Vertebral spine osteoporosis treatment efficacy in local population: A clinical study

Muhammad Asadullah[1], Rahila Ikram[2] and Shazza Qazi [3]
[1]Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
[2]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[3]Hamdard College of Medicine and Dentistry, Hamdard University, Karachi, Pakistan

Abstract: In Pakistani population the prevalence of Calcium and vitamin D deficiency is at alarming rate. Previous studies show that globally vertebral osteoporosis is most commonly recognized site causing deterioration to personal life satisfaction. It is very unfortunate that in Pakistan ample amount of research work has not been done in the area, consequently, information on rate of vertebral osteoporosis & fracture are rare in Pakistan. There is no reduction in T-score on supplementation with calcium and vitamin D3 administration. There is reduction in T-score on supplementation with calcium and vitamin D3 administration. the prime objective of the current work was to determine vertebral spine osteoporosis treatment efficacy in local population. This is an intervention experimental study with no control. The study population was selected from the local community; consisting of individuals with vertebral spine osteoporosis, further they were followed for up to 6 months. Data was analyzed by SPSS–22. Tabs Chewable: Calcium: 1250 mg, Cholecalciferol: 125 IU, BD/Day was advised. The mean T-score before and after treatment were recorded as; Mean ±S.D: 2.890 ±1.7217 and Mean ±S.D: -2.456±0.8064 respectively. The findings of the current work do not provide support for routine supplementation with calcium and vitamin D3 orally for osteoporosis.

Keywords: Pakistan, vertebral spine, osteoporosis.

INTRODUCTION

Bones tissues are no longer considered as a static part of the organs. Like different bones in the human skeleton, the spinal segment perseveres through numerous bone-related pathologies. Past investigations recommend that on the average the risk of lifetime experiencing vertebral fractures above age of 50 years is around 16% in postmenopausal ladies and 5% in men. Low bone density (LBD); the characterizing issue of osteoporosis, remains the most well-known reason for spine fractures (Melton, 1997). Additionally, age-related factors or idiopathic determinants for osteoporosis play vital role in increasing the risk of bone fracture. To mention about, some recent reviews revealed that among those diagnosed with the problem up to 60% of men, half of premenopausal and 30% of postmenopausal women experience the ill effects of osteoporosis optional to a systemic condition**.** Globally Population with age group of above 50 years are more prone to global health problems consequently posing significant burden on through the increase in disabilities and deaths associated with delicacy breaks (NIH, 2001)**.** Commonness among white postmenopausal women in Rochester is 33% and 8% at wrist and lumbar spine individually. A review led by Canadian Multicentre Osteoporosis (CMO) for the pervasiveness at lumbar vertebra was evaluated 12 % (Tenenhouse *et al*., 2000)**.**

When DEXA scan was made laterally of spine, lumbar osteoporosis prevalence was found to be 33% in Rochester ladies. In light of ordinary sweeps of the aggregates hip, wrist and AP spine, 35% white postmenopausal ladies have osteoporosis at least of one site as indicated by the WHO (Melton *et al*., 1998; Consensus, 1993). Cranney *et al.* (2008) conducted an inquire about the utilization of vitamin D as an adjuvant treatment in white population, his study concluded that daily intake of 700 IU of vitamin D or more with calcium of 500 to 1,200 mg anticipates lumbar spine bone fracture contrasted with a placebo treatment in the study populations. Shockingly, randomized controlled trials of vitamin D supplements are reliably conflicting. They neglect to explore contrasts by either sex, further; no clear meaning of decrease serum levels of vitamin D has been set up. Besides, just a couple of researches mentioned about the impacts of various dosages of vitamin D on bone wellbeing (Winzenberg *et al*, 2011) (Binkley *et al*, 2004). Decrease serum calcium combines with vitamin D inadequacy; increase break chance both by upgrading bone digestion and by expanding the risk of falling. Replacement with calcium and vitamin D diminishes bone fracture and danger of falling and consequently is prescribed as first-line system in the aversion of osteoporosis (NOF, 2014).

Rules commonly apply this proposal to men and women, though most investigations just have or essentially included women, with restricted information in males (Boonen *et al*, 2006)**.** Evidence based empirical research is not available in ample amount to provide support for

**Corresponding author:* e-mail: aarahila18@gmail.com

utilization of supplements, for example, vitamin D and calcium and its consequences for bone mineral density in local population, have not been well documented. Sufficient supplementation of vitamin D and calcium may streamline bone wellbeing, diminish bone fracture, and limit danger of delicacy breaks (Parfitt, 2004). Further, Calcium nutrition is additionally essential obviously; however, the confirmation that calcium alone averts breaks is negligible. As research shows that Bone density decreases more rapidly in winter than summer hence, Vitamin D3 supplements (around 20 mg (800 IU) every day) consolidated with calcium is recommended, as it will wipe out the faster fall in bone density during winter (Rosen *et al*, 1994). Besides, three reviews demonstrated that calcium and vitamin D3 together lower fracture hazard in peoples with age group above 65 years (Chapuy *et al*, 2002). Vertebral discomfort are now the most broadly perceived osteoporotic consequences (Felsenberg *et al*, 2002), just 1/3rd of them are recognized by an obvious clinical occasion, for example, intense backache (Cooper *et al*, 1992). Vertebral fractures observed by X-ray or other imaging methodologies are not declared. 46.5% of vertebral breaks identifiable by X-ray are not perceived in Latin America (Delmas *et al*., 2005). Regardless of the possibility, asymptomatic vertebral fractures however, are connected with life debilitating outcomes (Jalava *et al*., 2003; Kado, 2003). It is assessed that one in three women and one in twelve men belong to age group of 50 years and above will experience the ill effects of osteoporosis in their lifetime, which generally compares to three million people (Van Staa *et al*, 2001). In Europe, the cost of treating osteoporotic fractures is significantly high among all types of osteoporosis treatments (Dennison & Cooper, 2007). Jejunum, ileum and colon are the predominant locations of calcium absorption. Its uptake occurs by two mechanisms: (i) active transport and (ii) simple passive diffusion. At the point when there is low intake of Ca, active transport prevails and as intake increases, it consumed by non-specific pathways (Department of Health, 1998). 1, 25-dihydroxycholecalciferol, metabolite of vitamin D fortifies Ca transport over the intestinal cells by initiating CBP (Calcium Binding Protein) generation. This system happens inside the villus cells through the ordinary procedure of binding with receptor, DNA association and RNA generation.

Consequently, vitamin D is basic for effective Ca absorption (Food & Nutrition Board of the Institute of Medicine, 1997). Stimulation of vitamin D causes improvement in bone matrix formation and development. It additionally improves osteoclastic action and there is little information to propose that it might impact separation of bone cell precursors (Underwood & De Luca, 1984). Together with parathyroid hormone, it directs Calcium and Phosphorus metabolism and advances Ca absorption from the gut and renal tubules (Boland, 1986). With serum 25 OHD concentrations inside the reference range, partial Ca absorption demonstrates increments (Heaney *et al*, 2003).

Hypothesis

N / Hypothesis

There is no reduction in T-score on supplementation with calcium and vitamin D3 administration.

Alternate hypothesis

There is reduction in T-score on supplementation with calcium and vitamin D3 administration.

MATERIALS AND METHODS

IRB (institutional review board) JPMC, Karachi Pakistan, approved this study.

Study design, setting and population

This is an intervention experimental study with no control for the duration of six months (from Jan 2016 to Dec 2016) conducted at outpatient clinic (OPD), Medical unit 6, JPMC, Karachi, Pakistan. Participants were randomly advised two chewable tablets every day comprising of calcium and vitamin D3. All materials were delivered each month and participants were made a request to take tablets until trial conclusion. Population studied was 350, out of which 87 were diagnosed as osteoporotic through DEXA scan. Out of these 87, only 20 patients completed our study protocols. The study outline and sample size of the investigation relied upon a factorial intend to test calcium and vitamin D3 impact.

Intervention

Subjects (n = 20) completed 6 months of oral calcium and vitamin D (daily) was administered. Lumbar spine Dexa scan was performed at baseline and at twenty four weeks. Oral vitamin D3 (125 IU) daily and Calcium Carbonate 1250 mg) BD/Day was advised. Analyzation of data was done six months after the last individual was enrolled and depended on expectation to-treat.

Inclusion criteria

All those healthy individuals who have not been diagnosed with obvious disorder. The age group was of the study population was 19-96 years, who are postoperative at least of three months.

Exclusion criteria

Previous fractures, patient already on vitamin D supplements plus calcium supplements, pregnant women, women in stage of lactation, smoker, former smoker, and individuals treated with bisphosphonate were excluded.

RESULTS

The mean T-score before and after treatment were recorded as; Mean ± S.D:-2.890±1.7217 and Mean ± S.D:

-2.770±0.6441 respectively. On the average -0.1200 decrease was observed in Mean T-score values after treatment, with t-statistic -3.330, exact probability value of P=0.745. The 95% CI of mean difference is -0.8819 to -0.6419. The study target population was 350 individuals. Out of which 87 participants were diagnosed as osteoporotic through DEXA scan. Among these 87 participants diagnosed with osteoporotic, only 20 patients have completed the study protocols. At the end of the study 8 participants were diagnosed with Osteopenia whereas the remaining 12 were diagnosed with osteoporotic and -0.1200 reduction was recorded in their Mean T-scores.

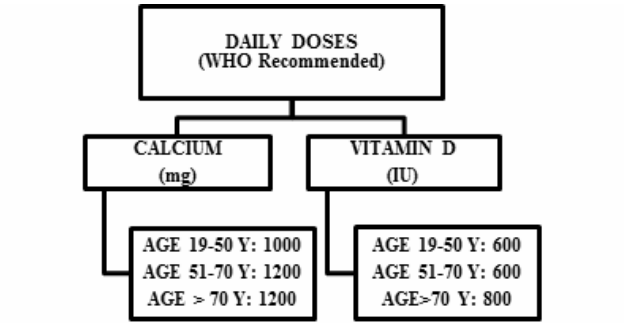

Fig. 1: Flow chart of daily doses of Calcium and Vitamin-D, recommended by WHO (WHO, 2004, Ross *et al*, 2011).

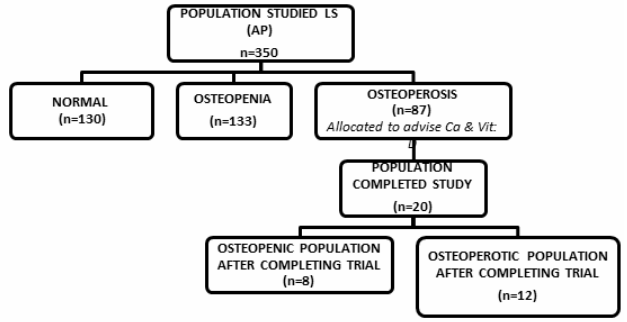

Fig. 2: Flow chart of studied population (Lumbar spine).

Summary statements

A sample size of 19 from a population of 350 achieves 79% power to detect non-inferiority using a one-sided Wilcoxon test assuming that the actual distribution is normal when the margin of equivalence is 2.100 and the true difference between the mean and the reference value is 0.500. The data are drawn from a single population with a standard deviation of 1.700. The significance level (alpha) of the test is 0.05000 (Al-Sunduqchi 1990; Chow *et al.*, 2003).

DISCUSSION

Last decade has experience ample body of research dedicated to examine the impact of growing supplementation with calcium and vitamin D advice on bone loss and break rates. The studies show that those postmenopausal women are more prone to Osteoporosis. Both supplements adjust calcium controlling hormone regulations and bone mass. Insufficient intake of either of these supplements brings about a lessened amount of ingested calcium bringing down ionized calcium concentration in the circulation and enhances PTH levels. PTH gives back the calcium level to typical by lessening release of calcium from the kidneys, by propelling the activation of vitamin D and by advancing bone resorption. For bone resorption in old aged individuals, bone remodelling is uncoupled. Consequently, the PTH-induced increased remodelling of bones in those people brings about an expanded rate of bone loss. Recent work has offer help to support an expansion in the bone turnover rate, autonomous of its impact on bone mineral density (BMD), builds danger of fracture. Calcium ingestion effectiveness decreases with maturing. Vitamin D is formed in the epidermal layer of the skin on exposure to bright ultraviolet beams of the sun. Meta-analyses contrasting the impact of calcium alone with placebo treatment uncovered that calcium as mono therapy does not fundamentally diminish fracture risk (Bischoff-Ferrari *et al*, 2005)**.** Boonen and co - workers (2007), in a meta - analysis of (n= 9083) four randomized controlled trials, reached to the conclusion that 700-800 IU of vitamin D alone daily for prevention purpose was deficient instead of utilizing high doses (Boonen *et al*., 2007). DIPART Group in 2010, in a meta-analysis concluded that regardless of gender, 400-800 IU of vitamin D3 is not effective in fracture prevention (DIPART Group, 2010). A new concept has also been developing about the confirmation that mild vitamin D inadequacy can detrimentally affect bone mineral mass in adolescent females and children (Outila *et al*, 2001)**.** A 3-year prospective cohort investigation in 171 healthy Finnish young ladies developed having a place with age group of 9-15 years differentiating subjects and genuine hypovitaminosis D (25 OHD <20 nmol/l) with those with typical vitamin D status (25 OHD 37.5 nmol/l) has discovered a refinement of 4% in BMD accumulation (Cheng *et al*., 2003). Balanced change in spine BMD has been recorded to be 27% more for subjects in the most significant tertile of vitamin D consumption separated and those in the least tertile. Pakistan additionally confronts expanding rates of osteoporosis. As indicated by Alam F, *et al*., (2016), right now 9.91 million individuals experiencing the ailment; the number is projected to increase to 11.3 million by 2020 and 12.91 million by 2050. In any case, Pakistani population is likewise tested with an endemic of vitamin D lack. Giving to two or three reviews directed in Karachi, Vitamin D lack is 70-97% in asymptomatic people (Alam F, *et al*., 2016). Outcomes were accounted for to be critical just for young girls at the post pubertal stage, without any distinctions for young girls of less maturity (<12 years); a finding that has been accounted previously (El-Hajj Fuleihan & Veith, 2007). To attain a low bone mass peak, decrease vitamin D level

status is a risk factor (El-Hajj Fuleihan *et al*., 2001). These outcomes took after a dose response relationship for the individuals who were Ca replete subjects. A qualification was similarly watched for lumbar spine bone density among the gathering supplemented at 10 mg daily and the placebo treatment bunch. Besides, vitamin D supplementation was found to basically decrease bone resorption (as assessed by urinary deoxypyridinoline release). Results were simply observed to be basic when the data were inspected by the consistence - based procedure (which joins just individuals who consented to guidelines) instead of the objective to-treat method (which incorporates all individuals who entered the trial). More inquires about are required on the merged effect of Ca and vitamin D on boosting amplifying bone mass achievement; particularly in youths. With maturing, the skin thins and contains less of the 7-dehydrocholesterol (vitamin D substrate). Various specialists have handled the subject of how much calcium and vitamin D is expected to limit bone loss. The effect of calcium and vitamin D on rates of bone loss has been comprehensively inspected in postmenopausal ladies.

It is now clear that this combination of supplementation will not counteract oestrogen insufficiency related bone loss. Meanwhile at skeletal sites, the rate of loss rich in cortical bone can be unassumingly lessened with calcium supplementation. At trabecular rich skeletal sites, for example, the spine, supplementation may improve BMD at first by bringing down the bone turnover rate; however, after the first year of treatment, no further favourable position to BMD is observed. A current Meta - analysis showed that in early postmenopausal women, BMD rises up from hormone replacement therapy (HRT) were altogether more significant in women who likewise took calcium supplements than they were in women who took HRT without included calcium. In this investigation, the calcium-supplemented women had an ordinary intake of around 1200 mg every day as appeared differently in relation to a large portion of that admission level in the unsupplemented ladies. This examination underscores the centrality of adequate calcium allow in ladies taking HRT. Numerous randomized controlled trials examining the effect of included calcium or conceivably vitamin D on rates of bone loss have been coordinated in ladies since most recent 6 years of menopause. In these ladies, calcium conventionally dependably cuts down rates of bone loss at cortical and trabecular rich skeletal goals. A couple of audits have attempted supplementation with vitamin D. In one such survey, all ladies were put on calcium supplements and the ladies were then randomized to either a vitamin D supplement or a placebo treatment. Vitamin D supplemented women had cut down rates of bone loss than did the women taking calcium as it were. Different recent audits have taken a looked at the effect of calcium as well as vitamin D on rate of low damage fractures. It is accounted for that vitamin D oral (400 IU) daily had no effect on fracture in this way the effects of merged calcium and vitamin D supplementation on fracture was watched. In elderly French women living in nursing homes, combined supplementation with 1200 mg of calcium and 800 IU of vitamin D consistently cut down fracture rates. These ladies had normal calcium intakes of around 500 mg daily with very low vitamin D levels. The impact of joined supplementation was assessed recently in active men and women, age 65 and older, who resided at home in Boston. Study subjects who took 500 mg calcium and 700 IU vitamin D daily had slow bone loss and fewer clinically manifested fractures over the three years investigation duration than those who availing placebo treatment. (Thomas *et al*., 2001).

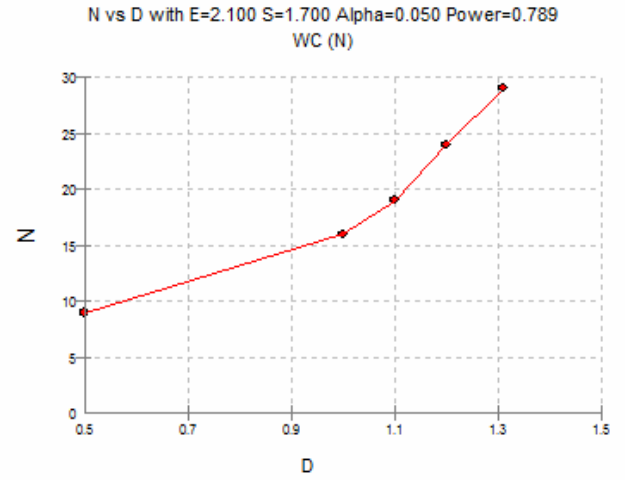

Fig. 3: non-inferiority test of one mean power analysis

U.S. Preventive Administrations Team (USPSTF) infers that the present proof is lacking to assess the modify of the focal points and harms of merged vitamin D and calcium supplementation for the fundamental balancing activity of fracture in light of osteoporosis in premenopausal ladies or in men. The USPSTF construes that the present affirmation is missing to assess the balance of the preferences and damages of every day supplementation with more prominent than 400 IU of vitamin D3 and more prominent than 1,000 mg of calcium for the essential preventive activity of fractures in non-institutionalized postmenopausal ladies. They proposed against daily supplementation with 400 IU or less of vitamin D3 and 1,000 mg or less of calcium for the evasion of fractures in such ladies (US Preventive Services Task Force, 2014).

One portion of all postmenopausal ladies will have an osteoporosis-related fracture during their lifetime. Points of interest and damages of every day supplementation of vitamin D3 and of calcium osteoporosis are not caught on. In premenopausal ladies and in men, there is lacking confirmation to choose the effect of merged vitamin D and calcium supplementation on osteoporosis. In postmenopausal women, there is adequate proof that

Table 1: AP LS Calcium Vit D3

			Paired t-test			
	Mean ± S.D	n	Diff of Mean	95% CI of Difference	Paired t-test	p-value
T-score (Pre-exposure)	-2.890±1.7217	20	-0.1200	-0.8819 to -0.6419	-3.330	0.745*
T-score (Post-exposure)	-2.770±0.6441	20				

*Non significant

Table 2: non-inferiority test of one mean power analysis

Margin Power	Actual Significance Difference Level					Standard Deviation
	N	(E)	(D)	Alpha	Beta	SD
0.79204	19	2.1	1.1	0.05	0.20796	1.7
0.79848	24	2.1	1.2	0.05	0.20152	1.7

Numeric Results for Non-Inferiority Test (H0: D >= |E|; H1: D < |E|)
Test Statistic: Wilcoxon Test (Normal Distribution)
Population size = 350.

supplementation with 400 IU of vitamin D3 combined with 1,000 mg of calcium daily has no effect on osteoporosis. There is deficient verification about the effect of higher dosages of consolidated vitamin D and calcium supplementation on fracture rate in non-institutionalized postmenopausal women. Vitamin D and calcium supplementation is as often as possible proposed for women, especially postmenopausal women, to avert breaks. Studies assess that 56% of women with age equal to greater than 60 years established take supplemental vitamin D, and 60% take supplement, containing calcium (Gahche *et al*., 2011). Our study participants were adaptable and group staying so in this manner less likely to profit by substitution which may have contributed essentially to the negative after effects of our study. Our outcomes finding is as per Boonen *et al*, (2006). Combination of calcium and vitamin D supplementation was observed non-significant at the spine in another study (Jackson *et al*, 2006**).** These debatable findings could be for the most part cleared up by variations in dosages and differences in dosing (Chel *et al*., 2008). Also, additionally contrast in vitamin D preparations and the heterogeneity of study populations may confound the outcomes. There is still no complete and definitive answer about how vitamin D and calcium supplementation can influence bone status, and especially, there are no masses based trials to institutionalize such issues in Pakistan. Chapuy and colleagues' (Chapuy *et al*., 2002) and Dawson-Hughes' (Dawson-Hughes *et al*., 1997) in primary-prevention trials, did not find a significant impact of consolidated calcium and vitamin D3 on osteoporosis prevention and fracture. Our trial was not proposed to specifically address whether supplementation ought to be used as a basic avoidance measure or in the people who live in a care-home condition. Elucidation of the part of supplementation in these settings foresees the results of various trials. The daily cholecalciferol 800 IU and calcium carbonate 1,000-mg supplementation expanded the aggregate body BMD in women aged 66 to 71 years in this randomized three years population-based trial. No site-particular changes at lumbar spine were recognized in the fundamental investigations (Kärkkäinen *et al*., 2010). Proposals for how to rethink for osteoporosis are sketchy and may lead nowhere. Nevertheless, they represent exactly the sort of exciting new avenues for researchers' dream of exploring. So why not divert more attention in this matter to the hunt for its nemesis? If such results exist, it will withstand the challenge and emerge vindicated.

CONCLUSIONS

The outcomes of the study do not support oral supplementation with calcium and vitamin D3 for osteoporosis. Hence, it is recommended that more than six months duration of the studies will be required in order to get standardized recommendations according to the DEXA scan results.

REFERENCES

Al-Sunduqchi and Mahdi S (1990). Determining the Appropriate Sample Size for Inferences Based on the Wilcoxon Statistics. Ph.D. dissertation under the direction of William C. Guenther, Dept. of Statistics, University ofWyoming, Laramie, Wyoming.

Binkley N, Krueger D and Cowgill CS *et al* (2004)**.** Assay variation confounds the diagnosis of hypovitaminosis D: A call for standardization. *J. Clin. Endocrinol. Metab.*, **89**: 3152-3157.

Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T and Dawson-Hughes B (2005) Fracture prevention with vitamin D supplementation: a meta-analysis of randomised controlled trials. JAMA., **293**: 2257-2264.

Boland R (1986) Role of vitamin D in skeletal muscle function. *Endocr. Rev.,* **7**: 434-447.

Boonen S, D Vanderschueren, P Haentjens and P Lips (2006). Calcium and vitamin D in the prevention and treatment of osteoporosis a clinical update. *J. Intern. Med.,* **259**(6): 539-552.

Boonen S, P Lips, R Bouillon, HA Bischoff-Ferrari D Vanderschueren and P Haentjens (2007). Need for additional calcium to reduce the risk of hip fracture with vitamin D supplementation: Evidence from a comparative meta analysis of randomized controlled trials. *J. Clin. Endocrinol. Metab.,* **92**(4): 1415-1423.

Chapuy MC, Pamphile R and Paris E *et al* (2002). combined calcium and vitamin D3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: The Decalyos II study. *Osteoporosis Int*., **13**: 257-264.

Chapuy MC, Pamphile R, Paris E, Kempf C, Schlichting M and Arnaud S *et al* (2002). Combined calcium and vitamin D3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: the Decalyos II study. *Osteoporos. Int.,* **13**: 257-264.

Chel V, Wijnhoven HAH, Smit JH, Ooms M and Lips P (2008). Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. *Osteoporos Int.,* **19**: 663-671.

Cheng S, Tylavsky F and Kroger H *et al*. (2003). Association of low 25-hydroxvitamin D concentrations with elevated parathyroid hormone concentrations and low cortical bone density in early pubertal and prepubertal Finnish girls. *Am. J. Clin. Nutr.,* **78**: 485-492.

Chow SC Shao J and Wang H (2003). Sample Size Calculations in Clinical Research. Marcel Dekker. New York.Julious, Steven A. 2004. Tutorial in Biostatistics. Sample sizes for clinical trials with Normal data. *Statistics in Medicine*, **23**:1921-1986.

Consensus, A (1993). Consensus development conference: Diagnosis, prophylaxis and treatment of osteoporosis. *Am. J. Med.,* **94**: 646-650.

Cooper C, Atkinson EJ, O Fallon WM, Melton LJ 3rd (1992). Incidence of clinically diagnosed vertebral fractures: A population-based study in Rochester, Minnesota, 1985-1989. *J. Bone. Miner. Res.,* **7**: 221-227.

Cranney A, Weiler HA, O'Donnell S and Puil L (2008)**.** Summary of evidence based review on vitamin D efficacy and safety in relation to bone health. *Am. J. Clin. Nutr.,* **88**: 513S-519S.

Dawson-Hughes B, Harris SS, Krall EA and Dallal GE (1997). Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N. Engl. J. Med*., **337**: 670-676.

Delmas PD, van de Langerijt L, Watts NB, Eastell R, Genant H and Grauer A *et al* (2005). IMPACT Study Group. Under diagnosis of vertebral fractures is a worldwide problem: The IMPACT study. *J. Bone Miner Res.,* **20**(4): 557-563.

Dennison E and Cooper C (2007). Lifestyle and constitutional risk factors. In Managing Osteoporosis, pp. 69-80 [SA Lanham-New, T O'Neill, R Morris, D Skeleton and A Sutcliffe, editors]. Oxford: Clinical Publishing.

Department of Health (1998). Nutrition and Bone Health: With Particular References to Calcium and Vitamin D. Report on Health and Social Subjects 49. London. H. M. Stationery Office.

DIPART Group (2010). Patient level pooled analysis of 68 500 patients from seven major vitamin d fracture trials in us and Europe. *BMJ*, **340**: b5463.

El-Hajj Fuleihan G, Nabulsi M, Choucair M, Salamoun M, Hajj Shahine C, Kizirian A and Tannous R (2001). Hypovitaminosis D in healthy schoolchildren. *Pediatrics,* **107**: E53.

El-Hajj Fuleihan G, Veith R, Netelenbos JC, Lips P, van Ginkel FC, Khoe E, Leeuwenkamp OR and Hackeng Elders PJM (2007). Vitamin D insufficiency and musculoskeletal health in children and adolescents. In Nutritional Aspects of Osteoporosis, pp.91-108.

Faiza Alam, Salwa Nihal, Alizeh Abbas, Muhammad Abdullah, Sana Shah, Syed Arish Haider and Syeda Sadia Fatima (2016). Reciprocal Relation of Fetuin- A and Beta Crosslaps with Bone Health. *J. Orthop. Res. Physiother.,* 2: 024.

Felsenberg D, Silman AJ, Lunt M, Armbrecht G and Ismail AA and Finn JD *et al* (2002). European Prospective Osteoporosis Study (EPOS) Group Incidence of vertebral fracture in Europe: results from the European Prospective Osteoporosis Study (EPOS). *J. Bone Miner. Res.,* **17**(4): 716-724.

Food and Nutrition Board of the Institute of Medicine (1997). Dietary Reference Intakes: Calcium, Phosphorus, Magnesium, Vitamin, and Fluoride. Washington, DC: National Academy Press.

Gahche J, Bailey R and Burt V *et al* (2011). Dietary supplement use among U.S. adults has increased since NHANES III (1988-1994). *NCHS Data Brief*., **61**: 1-8.

Heaney RP, Dowell MS and Hale CA (2003). Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J. Am. Coll. Nutr.,* **22**: 142-146.

Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, Bassford T, Beresford SA, Black HR, Blanchette P, Bonds DE, Brunner RL, Brzyski RG, Caan B, Cauley JA, Chlebowski RT, Cummings SR, Granek I, Hays J, Heiss G, Hendrix SL, Howard BV, Hsia J, Hubbell FA, Johnson KC, Judd H, Kotchen JM, Kuller LH, Langer RD, Lasser NL, Limacher MC, Ludlam S, Manson JE, Margolis KL, McGowan J, Ockene JK, O'Sullivan MJ, Phillips L, Prentice RL, Sarto GE, Stefanick ML, Van Horn L, Wactawski-Wende J, Whitlock E, Anderson GL, Assaf AR and Barad D (2006). Women's Health Initiative Investigators Calcium plus vitamin D supplementation

and the risk of fractures. *N. Engl. J. Med.*, **354**(7): 669-683.

Jalava T, Sarna S, Pylkkänen L, Mawer B, Kanis JA and Selby P *et al* (2003)**.** Association between vertebral fracture and increased mortality in osteoporotic patients. *J. Bone Miner Res.*, **18**(7): 1254-1260.

Kado DM, Duong T, Stone KL, Ensrud KE, Nevitt MC and Greendale GA *et al* (2003). Incident vertebral fractures and mortality in older women: a prospective study. *Osteoporos. Int.*, **14**(7): 589-594.

Kärkkäinen M, M Tuppurainen K, Salovaara L, Sandini, T Rikkonen, J Sirola, R Honkanen, J Jurvelin, E Alhava and H Kroger (2010). Effect of calcium and vitamin D supplementation on bone mineral density in women aged 65-71 years: A 3-year randomized population-based trial (OSTPRE-FPS); *Osteoporos Int.*, **21**: 2047-2055.

Melton LJ II (1997). Epidemiology of spinal osteoporosis. *Spine*, **22**: 2S-11S.

Melton LJ III, Atkinson EJ, O'Connor MK *et al* (1998). Bone density and fracture risk in men. *J. Bone Miner Res*., **13**: 1915-1923.

NIH (2001). Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy. *JAMA.*, **285**: 785-795.

Outila TA, Kakkainen MU and Lamberg-Allardt CJ (2001) Vitamin D status affects serum parathyroid hormone concentrations during winter in female adolescents: Associations with forearm bone mineral density. *Am. J. Clin. Nutr.*, **74**: 206-210.

Parfitt AM (2004). The evolution of vitamin D-related bone disease: The importance of an early stage of increased bone turnover without impaired mineralization. In: Burckhardt P, Dawson-Hughes B, Heaney R, editors. Nutritional Aspects of Osteoporosis. New York: Academic Press, pp.197-208.

Rosen CJ, Morrison A, Zhou H, Storm D, Hunter SJ and Musgrave K *et al* (1994). Elderly women in northern New England exhibit seasonal changes in bone mineral density and calciotropic hormones. Bone Miner., **25**: 83-92.

Ross CA, Taylor CL, Yaktine AL, Del Valle HB and eds (2011). Committee to Review Dietry Referance Intakes for Vitamin D and Calcium; Institute of Medicine. Dietry Referance Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press.

Tenenhouse A, Joseph L and Kreiger N *et al* (2000)**.** Estimation of the prevalence of low bone density in Canadian women and men using a population-specific DXA reference standard: The Canadian Multicentre Osteoporosis Study (CaMos). *Osteoporos. Int.*, **11**: 897-894.

Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, Vamvakas Torgerson DJ, Iglesias C and Reid DM (2001) Economics of Osteoporosis. Key Advance Series. London: Aesculapius Press.

U.S. Preventive Services Task Force (2014). Vitamin D and Calcium Supplementation to Prevent Fractures in Adults: Recommendation Statement, American Family Physician, **89**: 896CDE.

Underwood JL and DeLuca HF (1984) Vitamin D is not directly necessary for bone growth and bone mineralization. *Am. J. Physiol. Endocrinol. Metab.*, **246**: E492-E498.

Van Staa TP, Dennison EM, Leufkens HG and Cooper C (2001). Epidemiology of fractures in England and Wales. *Bone*, **29**: 517-522.

Winzenberg T, Powell S, Shaw KA and Jones G (2011)**.** Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. *BMJ.*, **342**: c7254.

World Health Organization (2004). and Food and Agriculture Organization of the United Nations. Vitamin and Mineral Requirements in Human Nutrition. 2nd ed. World Health Organization, Geneva, Switzerland, pp.45-85.