April 2012

Response of a single 'mega intramuscular dose' of vitamin D on serum 25OHD and parathyroid hormone levels

Aysha Habib
Aga Khan University, aysha.habib@aku.edu

Dileep K Rohra

Shakil A Saghir

Shamsa K Udani

Richard Wood

See next page for additional authors

ORIGINAL ARTICLE

Response of a Single 'Mega Intramuscular Dose' of Vitamin D on Serum 25OHD and Parathyroid Hormone Levels

Aysha Habib Khan[1,3], Dileep K. Rohra[2,5], Shakil A. Saghir[2], Shamsa K. Udani[1], Richard Wood[4] and Abdul Jabbar[3]

ABSTRACT

Objective: To determine the changes produced in serum 25OHD and iPTH levels after 600,000 IU of injection cholecalciferol in volunteers.

Study Design: Interventional study.

Place and Duration of Study: Section of Chemical Pathology, Department of Pathology and Microbiology, the Aga Khan University Hospital, Karachi, from June 2009 - June 2010.

Methodology: Volunteers of either gender aged 18-40 years with known 25OHD, calcium (Ca), creatinine (Cr) and phosphorous (P) levels were included in the study. Subjects on therapy like vitamin D and calcium supplements, corticosteroids or anti-epileptic medicines, primary hyperparathyroidism and hypercalcaemia, with co-morbidity like renal failure, liver disease and history of malabsorption, diarrhea or hyperthyroidism were excluded. All volunteers were given an intramuscular injection of vitamin D3 (cholecalciferol, 600,000 IU). After 8 weeks, serum 25OHD, iPTH, Ca and P levels were determined again. For 25OHD level, cut-off of ≤ 50 nmol/l was defined as deficient, 50-75 nmol/l as insufficient and ≥ 75 as optimal level.

Results: Mean 25OHD and iPTH levels were 35.06 ± 16.6 nmol/l and 81.15 ± 76.78 pg/ml respectively at baseline. Seventeen volunteers were 25OHD deficient. Five had high iPTH levels (25%) (mean 156 ± 123.7 pg/ml). 25OHD and iPTH showed a significant inverse correlation at baseline (< 0.01). After 8 weeks of injection vitamin D 25OHD levels became optimal in 6 subjects (35%) [mean 92.9 ± 16.6 nmol/l]. It remained low in 5 volunteers (25%) [mean 41.6 ± 9.6 nmol/l] while insufficient levels were seen in 9 volunteers (40%) [mean 63.3±5.8 nmol/l]. Follow-up mean Ca, P and iPTH were 2.25 mmol/l (± 0.09), 1.1 (± 0.1) and 47.52 pg/ml (± 22.56) respectively. A significant increase in mean 25OHD level was seen at follow-up (p < 0.01), while the change in PTH was insignificant (p=0.05).

Conclusion: Single mega-dose of cholecalciferol achieved optimal levels of 25OHD in 35% of subjects after eight weeks of supplementation.

Key words: *Vitamin D (25OHD). Vitamin D deficiency (VDD). Parathyroid hormone (PTH). Secondary hyperparathyroidism (sHPTH). Cholecalciferol (vitamin D3).*

INTRODUCTION

Adequate vitamin D (25OHD) and parathyroid hormone (PTH) levels are crucial for bone health. Vitamin D deficiency (VDD) causes a decrease in intestinal calcium absorption and produces secondary hyperparathyroidism (sHPTH) which is the primary mediator of the detrimental effects of VDD on skeleton.[1] Recently, role of sHPTH in producing hypertension and ventricular hypertrophy has been shown. sHPTH has been linked to cardiovascular mortality in patients with heart failure. Potential benefits have been observed after raising the levels of 25OHD and thus lowering of PTH levels in many chronic diseases.[2-7]

A high prevalence of VDD has been identified in Pakistan in healthy volunteers and medical clinic patients. A great concern was that 94.6% of adults were between 20-30 years old; the age at which peak bone mass is achieved and sHPTH was present in 31% of individuals.[8,9] Recent reports from two different public hospitals in Karachi, demonstrated high prevalence of VDD in pregnant women and their newborns urging for an immediate action to the problem.[10,11]

sHPTH is reversible when 25OHD levels rises, however, the optimal dose of 25OHD needed to normalize PTH is currently taken as 75 nmol/l (32 ng/ml) by most experts.[1,12] In order to correct VDD, the practice pattern of physician varies. Different protocols have been proposed and strategies studied recently for vitamin D supplementation. However, there is no universally accepted regimen and treatment practice.[13-18] A recent trial demonstrated that the dose-response curve of serum 25OHD concentration to the cholecalciferol (vitamin D3) supplementation is widely variable, suggesting that people may respond differently in terms

[1] *Department of Pathology and Microbiology / Biological and Biomedical Sciences[2] / Medicine[3], The Aga Khan University Hospital, Karachi.*

[4] *Department of Nutrition, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA 01003.*

[5] *College of Medicine, Alfaisal University, Riyadh, Saudi Arabia.*

Correspondence: *Dr. Aysha Habib Khan, Department of Pathology and Microbiology and Medicine, The Aga Khan University Hospital, Karachi.*
E-mail: aysha.habib@aku.edu

Received September 12, 2011; accepted February 20, 2012.

of serum 25OHD increases to a fixed dose of cholecalciferol.[16]

Studies published in recent years have focused mainly on hypovitaminosis D and on the optimal treatment capable of normalizing 25OHD and the dose of cholecalciferol needed to normalize sHPTH.[13-15]

The present study was conducted with the aim of determining the changes produced in serum vitamin D and iPTH levels after a single intramuscular injection of 600,000 IU of cholecalciferol in patient with VDD.

METHODOLOGY

An interventional study was conducted in the Department of Pathology and Microbiology, the Aga Khan University (AKU) from June 2009 to June 2010. The study was funded by Higher Education Commission (HEC) of Pakistan and was approved by Ethical Review Committee (ERC) of the Aga Khan University Hospital (AKUH).

Volunteers of either gender aged 18-40 years whose 25OHD level had been checked within last one week at the clinical laboratory of the AKUH, Karachi were contacted by telephone. The aims of the study were explained to them. On a pre-determined day, the participants were requested to visit the Clinical Laboratory of AKUH. A written informed consent was obtained from all the participating individuals. Before inclusion in the study, it was ensured that the serum creatinine (Cr), calcium (Ca) and phosphorous (P) of the volunteers were within normal limits.

Those volunteers who were taking any drug which could confound the results like vitamin D supplements, corticosteroids or anti-epileptic medicines were excluded from the study. Other exclusion criteria were primary hyperparathyroidism and hypercalcaemia, presence of any other co-morbidity like renal failure, liver disease and history of malabsorption, diarrhea or hyperthyroidism.

All volunteers irrespective of their 25OHD status were given an intramuscular injection of vitamin D3 (Cholecalciferol, 600,000 IU). Two months later, volunteers were contacted and invited again to come to Clinical Laboratory. During this visit their serum 25OHD, intact-PTH (iPTH), Ca and P levels were determined again.

Venous blood was drawn in fasting volunteers between 8:00 - 10:00 am in plain serum tubes for Ca, P and 25OHD, and in EDTA tubes for iPTH analysis. Immediately after phlebotomy, serum was separated from the blood samples by centrifugation, aliquot and preserved for 25OHD and iPTH levels at -20ºC until analysis.

25OHD was measured by an electrochemiluminescence immunoassay (ECLIA) performed on Elecys 2010 (Roche Diagnostics, USA). For quality control, Elecsys Preci Controls in various concentration ranges was run with each batch. Results were accepted only if the concentration was within the defined limits of the pre-control.

iPTH was measured by a solid-phase, two site chemiluminescent enzyme-labelled immunometric assay on Immulite (Siemens Medical Solutions Diagnostics, USA). Low and high levels of quality control samples of iPTH were run with each batch. Interassay precision (pg/ml) was found to have a range of 8.6%-9.0%.

Ca, P and Cr levels were assessed on Beckman Synchron Cx7 by enzymatic methods; Arsenazo dye method for C, Jaffe reaction for Cr and Phosphomolybdate reaction for P. Low, medium and high Beckman commercial controls were run with every batch as quality control.

Statistical Package for Social Sciences (SPSS) [release 13.0] standard version, copyright © SPSS, was used for data analysis. For 25OHD, cut-off of ≤ 50 nmol/l was defined as deficient, 50-75 nmol/l as insufficient and ≥ 75 as optimal levels.

Data was checked for normality by histogram. Descriptive analysis was done for demographic and laboratory tests. Percent increase and decrease in 25OHD and iPTH were calculated using standard equations. The data are presented as mean ± standard deviation. The association between serum 25OHD and iPTH concentrations was studied by linear regression and correlation. The statistical analysis for the differences in the levels of 25OHD, iPTH, Ca and P before and after vitamin D supplemen-tation was made using paired t-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Twenty volunteers (6 males and 14 females) participated in the study. The mean age of the participants was 27 ± 3.5 years. The mean BMI of males and females was 26.8 ± 2.7 and 24.1 kg/m^2, respectively. Change in serum 25OHD and iPTH values in individual volunteers and their mean values at baseline and at follow-up, 8 weeks after injection Cholecalciferol are shown in Table I and Figure 1.

At baseline, 17 volunteers were deficient in 25OHD with serum levels < 50 nmol/l, one had serum levels of 76.97 nmol/l (optimal ≥ 75 nmol/l) and 2 had insufficient 25OHD serum levels (50-75 nmol/l). Mean serum 25OHD levels and iPTH levels were 35.06 ± 16.23 nmol/l and 81.15 ± 76.78 pg/ml, respectively. Five of the volunteers (25%) had high iPTH levels with mean serum levels of 156 ± 123.7 pg/ml. Baseline serum Ca and P levels were within normal range; 2.3 ± 0.12 mmol/l and 0.9 ± 0.13 mmol/l. Serum 25OHD and iPTH showed significant inverse correlation (r^2 = 0.187, $p < 0.01$) at baseline (Figure 2A).

Table I: Percent change in serum 25OHD (nmol/l) and iPTH (pg/ml) levels after 600,000 IU intramuscular injection of cholecalciferol (N = 20).

Subjects	Pre-treatment 250HD	Post treatment 250HD	% change	Pre-treatment iPTH	Post-treatment iPTH	% change iPTH
1	9.98	63.09	532.0	377.0	34.0	90.0
2	21.71	87.98	30.5	79m .3	47.3	40.0
3	42.80	76.62	79.0	94.3	52.3	44.0
4	22.61	92.85	310.0	68.0	62.2	8.5
5	37.81	66.44	75.7	73.4	71.4	2.7
6	38.68	48.69	25.81	107.0	42.1	60.0
7	22.16	84.88	232.9	111.0	85.5	22.0
8	40.01	72.55	81.34	58.9	42.2	28.0
9	14.52	59.47	309.45	61.2	35.0	42.0
10	28.10	48.69	73.26	49.8	97.0	-94.0
11	24.26	42.43	74.89	39.3	45.0	-14.0
12	18.37	53.04	188.7	62.6	60.5	3.362
13	76.97	124.84	62.19	66.0	24.8	62.0
14	54.01	67.49	24.95	54.7	18.0	67.0
15	23.33	42.45	81.92	54.0	45.3	16.0
16	39.23	57.40	46.3	67.5	33.7	50.0
17	49.34	25.20	-48.91	91.6	11.6	87.0
18	44.90	89.23	98.72	36.8	36.8	0.0
19	39.33	63.79	62.18	60.3	46.5	22.0
20	52.99	65.39	23.41	21.5	30.7	42.0
Mean±SD	35.06±16.23	66.63±24.99	134.4±141.87	81.0	46.0	43.0
p-value	< 0.01		-	0.05		-

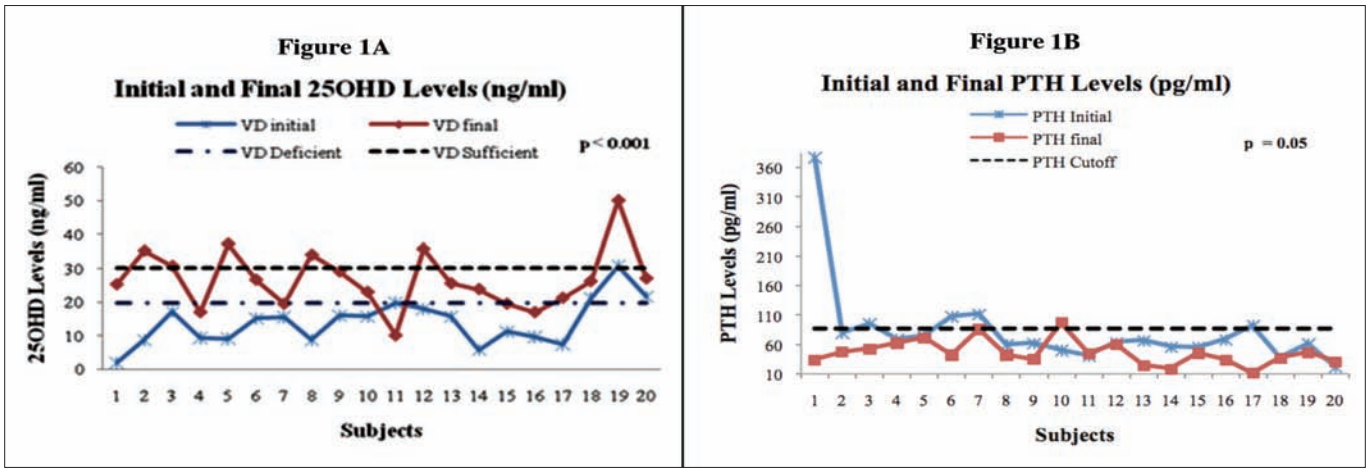

Figure 1 (A,B): Response of 25OHD (nmol/l) and iPTH levels (pg/ml) to 600,000 IU of injection cholecalciferol in healthy volunteers in Karachi, Pakistan (n = 20).

Table II: Response of 25OHD (ng/ml) and iPTH (pg/ml) to different dosing regimen of Cholecalciferol supplementation in literature.

VD (IU)	N	Route	Dosing interval	Baseline		2 months		3 months		4 months		6 months		1 year		Ref
				250HD	PTH	250HD	PTH	250HD	PTH	250HD	PTH	250HD	PTH	250HD	PTH	
1000	30	oral	Daily	23.4	92.5	-	-	49.6	70.2	-	-	57.6	66.0	-	-	16∞
60,000	23	oral	Weekly *	13.5	54.0	82.4	29.0	-	-	-	-	-	-	24.7	10.9	13
60,000	40	oral	Weekly***	25.4	-	94.5	-	-	-	-	-	56.0	-	-	-	17
300,000	30	oral	Monthly**	22.0	99.9	-	-	51.4	78.0	-	-	78.2	72.9	-	-	16
600,000	50	IM	Single	32	7.4	-	-	-	-	112.0	6.0	-	-	73.0	5.2	14
600,000	20	oral	Single	42.9	57.0	106.8	42.8	79.6	37.7	-	-	-	-	-	-	18∞
600,000	40	IM	Single	22.8	122.0	-	-	69.1	24.8	-	-	-	-	-	-	15∞
600,000	20	IM	Single	35.06	81.0	66.7	46.0	-	-	-	-	-	-	-	-	This study

** Weekly for 4 months; ** Once at baseline and once at 3 months; *** Weekly for 8 weeks followed by 60,000 IU/month for 4 months.*
∞ Values of 25OHD from reference 15, 16 & 18 are converted from ng/ml to nmol/l; ∞ Value of i PTH from reference 14 are in pmol/l.

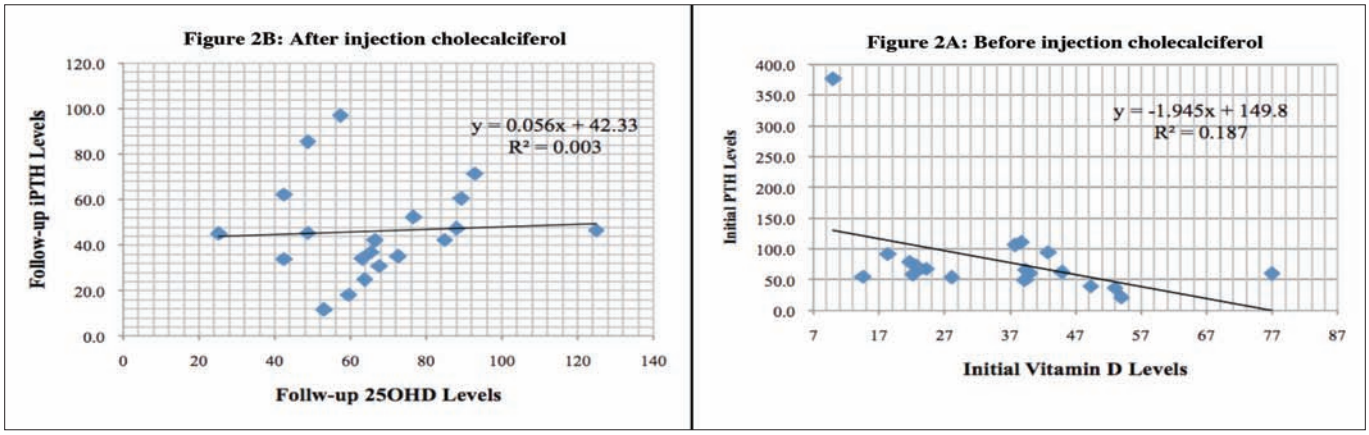

Figure 2 (A,B): Association between 25OHD (nmo/l) and iPTH (pg/ml) before and after 8 weeks of injection cholecalciferol (600,000 IU).

After 8 weeks of injection vitamin D (600,000 IU), serum 25OHD levels became optimal (≥ 75 nmol/l) in 6 volunteers (35%) (mean 92.72 ± 16.65 nmol/l). It remained low in 5 volunteers (25%) (mean 41.48 ± 9.62 nmol/l), while insufficient levels were seen in 9 volunteers (40%) (mean 63.19 ± 5.88 nmol/l). Follow-up mean serum Ca, P and iPTH were 2.5 ± 0.10 mmol/l, 1.16 ± 0.13 mmol/l and 47.52 ± 22.56 pg/ml respectively. In one individual, despite increase in 25OHD levels, the iPTH levels increased.

Mean baseline 25OHD levels (35.06 ± 16.23 nmol/l) were significantly different from follow-up (66.63 ± 24.99 nmol/l) 25OHD levels ($p < 0.01$) while significant difference was not seen in baseline (81.15 ± 76.78 pg/ml) and follow-up (47.52 ± 22.56 pg/ml) iPTH levels ($p = 0.05$) (Figure 1A and B).

Mean follow-up serum Ca (2.5±0.10 nmol/l) and P (1.1 ± 0.13 nmol/l) did not show significant change ($p = 0.47$ and 0.46 respectively) from baseline values of Ca (2.3 ± 0.11 nmol/l) and P (0.9 ± 0.13 nmol/l). The inverse correlation seen in the baseline samples between iPTH and 25OHD changed to $r^2 = 0.03$ (Figure 2B).

DISCUSSION

In Pakistan, VDD is widely prevalent despite adequate sunshine throughout the year. Serum 25OHD levels observed at baseline in the current study and sHPTH in 25% of the study participants further supported the previous findings and endorsed the wide spread existence of 25OHD deficiency along with sHPTH.[7,8,19-21] With recent greater awareness about VDD, testing for 25OHD has become common in diagnosing and treating VDD.

A controvesial aspect in the setup is the treatment strategies of correcting VDD. Several strategies have been reported recently in literature using oral and injectable vitamin D preparations of 1,000-600,000 IU and with variable outcomes (Table II). In a report from India, pattern of 25OHD response at 2 months and one year interval of giving 60,000 IU of cholecalciferol per week was evaluated in 28 subjects. VDD was corrected in all subjects at 8 weeks with normalization of PTH. However, at one year follow-up all subjects develop VDD with sHPTH, indicating need for ongoing supplementation for sustaining 25OHD levels.[13]

In Karachi, oral vitamin D supplementations are available in combination with calcium supplement with most of the available oral preparations containing 100-125 units of cholecalciferol; only a few contains 400 IU of cholecalciferol. In addition, two depot intramuscular preparations of 200,000 and 600,000 IU of cholecalciferol are also available. Treatment of VDD with oral preparations is much more expensive as compared to injections.

Practice patterns of physicians in treating VDD are also widely variable,[20] with some physicians prescribing two injections one month apart while others prescribe 3 injections of vitamin D at two weekly intervals. Another practice is to give 600,000 units intramuscularly every 3 months; cases have been seen (personal observation) in which alternate day injections of 600,000 IU have been prescribed for 1-2 months. At the same time, some physicians treat the patients with severe VDD with low dose oral preparation and hence results in treatment failure. Very few physicians perform complete bio-chemical testing to assess the response to sHPTH associated with VDD and insufficiency to vitamin D replacement. The effects of these mega doses have not been studied in our setting and practices are based on individual experiences. Information regarding effective correction of VDD with currently available depot preparation is lacking in our population. In Pakistani immigrant in Denmark recently, effects of low dosages of 400 and 800 IU/day (10 and 20 µg/day) were studied at 6 and 12 months after intervention. Increases in 25OHD were observed in study participants, but optimal levels were not achieved. However, this degree of intervention did not have any significant effect on bone turnover

marker and DEXA measurement.[23] While, in another study a dose of 22 µg/day of vitamin D increased the serum concentration to optimal levels in white young adult women residing in Orono, Maine.[24] Higher intakes are recommended for South Asian populations,[12,23] but the controversy remains about which concentration of serum 25OHD should constitute the lower limit of adequacy especially for South Asian population. Deficiency is defined as the 25OHD level below which serum PTH concentration increases. Estimates of 25OHD required for maximal suppression of PTH varies. However, the most widely acceptable cutoff is 75 nmol/l (30 ng/ml).

In a study conducted in Sydney to assess the efficacy and safety of annual 600,000 IU of cholecalciferol for treatment of VDD, 50 vitamin D-deficient participants were evaluated at baseline and after 4 and 12 months of therapy. Significantly higher levels of 25OHD were seen at 4 months, and 12 months compared with baseline while there was a 30% decline in PTH levels at 12 months. 25OHD levels increased by 128% over 12 months. Recommendations were made to further assess the efficacy of this mega dose as it is cost effective as compared to oral forms of treatment, and have simple dosing regimen thus improving patient compliance.[14]

On the other hand, in another study conducted in Qatar, Doha, 37 out of 40 adolescents before, 3 and 6 month after treatment with a single mega dose of cholecalciferol showed mean levels of 67.3 nmol/l with significant improvements in symptoms for 3 months. Recommendations from this study support the effectiveness of this dose of VD for 3 months.[15]

In this study, vitamin D levels were optimally achieved in 35% of the subjects post-injection. There is wide variation in the biochemical response to vitamin D injections (Table I). Though the change in 25OHD level in baseline and follow-up is statistically significant; but the baseline and post-injection mean change in iPTH is not statistically significant with a single mega dose.

Further research is needed at different follow-up intervals to assess the effectiveness of vitamin D therapy and to determine how much vitamin D is required to optimize serum 25OHD status throughout the year for optimal physiological functioning.

We recommend that a single mega dose of vitamin D may not achieve optimal levels of 25OHD in all individuals. In order to confirm the adequacy of treatment a repeat testing should be performed to assess 25OHD levels between 8-12 weeks of supplementation to confirm that the target 25OHD level has been reached. It is important to acquaint physicians in Pakistan that repletion dose of vitamin D may vary among individuals based on their initial levels, body mass index and effective sunlight exposure. The required dose to acquire 75 nmol/l can be estimated from measured levels; as each 100 IU of vitamin D increases the 25OHD levels to 1.0 ng/ml. It is equally important to emphasize on life style measures like safe exposure to sunlight and diet and supplements to acquire the recommended daily allowance of 800-1000 IU for older age groups. It is important to understand that over-the-counter active metabolites are not a substitute for adequate vitamin D intake and should not be used to treat vitamin D deficiency. Long-term follow-up to assess the relationship between VDD and iPTH and potential to develop hypercalciuria with excess use of VD is needed in our population.

CONCLUSION

Single mega-dose of cholecalciferol achieve optimal levels of 25OHD in 35% of subjects after 8 weeks of supplementation.

REFERENCES

1. Hollick MF. Vitamin D deficiency. *N Engl J Med* 2007; **357**:266-81.
2. Cozzolino M, Ketteler M, Zehinder D. The vitamin D system: a cross talk between the heart and kidney. *Eur J Heart Fail* 2010; **12**:1031-41.
3. Sugimoto T, Tangiwa T, Onishi K, Fujimoto N, Matsuda A, Nakamori S, *et al*. Serum intact parathyroid hormone levels predict hospitalization for heart failure. *Heart* 2009; **95**:395-8.
4. Schierbeck LL, Jensen TA, Bang U, Jensen G, Kober L, Jensen JE. Parathyroid hormone and vitamin D markers for cardiovascular and all cause mortality in heart failure. *Eur J Heart Fail* 2011; **13**:626-32.
5. Von Restroff C, Broschoff-Ferrari HA, Theilar R. High-dose oral vitamin D3 supplementation in rheumatology patients with severe vitamin D3 deficiency. *Bone* 2009: **45**:747-9.
6. Hollick MF, Chen Tai C. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 2008; **87**:1080S-6S.
7. Martineau AR, Tumors PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, *et al*. High-dose vitamin D3 during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomized controlled trial. *Lancet* 2011; **377**:242-50.
8. Lubna Z, Aysha HK, Jabbar A. High prevalence of vitamin D deficiency in medical clinic patients. *JPMA* 2008; **58**:482-4.
9. Mansoor S, Khan AH, Ghani F, Fatimi Z, Badruddin S, Mansoor S, *et al*. Prevalence and significance of vitamin D deficiency and insufficiency among apparently healthy adults. *Clin Biochem* 2010; **4**:1431-5.
10. Karim SA, Nusrat U, Aziz S. Vitamin D deficiency in pregnant women and their newborns as seen at a tertiary-care centre in Karachi, Pakistan. *Int J Gynaecol Obstet* 2011; **112**:59-62.
11. Hossain N, Khanani R, Hussain-Kanani F, Shah T, Arif S, Pal L. High prevalence of vitamin D deficiency in Pakistani mothers and their newborns. *Int J Gynaecol Obstet* 2011; **112**:229-33.
12. Dawsen-Hughes B, Mithal A, Bonjour JP, Boonem S, Buckhardt P, Fuleihan GE, *et al*. IOF position statement: vitamin D recommendations for older adults. *Osteoporosis Int* 2010; **21**: 1151- 4.

13. Goswami R, Gupta N, Ray D, Singh N, Tomar N. Pattern of 25-hydroxy vitamin D response at short (2 month) and long (1 year) interval after 8 weeks of oral supplementation with Cholecalciferol in Asian Indians with chronic hypovitaminosis D. *Br J Nutr* 2008; **100**:526-9.

14. Terrence HD, Kenneth HO, Peter GR, Matthew M. Annual intramuscular injection of a mega-dose of Cholecalciferol for treatment of vitamin D deficiency: efficacy and safety data. *Med J Aust* 2005; **183**:10-2.

15. Ashraf TS, Ashraf A, Magda W, Maryam A, Elsaid M, Aziz B. Manifestation of severe vitamin D deficiency in adolescents: effects of intramuscular injection of a mega dose of cholecalciferol [Internet]. *J Trop Pediatr* 2010; Available from: http://tropej.oxfordjournals.org/content/early/2010/04/28/tropej.fmq028.abstract

16. Guisti A, Barone A, Pioli G, Girasole G, Razzano M, Pizzonia M, *et al*. Heterogeneity in serum 25-hydroxy-vitamin D response to Cholecalciferol in elderly women with secondary hyperparathyroidism and vitamin D deficiency. *J Am Geriatr Soc* 2010; **58**:1489-95.

17. Gupta A, Gupta N, Singh N, Goswani R. Presence of impaired intestinal calcium absorption in chronic hypovitaminosis D and its change after Cholecalciferol supplementation: assessment by the calcium load test. *J Hum Nutr Diet* 2010; **23**:54-60. Epub 2009 Oct 15.

18. Cipriani C, Romagnoli E, Scillitani A, Chiodini I, Clerico R, Carnevale V, *et al*. Effect of a single oral dose of 600,000 IU of Cholecalciferol on serum calcitropic hormones in young subjects with vitamin D deficiency: a prospective intervention study. *J Clin Endocrinol Metab* 2010; **95**:4771-7. Epub 2010 Jul 21.

19. Khan AH. Vitamin D deficiency: are we still ignoring the evidence. *JPMA* 2008; **58**:482-4.

20. Khan AH, Jabbar A, Baqir R. Vitamin D deficiency osteomalacia: the continuing challenge. *J Coll Physicians Surg Pak* 2008; **18**:666.

21. Khan AH, Iqbal R. Vitamin D deficiency in an ample sunlight country. *J Coll Physicians Surg Pak* 2009; **19**:267-8.

22. Khan AH, Jabbar A, Baig R, Haq NU, Sutton R. Vitamin D deficiency osteomalacia: the continuing challenge (case report). *Pak J Pathol* 2008; **19**:146-8

23. Rikke A, Christian M, Lene TS, Christine B, Kevin DC, Jette J, *et al*. Effect of vitamin D supplementation on bone and vitamin D status among Pakistani immigrants in Denmark: a randomised double-blinded placebo-contolled intervention study. *Br J Nutr* 2008; **100**:197-207.

24. Nelson ML, Blum JM, Hollice BW, Rosen C, Sullivan SS. Supplements of 20 µg/d cholecalciferol optimized serum 25-hydroxy vitamin D concentration in 80% of pre-menopausal women in winter. *Am Inst Nutr* 2009; **139**:540-6.

.....✯.....