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Preventive use of calcitriol versus cholecalciferol on biochemical markers of Metabolic Bone Disease (MBD) in very low birth weight infants: a pilot randomized clinical trial

Mohammad Bagher Hosseini[1]. Nafiseh Hosseini[2]. Taher Entezari-Maleki[*]. Zakieh Salimi[4]

1- Pediatric Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

2- Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

3- Assistant professor, Department of Clinical Pharmacy, Faculty of Pharmacy, Tabriz University of Medical Sciences.(corresponding author)

4- Neonatal Intensive Care Unit, Alzahra Teaching Hospital, Tabriz University of Medical Sciences, Tabriz, Iran

* Corresponding author: btentezari@gmail.com

Address: Department of Clinical Pharmacy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Daneshgah Street, Tabriz, Iran

Phone Num: 00989126350900

Abstract

About 55% of extremely-low-birth-weight (birth weight < 1000 g) and 23% of very-low-birth-weight infants (birth weight < 1500 g) suffer from metabolic bone disease (MBD). There are limited data on the use of calcitriol (1, 25-dihydroxycholecalciferol) to prevent or treat MBD in preterm infants. Therefore, this study aimed to compare the preventive effect of calcitriol and cholecalciferol on the biochemical markers of MBD in preterm infants.

This study was a pilot randomized controlled trial conducted in the Alzahra teaching hospital of Tabriz University of Medical Sciences. we randomized 72 very-low-birth-weight infants in two groups of calcitriol 0.25 µg/day and cholecalciferol 400 IU/day. Biochemical markers, including serum 25-hydroxyvitamin D, Alkaline phosphatase (ALP), Phosphorus (P), calcium (Ca), Parathyroid hormone (PTH), and tubular reabsorption of phosphate (TRP) levels were checked at baseline, three, and five weeks after medication, consecutively.

After three weeks of supplementation, infants in the cholecalciferol group had higher levels of serum 25-hydroxyvitamin D (P=0.001) and lower levels of urine phosphate (P=0.009); There were no significant differences in other biochemical markers. At the end of the fifth week, there was no significant difference between the two groups in terms of biochemical markers.

Conclusion: The study indicated that the use of cholecalciferol caused a lower urinary loss of phosphate in very-low birth-weight infants at a short time; however, these findings were not sustained during the study period.

Keywords: Metabolic Bone Disease, neonatal prematurity, calcitriol, cholecalciferol, 25-hydroxyvitamin D, biochemical markers

Introduction

Neonatal Metabolic Bone Disease (MBD) or osteopenia of prematurity is a condition characterized by the bone mineral content reduction in preterm infants. It may affect biochemical markers of bone metabolism.[1] This medical condition diagnosed with changes in biochemical markers, such as serum calcium (Ca), Phosphorus (P), and alkaline phosphates (ALP) levels usually presents within 6–16 weeks post-birth.[2,3] Prevalence of MBD is inversely associated with birth weight as well as gestational age. About 55% of the extremely-low birth-weight (birth weight < 1000 g) and 23% of the very-low-birth-weight infants (birth weight < 1500 g) suffer from MBD.[2]

Pathogenesis of MBD is complex and includes prenatal minerals deprivation, insufficient postnatal intake of Ca, P, and vitamin D, extended time of total parenteral nutrition (TPN), decrease in tubular reabsorption of phosphate (TRP), and prolonged-time of immobilization. Furthermore, the use of some medications, such as diuretics and corticosteroids in neonatal intensive care unit (NICU) may contribute to the pathogenesis of the disease.[1,4] Inadequate intake of vitamin D during pregnancy is associated with reduced intrauterine bone growth and subsequently leads to the prevalence of osteopenia in different communities.[5]

Vitamin D is being supplied through either nutrient content or the conversion of 7-dehydrocholesterol in the skin upon exposure to ultraviolet B radiation.[6] This Vitamin is converted to 25-hydroxyvitamin D (25 (OH)D) in the liver, which is then activated to become 1, 25-dihydroxy vitamin D (1,25(OH) D) in the kidneys. The second hydroxylation can also occur in other organs. Vitamin D increases both intestinal absorption of Ca and phosphorus and bone mineralization.[7] Since the daily use of 160-180 ml/kg breast milk could only provide 4 IU/kg of vitamin D, it is highly recommended to take vitamin D supplementation after birth.[5] There are different definitions for vitamin D deficiency, but there is an agreement on the institute of

medicine's (IOM) suggesting the serum level of 1,25(OH) D < 20 ng/ml as insufficient vitamin D level.[8]

Several types of research have been performed on vitamin D analogs, especially ergocalciferol (vitamin D2) and cholecalciferol in neonates with MBD. However, there are few studies on the preventive effect of calcitriol on the biochemical marker of MBD in preterm infants. Cholecalciferol is a usual drug for the prevention of MBD in Neonatal intensive care units (NICUs), but calcitriol, the active form of vitamin D, has several direct effects on end organs that make it an attractive alternative treatment in the prevention of MBD. According to a hypothesis, calcitriol could also prevent MTB and its biomarkers.

So, in this study, we compared the preventive effect of calcitriol and cholecalciferol on the biochemical markers of MBD in preterm infants. To the best of our knowledge, this was the first study comparing the effect of these two drugs on the biochemical markers of MBD in preterm infants.

Method

Study Design and Setting

This study was a pilot randomized controlled trial conducted in the Alzahra teaching hospital of Tabriz, the largest perinatal referral center at the northwest of Iran, for seven months from December 2016 to May 2017.

Inclusion criteria, screening, and enrollment

In the study, 111 Very-low-birth-weight infants with gestational age between 26 and 32 weeks were enrolled. The exclusion criteria included the major congenital anomalies, history of familial bone disease, being nothing by mouth (NPO) for more than five days, receiving parenteral nutrition for more than two

weeks, and infants were given birth to from mothers receiving anticonvulsant medications, such as phenytoin or phenobarbital. Parents who either could not complete or understand the consent form or wanted to leave the study at any time were also excluded.

According to the protocol of Parenteral Nutrition of preterm infants in the NICU, all infants received elemental calcium with dose of 60-80mg/Kg/ day of as Calcium (Gluconate Calcium10 %) and elemental phosphor with a dose of 46-60 mg/Kg/day as phosphor (Glycophose by Fresenius Kabi USA) from the very first postnatal days with a molar Ca:P ratio 1.3:1 in mg. Moreover, all infants received minimal enteral feeding by breast milk from the second day after delivery. Breastmilk increased step by step according to the feeding protocol of the NICU. By two weeks postnatal age, when the infants had received at least 100 ml/kg/day of enteral feedings, calcitriol 0.25 µg/day or cholecalciferol 400 IU/day were started in each of two groups of infants.

Study Protocol

The calcitriol group received their medication (0.25 µg/day) (Dana Pharmaceutical Company, Tabriz, Iran). Of note, we used calcitriol based on a previous study in which the researchers used calcitriol at a dose of 0.25 µg three times a day for treatment of a case of MBD of prematurity .[10,11] This dose was the lowest in the suggested dose range for the treatment of rickets. On the other hand, the cholecalciferol group received 400 IU/day of cholecalciferol drop (Vitabiotics, British Nutraceutical Company) based on the recommendation of the American Academy of Pediatrics (AAP) for the prevention and management of rickets.[12]

After preparing the medications in an insulin syringe by a pharmacy technician in a cleanroom, the syringes were sent to the NICU. The nursing staff was blinded to the content of syringes and it is obvious that neonates are not aware of the receiving medication. The medications were

administered through a nasogastric tube during breast milking. Vitamin D Supplementation was also given to the infants followed until discharge.

Furthermore, demographic data of infants including maternal risk factors, gestational age, birth weight, The Apgar score, hospitalization time, sex, and laboratory data were recorded in a data collecting form.

Blood Sampling

Biochemical markers of MBD including serum ALP, 25-hydroxyvitamin D, phosphorus, Ca, PTH, and TRP were checked at baseline, at the end of the third and fifth weeks.

Renal tubular reabsorption of phosphate (TRP) is the fraction of phosphate in the glomerular filtrate that is reabsorbed in the renal tubules. Hence, we measured the infants' Serum, urine phosphate and serum, urine creatinine. Their TRP was calculated with the following fraction:

$$TRPi = \left(1 - \frac{PO_4\,(U) \times Cr\,(S)}{PO_4\,(S) \times Cr\,(U)}\right) \times 100$$

The normal range of TRP is 78-91% and a value above 95% is a significant marker of insufficient P supplement.[13] In the current work, Serum Ca, ALP, and Phosphorus were analyzed with the enzymatic method using Selectra auto analyzer E (made in the Netherlands) and Selectra auto analyzer prom (made in France) 25-hydroxyvitamin D was also analyzed using Electrochemiluminescence assay (ECL) in Cobas-e 2010. Besides, PTH was analyzed using ELISA in Cobas-e 411.

Primary Outcomes

Primary outcomes included biochemical markers of MBD serum ALP, phosphorus, Ca, PTH, and TRP at baseline as well as three and five weeks after medication.

Study Sample Size Calculation

Because of lacking study related to our topic we decided to choose a pilot sample of 35 patients in each group.

Statistical Analysis

Data analysis was performed using SPSS software Ver.16.0 (Chicago, SPSS Inc., 2007). First, the Kolmogorov–Smirnov test was performed to determine the distribution of data. Repeated measures analysis of variance (ANOVA) by Bonferroni adjustment for pairwise comparisons (within and between subjects) was further performed. In addition, the independent sample t-tests were used to compare means between the groups. The Chi-square and/or Fisher's exact tests were applied to sets of categorical data, Intergroup comparison was performed, Continuous data were shown as mean ± standard deviation (SD), and p-values less than 0.05 were assumed as statistically significant.

Results

Totally, 111 preterm infants were screened for their eligibility. Among them, three cases were excluded because of their congenital anomalies (n=1) and a history of taking anticonvulsant medications by their mothers (n=2). 108 preterm infants were randomized 1:1 into calcitriol (n=54) and cholecalciferol groups (n=54). Of them, 13 cases were excluded because of being NPO for more than five days, nine other cases were excluded because of difficulty in urine collection, and

14 preterm infants were discharged or transfer before completing the research. Finally, 72 preterm infants who met the inclusion criteria completed the study (37 preterm infants in the calcitriol group and 35 in the cholecalciferol group).

The baseline demographic and clinical data of patients were similar in both groups ($p> 0.05$), which is shown in Table I. Mean gestational age in calcitriol and cholecalciferol groups was 28.21±2.35 and 27.98±1.49 weeks, respectively. Moreover, the mean birth weight in the calcitriol group was 1029.03±163.44 gr, while it was 983.87±163.51 gr in the cholecalciferol group. Table II shows maternal characteristics and indicates no significant differences in maternal risk factors of the two groups ($p> 0.05$).

Biochemical markers of MBD are also presented in Table III. Mean serum vitamin D was significantly higher in the cholecalciferol group and had an increasing trend in the two next measurements (P=0.001). Infants in cholecalciferol group also had a significantly lower loss of phosphate in urine after three weeks of supplementation (P=0.009). Increasing of mean serum phosphate level seemed to be a late finding and happened after eight weeks (P=0.049). TRP level was also significantly higher in the cholecalciferol group after three weeks of supplementation (P = 0.037). Intergroup comparison of serum vitamin D, PTH, phosphorus, and urine phosphorus indicated changes between baseline, the 5^{th}, and the 8^{th} weeks. Figures 2 and 3 show the trend of serum vitamin D and urine phosphate during the study time.

Other biochemical markers were not significantly different in the two groups. For example, the mean ALP level in both groups was less than 900 (IU/L), which can be related to insufficient intake of calcium and phosphor. It is noteworthy that biochemical markers in the second test (after three

weeks of supplementation) were better than the third test. It is suggested that it may happen because of quick growth after the fifth week and higher demands for micronutrients.

Discussion

In this study, we compared the effects of calcitriol and cholecalciferol on the prevention of MBD for the first time. The results also showed a lower loss of phosphate in urine as well as higher TRP levels in the cholecalciferol group after three weeks of supplementation. In the present study, 79% of infants had vitamin D deficiency/insufficiency at the time of hospitalization.

Several researches have evaluated the effect of different doses of cholecalciferol on the prevention of MBD. Different doses (400 – 1000 IU/day) have been suggested[2,5,14,15] ; however, AAP has recommended 400 IU/day of cholecalciferol for the prevention of rickets.[12]

Rustico and collogues investigated the effect of 0.05-0.08 µg/kg/day calcitriol on the treatment of MBD of prematurity and secondary hyperparathyroidism in preterm infants. They showed significant decrease in PTH levels from a median level of 220 to 25 pg/ml and increase in Ca (9.9 to 10.3 mg/dl), phosphorus (4.3 to 5.4 mg/dl), and TRP (81% to 91.5%). However, no differences in ALP was observed.[11]

Results of the current study demonstrated that cholecalciferol caused a lower loss of phosphate in the urine, which is in contrary to Rustic's study. Of note, there have been few research works studying the effect of calcitriol on the treatment or the prevention of osteopenia of prematurity. This study, however, had some differences with Rustic's et al study. First of all, they investigated the effect of calcitriol on the treatment of infants with Proven MBD with radiographic finding and secondary hyperparathyroidism, while we evaluated the effect of calcitriol on the prevention of MBD. We did not evaluate radiologic finding. Also, based on Yuri Dowa's study, There was a significant correlation between ALP and TRP with increased PTH levels.[16]

Second of all, in our study, the dose of calcitriol, was higher than Rustic's et al. study (0.25 µg/day vs.0.05-0.08 µg/kg/day). They used calcitriol for a longer time (1-12 month/s) than this study (2 months).

In 2012, in a case study performed by Hang Yi Chen, two preterm infants suffering from rickets were included. Case 1 received 0.125-0.3 mg/kg/day of calcitriol for 40 days and case 2 received the same dose for 37 days. In case 1 serum ALP level decreased from 1640 IU/L to 637 IU/L and phosphorus level increased from 3.75 mg/dl to 6 mg/dl. In case 2 serum ALP level decreased from 1463 IU/L to 475 IU/L and phosphorus level increased from 2.7 mg/dl to 5.8 mg/dl. Finally, both infants gradually recovered and were discharged.[17]

The present study investigated the effect of calcitriol in the prevention of osteopenia of prematurity but not the treatment of Rickets. Rickets of prematurity is more severe than osteopenia or MBD of prematurity and there are radiologic findings of rickets beside biomarker findings of osteopenia. Infants in this study were also younger than those in Hang Yi Chen's study. Moreover, several studies have indicated the incomplete development of Vitamin D metabolism in the preterm infants' liver system and the fact that it is completed as the infant grows.[5,17,18]

In another study performed by Lucas & OG brook, it was mentioned that preterm infants may have insufficient 1-αhydroxylase so that they can't produce the active form of vitamin D. They also confirmed the idea that alfacalcidol should not be used to prevent or treat MBD in preterm infants.[18]

Chen also preferred calcitriol to alfacalcidol as the first-line treatment of rickets of prematurity because preterm infants are not completely able to convert alfacalcidol to calcitriol.[17]

Unlike the results of the two mentioned studies, this study showed that Cholecalciferol had a better effect on biomarkers of bone metabolism in comparison with calcitriol. This may suggest that preterm infants don't have significant insufficiency in α hydroxylation.

Interestingly, in a review article in 2006, it was indicated that 1, 25-dihydroxy vitamin D can also be produced in other organs, which is in agreement with the results of the present study.[19]

The review article indicated that tissues and cells having vitamin D receptor (VDR) can express cyp27B1. For instance, skin, colon, prostate, lungs, brain, and placenta can produce 1, 25-dihydroxy vitamin D.[19, 20] This may be the reason why preterm infants having immature liver and kidney enzymatic system can produce enough 1, 25-dihydroxy vitamin D.

Study limitations

In the study, Because of some limitations, the result should be interpreted cautiously. First, because of cost limitations, we could not continue the study for a longer duration of time. A longer time of supplementation and checking biomarkers might show more exact results.

Second, our study sample size was limited and a larger sample size is required to show out precise effect of the study. Importantly, radiologic changes in bone could be followed up with DEXA method to confirm further outcomes. Because of our limited fund, we couldn't do it either.

The current study was the first clinical randomized trial comparing the effect of Calcitriol and Cholecalciferol on biochemical markers MBD in very-low-birth-weight infants.

Moreover, the higher levels of serum 25-hydroxyvitamin D and lower urinary loss of phosphate caused by cholecalciferol indicated that preterm infants can produce an active form of vitamin D despite having an immature liver. This can address further studies to investigate vitamin D metabolism in preterm infants.

Conclusion

The results of this study suggested that cholecalciferol caused a lower urinary loss of phosphate in very-low-birth-weight infants. There was no significant difference in other biochemical markers. Further studies are recommended to obtain the precise clinical outcome.

Ethics

The Institutional Review Board (IRB) of Tabriz University of medical sciences approved the study protocol. Then, it was registered in WHO clinical trial registry platform under ID: RCT20111206008307N28 (available on: https://en.irct.ir/trial/30580). All eligible parents of children completed the written informed consent form. The study was performed in adherence to the Declaration of Helsinki and later revisions (2013) on ethical principles for medical research.[21]

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The authors have declared that they have no conflicts of interest. All patients' parents completed the written informed consent form before the study. The study was performed in adherence with the Declaration of Helsinki and later revisions on ethical principles for medical research. The Institutional Review Board (IRB) of the university approved the study protocol. (IR.TBZMED.REC.1395.1035)

Conflict of interest

None

Founding

None

Author Contribution

MBH and TEM designed, and directed and supervised the study. They wrote and edited the manuscript, and performed data analysis. NH conducted the study, collected data and wrote and revised manuscript. ZS evaluated patients, collected data, administered medications, and followed up the patients. All authors read and confirmed the manuscript.

Data Sharing Statement

It would be done per request from the corresponding author via email address

References

1. Rehman MU, Narchi H .Metabolic Bone Disease in the preterm infant: Current state and future directions, World J Methodol. 2015;5(3):115-121. doi: 10.5662/wjm.v5.i3.115

2. Backström MC, Kuusela AL, Mäki R.Metabolic Bone Disease of prematurity. Ann Med.1996;28(4):275-282. doi :10.3109/07853899608999080

3. Malcolm AJ. Metabolic Bone Disease. Diagn Histopathol. 2002;8(1):19–25. doi:10.1054/cdip.2001.0091

4. Dokos C, Tsakalidis C, Tragiannidis A, Rallis D. Inside the "fragile" infant: pathophysiology, molecular background, risk factors and investigation of neonatal osteopenia. Clin Cases Miner Bone Metab. 2013;10(2):86-90: PMID: 24133523

5. Pharande P, Pammi M, Collins CT, Zhou SJ, Abrams SA. Vitamin D supplementation for prevention of vitamin D deficiency in preterm and low birth weight infants. Cochrane Database of Systematic Reviews 2015; 2: CD011529. doi: 10.1002/14651858.CD011529

6. Aly H, Abdel-Hady H. Vitamin D and the neonate: An update. J Clin Neonato. 2015;4(1):1-7. doi: 10.4103/2249-4847.151155

7. Hagino H. Vitamin D3 analogs for the treatment of osteoporosis. Can J Physiol. Pharmacol. 2015;93(5):327-332. doi:10.1139/cjpp-2014-0419

8. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. Int J Clin Endocrinol Metab. 2011; 96(1):53-58. doi: 10.1210/jc.2010-2704

9. Mathur NB, Saini A, Mishra TK. Assessment of Adequacy of Supplementation of Vitamin D in Very Low Birth Weight Preterm Neonates: A Randomized Controlled Trial. J Trop Pediatr. 2016;62(6):429-435. doi:10.1093/tropej/fmv110

10. Khadilkar VV, Khadilkar AV, Joshi SS. Bone disease in preterm. J Indian Assoc Pediatr Surg. 2007;74(10):945-946. doi: 10.1007/s12098-007-0174-z.

11. Rustico SE, Kelly A, Monk HM, Calabria AC. Calcitriol treatment in Metabolic Bone Disease of prematurity with elevated parathyroid hormone: a preliminary study. J Clin Transl Endocrinol. 2015;2(1):14-20. doi:10.1016/j.jcte.2014.12.001

12. American academy of pediatrics (2017) Recommendations released on preventation , management of rickets available at http://www.aappublications.org/news/2017/02/10/Rickets021017

13. Acar DB, Kavuncuoğlu S, Çetinkaya M, Petmezci E, Dursun M, Korkmaz O, et al Assessment of the place of tubular reabsorption of phosphorus in the diagnosis of osteopenia of prematurity. Turk Pediatri Ars. 2015;50(1):45-50. doi: 10.5152/tpa.2015.1478

14. Rigo J, Pieltain C, Salle B . Enteral calcium, phosphate and vitamin D requirements and bone mineralization in preterm infants. Acta paediatr. 2007; 96(7):969-974. doi:10.1111/j.1651-2227.2007.00336.x

15. Natarajan CK, Sankar MJ, Agarwal R. Trial of daily vitamin D supplementation in preterm infants. Pediatrics. 2014;133(3): 628-634. doi.org/10.1542/peds.2012-3395

16. Dowa Y, Kawai M, Kanazawa H, Iwanaga K, Matsukura T, Heike T . Screening for secondary hyperparathyroidism in preterm infants. Pediatr Int. 2016; 58(10):988-999. doi:10.1111/ped.12961

17. Chen HY, Chiu LC, Yek YL, Chen YL. Detecting rickets in premature infants and treating them with calcitriol: experience from two cases. Kaohsiung J Med Sci. 2012;28(8):452-456. doi:10.1016/j.kjms.2012.04.009

18. Brooke OG, Lucas A. Metabolic Bone Disease in preterm infants. Arch Dis Child. 1985;60(7):682-685. doi: 10.1136/adc.60.7.682

19. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest. 2006;116(8):2062-2072. doi: 10.1172/JCI29449

20.Liu NQ, Hewison M. Vitamin D, the placenta and pregnancy. Arch Biochem Biophys. 2012;523(1):37-47. doi: 10.1016/j.abb.2011.11.018

21. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA.* 2013;310(20):2191–2194. doi:10.1001/jama.2013.281053

Table I: maternal risk factors in two groups of very low birth weight infants receiving cholecalciferol and calcitriol

Mother's risk factors	Group 1 n (%) (calcitriol n=37)	Group 2 n (%) (vit D n=35)	P Value
Hypertension *	9(24.3)	8(22.8)	0.884
Preeclampsia *	9(24.3)	7(20.0)	0.659
Diabetes Meletus **	2(5.4)	3(8.5)	0.597
Smoking**	1(2.7)	0 (0.0)	1.000
Thyroid disorder**	4(10.8)	2(5.7)	0.434
Complete antenatal	13(35.1)	13(37.1)	0.956
corticosteroid use*	8(21.6)	6(17.1)	0.768
Partial antenatal* corticosteroid use			

* conducted by chi-square.
** conducted by the Fisher's exact test.
Complete antenatal corticosteroid means full dose corticosteroid given to pregnant woman expecting preterm delivery.

Table II: Demographic data in two groups of very low birth weight infants receiving cholecalciferol and calcitriol

Study factors	Group 1 (mean ± SD) (calcitriol n=37)	Group 2 (mean ± SD) (cholecalciferol n= 35)	P Value
Birth Weight (gram)*	1029.0 ± 163.4	983.8 ± 163.5	0.281
Gestational age (week)*	28.2 ± 2.3	27.9 ± 1.4	0.622
Apgar score 1*	5.8 ± 1.6	5.5 ± 1.6	0.373
Apgar score 5*	7.9±1.3	7.51±1.0	0.455
Hospital stay (day)*	58.6 ± 16.4	55.9 ±14.3	0.512
Male/Female (ratio)**	19/18	17/18	0.810

*performed by independent t-test
**performed by chi-square test.
SD: standard deviation

Table III: comparison of biochemical markers measured as baseline, third week and fifth week after supplementation in two groups of very low birth weight infants

Study factors	Time	Group 1 (calcitriol n=37)	Group 2 (vit D n= 35)	P value
25(OH)-vitamin D(ng/mL)	Baseline	6.16 ±4.8	10.0 ±16.5	0.182
	5th week	7.0 ±6.6	37.3 ±20.2	0.001*
	8th week	4.1 ± 1.6	55.0 ±18.8	0.001*
Alkaline phosphatase (IU/L)	Baseline	633.0 ±225.7	576.5 ±224.7	0.299
	5th week	886.5 ±313.9	991.4 ±427.7	0.238
	8th week	1194.1 ±378.8	907.3 ±458.6	0.069
Calcium (mg/dl)	Baseline	9.3± 0.5	9.5 ± 0.5	0.061
	5th week	9.2 ±0.7	9.3 ± 0.4	0.485
	8th week	9.0 ±0.91	9.4 ± 0.5	0.164
Parathyroid hormone (pg/ml)	Baseline	56.1 ± 39.2	53.0 ± 39.7	0.745
	5th week	34.5 ± 39.7	29.5 ±26.3	0.533
	8th week	56.7 ± 51.4	50.0 ± 40.2	0.692
Serum phosphorus (mg/dL)	Baseline	4.5 ±1.0	4.9±1.0	0.218
	5th week	4.4 ±1.0	4.2 ±0.8	0.454
	8th week	4.4 ±1.8	5.8 ±1.9	0.049*
Urine Phosphorus (mg/dL)	Baseline	5.1 ±7.4	7.4 ±7.8	0.218
	5th week	6.1 ±9.0	1.7 ±3.2	0.009*
	8th week	18.3±25.8	7.2 ±9.7	0.122
tubular reabsorption of phosphate	Baseline	90.3 ±11.0	87.6 ±11.0	0.344
	5th week	86.2 ±20.4	95.1 ±10.8	0.037*
	8th week	84.4 ±14.1	88.2 ± 22.2	0.579

*statistically significant

Performed by the repeated measure ANOVA test

Table IV: Intergroup comparison of biochemical markers measured as baseline, third week and fifth week after supplementation in two groups of very low birth weight infants

			Group 1(calcitriol n=37)		Group 2 (cholecalciferol n=35)	
Study factors	Time A	Time B	Mean Difference	P value	Mean Difference	P value
25(OH) vitamin D(ng/mL)	baseline	5th week	-0.9	1.000	-27.3	.000*
		8th week	2.0	.0.650	-45.0	.000*
Alkaline phosphatase (IU/L)	baseline	5th week	-253.5	0.001*	-414.8	.000*
		8th week	-561.0	0.001*	-330.7	.013*
Calcium (mg/dl)	baseline	5th week	0.1	1.000	0.2	.187
		8th week	0.2	0.730	0.1	.776
Parathyroid hormone (pg/ml)	baseline	5th week	21.6	0.084	23.5	.020*
		8th week	-0.5	1.000	3.0	1.000
Serum phosphorus(mg/dL)	baseline	5th week	0.1	1.000	0.6	.076
		8th week	0.2	1.000	-.0.9	.041*
Urine Phosphorus (mg/dL)	baseline	5th week	-1.0	1.000	5.6	.003*
		8th week	-13.1	0.005	0.1	1.000
tubular reabsorption of phosphate	baseline	5th week	4.0	0.968	-7.4	.087
		8th week	5.8	0.744	-.0.5	1.000

*statistically significant

Performed by the repeated measure ANOVA test and Bonferroni adjustment