALLARD SCORING	GA AT DISCHARGE IN WEEKS	STEROID INTAKE	VITAMIN D LEVELS IN NG/ML	VITAMIN D DEFICIENCY	SILVERMAN ANDERSON SCORING	RDS	XRAY	VENTILATOR	CPAP	OXYGEN FU	SURFACTANT ADMINISTRATION	DURATION OF HOSPITAL STAY IN DAYS	BLOOD CULTURE POSITIVE SEPSIS	STARTING FEEDS DAY	FEEDS STARTED WITH	COMPLETE ENTERAL NUTRITION DAY	BIRTH WEIGHT IN GRAMS	DISCHARGE WEIGHT	HNNE SCORE AT 40 WEEKS
39	BABY OF ARCHANA 1	MALE	22	7	9	LSCS	33+4	24,32-34WEEKS	37+5	COMPLETE	19.9	+	0	-	-	-	-	-	-	21	NO	1	SF	3	1440	1560	31
40	BABY OF ARCHANA 2	MALE	22	7	9	LSCS	33+4	24,32-34WEEKS	37+5	COMPLETE	13	+	0	-	-	-	-	-	-	21	NO	1	SF	3	1520	1630	31
41	BABY OF SAMEEN BANU	MALE	34	7	8	LSCS	32+5	25,34WEEKS	35+5	COMPLETE	6.09	+	3	-	-	-	2	1	-	14	NO	2	RT	7	1500	1480	32
42	BABY OF PARIMALA	MALE	28	6	7	LSCS	32+1	24,32-34WEEKS	35+4	COMPLETE	17.8	+	5	+	HMD 3	-	2	1	-	20	NO	2	RT	6	1420	1400	32
43	BABY OF AISHWARYA 1	FEMALE	30	7	9	LSCS	31+5	20,32WEEKS	37+2	COMPLETE	40.1	-	0	-	-	-	1 IVO AOP	1	-	29	YES	1	RT	14	1500	1550	32
44	BABY OF AISHWARYA 2	MALE	30	7	9	LSCS	31+5	19,30-32WEEKS	37+2	COMPLETE	31.7	-	4	-	-	7DAYS IVO AOP AND EOS	2	1	-	29	YES	1	RT	14	1240	1580	33
45	BABY OF ROOPA MANJUNATH GEJJI	FEMALE	24	8	9	NVD	30+5	15,30WEEKS	33+5	COMPLETE	25	-	0	-	-	-	-	-	-	21	NO	1	RT	7	1360	1440	32
46	BABY OF SEVANTI	MALE	39	4	8	LSCS	31+3	17,30-32WEEKS	36+2	COMPLETE	113	-	9	+	HMD 3	3DAYS	1DAY	-	1 DOSE OF NEOSURF	21	NO	3	RT	12	1180	1350	31
47	BABY OF ANNAPOORNA 1	MALE	45	7	8	LSCS	31+4	20,32WEEKS	36+1	COMPLETE	34.7	-	0	-	-	-	-	-	-	30	NO	1	RT	4	1120	1300	32
48	BABY OF ANNAPOORNA 2	MALE	45	4	7	LSCS	31+4	17,30-32WEEKS	36+1	COMPLETE	10.5	+	8	+	HMD 3	1.5DAYS	2 DAYS	1DAY	1 DOSE OF NEOSURF	30	NO	2	RT	10	1450	1570	32
49	BABY OF MANISHA YETOJI MOHAN	FEMALE	27	7	9	LSCS	29	16,30-32WEEKS	33+3	COMPLETE	13.9	+	0	-	-	-	-	-	-	30	NO	1	RT	5	1170	937	29
50	BABY OF NIKITA PATIL	MALE	27	7	8	NVD	29+5	17,30-32WEEKS	32+6	INCOMPLETE	10	+	7	+	HMD 1	-	2 DAYS	-	-	22	YES	2	RT	9	1250	1340	32
51	BABY OF DEEPA DYAMANGOUDA PATIL	FEMALE	27	6	7	LSCS	31	18,30-32WEEKS	34+3	COMPLETE	19.4	+	9	+	HMD 3	4DAYS	3DAYS	1DAY	1 DOSE OF NEOSURF	18	NO	5	RT	12	1690	1690	33
52	BABY OF SUREKHA	MALE	28	5	7	LSCS	33+3	27,34-36WEEKS	34+6	INCOMPLETE	12.1	+	7	+	HMD 1	-	12HRS	-	-	10	NO	2	RT	3	1470	1240	33
53	BABY OF JYOTI GOPAL KILABANUR	MALE	26	6	7	LSCS	33+6	30,36WEEKS	34+6	COMPLETE	3	+	5	+	HMD 1	-	1DAY	2DAYS	-	7	NO	1	RT	3	2830	2850	33
54	BABY OF ZEBA	MALE	28	9	10	LSCS	30+6	14,28-30WEEKS	34	COMPLETE	14	+	1	-	-	-	-	-	-	22	NO	1	RT	3	1650	1600	31
55	BABY OF VARSHA 1	MALE	31	9	10	LSCS	32+6	23,32-34WEEKS	34+5	COMPLETE	3	+	0	-	-	-	-	-	-	13	NO	1	RT	3	1800	1730	30
56	BABY OF VARSHA 2	MALE	31	9	10	LSCS	32+6	23,32-34WEEKS	34+5	COMPLETE	3	+	1	-	-	-	-	1DAY	-	13	NO	1	RT	3	2160	1970	30
57	BABY OF AISHWARYA YOGESH BHOPALE	MALE	23	7	8	LSCS	32+6	23,32-34WEEKS	36+1	INCOMPLETE	3	+	0	-	-	-	-	-	-	23	NO	2	RT	5	2400	2390	32
58	BABY OF VAIJANTA	MALE	30	7	9	LSCS	31+1	14,28-30WEEKS	34	COMPLETE	27	-	0	-	-	-	-	-	-	20	NO	1	RT	4	1500	1580	32
59	BABY OF UMA MALENAHALLI	MALE	32	7	8	LSCS	31+2	17,30-32WEEKS	37+1	COMPLETE	11.9	+	6	+	HMD 1	-	1DAY	4HRS	-	35	YES	1	RT	4	1420	1630	32
60	BABY OF SHAHNAZ MALIK	MALE	38	8	9	LSCS	29+5	11,28-30WEEKS	35+1	COMPLETE	13.8	+	4	+	HMD 2	-	2DAYS	-	-	28	NO	1	RT	4	1010	1210	29
61	BABY OF NIKHAT	FEMALE	24	5	7	LSCS	28+1	8,26-28WEEKS	33+5	COMPLETE	32.1	-	0	-	-	-	-	-	-	38	NO	1	RT	3	725	1120	32
62	BABY OF POOJA BHOGAN	FEMALE	27	2	6	LSCS	33	23,32-34WEEKS	36+3	COMPLETE	8.44	+	2	-	-	-	8 DAYS IVO TEF	-	-	20	NO	9	RT	20	1530	1460	31
63	BABY OF NEELA LAXMAN	MALE	25	8	9	NVD	29	13,28-30WEEKS	31+3	INCOMPLETE	17.7	+	3	-	-	-	-	3DAYS	-	17	NO	2	RT	7	1420	1230	30
64	BABY OF MRUNAL PRAVEEN	MALE	40	6	8	LSCS	29+4	20,32WEEKS	33+4	COMPLETE	30	-	0	-	-	-	-	-	-	27	NO	1	RT	3	1510	1720	33
65	BABY OF JAYASHRI	MALE	36	7	8	LSCS	33	25,34WEEKS	35	COMPLETE	15.1	+	0	-	-	-	-	1DAY	-	14	NO	3	SF	5	1800	1570	32
66	BABY OF SHRIDEVI 1	MALE	22	4	7	LSCS	29+1	13,28-30WEEKS	32+3	COMPLETE	11.1	+	10	+	HMD 3	1	3	-	1 DOSE OF NEOSURF	23	YES	3	RT	13	1050	933	33
67	BABY OF SHRIDEVI 2	MALE	22	4	7	LSCS	29+1	19,28-30WEEKS	32+3	COMPLETE	10.3	+	10	+	HMD 3	1	3	1	1 DOSE OF NEOSURF	23	YES	2	RT	14	997	931	30
68	BABY OF SUPRIYA	FEMALE	26	8	9	LSCS	32	28,34-36WEEKS	35+3	INCOMPLETE	21	-	0	-	-	-	-	-	-	19	NO	2	RT	7	1310	1400	33
69	BABY OF KAREMMA	MALE	37	8	10	LSCS	34	25,34WEEKS	36+6	INCOMPLETE	14	+	8	+	HMD 3	3	2	-	1 DOSE OF NEOSURF	20	NO	4	RT	12	1457	1410	31
70	BABY OF SUPRIYA RAKESH KUTRE	MALE	28	6	10	LSCS	33	21,32-34WEEKS	33+5	NOT TAKEN	14	+	6	+	HMD 1	-	2	-	-	5	NO	1	RT	3	1860	1730	32