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Vitamin D Supplementation For Non Specific Musculoskeletal Pain Among Adults Attending Department Of Orthopaedics, Gauhati Medical College: A Randomized Controlled Study

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Abstract
Objectives: *The objective of this study was to measure the level of vitamin D in adult patients coming with nonspecific musculoskeletal pain and evaluate the effect of the correction of vitamin D deficiency on the pain, fatigue and quality of life.*
Methods: *A pragmatic prospective study was conducted in Orthopaedics out patient department at Gauhati Medical College and Hospital, between January 1 and June 30, 2016. All patients aged 18 to 60 years consulting for diffuse nonspecific musculoskeletal chronic pain and/or chronic unexplained fatigue, were enrolled in this study. They were randomised into study group who received the vitamin D supplementation and the control group who received a placebo.*
Results: *Eighty nine complete cases underwent the study. Out of this sixty eight patients were deficient, fifteen patients had insufficient vitamin D level and rest six had sufficient level. The correction of vitamin D deficiency resulted in a significant decrease in the level of pain intensity according to VAS (P=0.001) and the pain free duration (P<0.001), consumption of analgesics (P=0.002) and discomfort in carrying out activities of daily living following: the shopping, cleaning, walking for more than a kilometer (P<0.001) and dressing (P=0.012).*
Conclusion: *The correction of vitamin D has had a positive impact both physical, mental and social activities. Chroninc nonspecific musculoskeletal pain should be evaluated for vitain D deficiency. Supplementation in these patients brings overall improvement in these patients.*

I. Introduction

Vitamin D, the sunshine vitamin, is now recognized not only for its importance of bone health in children and adults, but also for other health benefits including reducing risk of chronic diseases including autoimmune diseases, common cancer and cardiovascular disease. Vitamin D made in the skin or ingested in the diet is biologically inert and requires two successive hydroxylations first in the liver on carbon 25 to form 25-hydroxyvitamin D [25(OH)D], and then in the kidney for a hydroxylation on carbon 1 to form the biologically active form of vitamin D, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. With the identification of 25(OH)D and $1,25(OH)_2D$, methods were developed to measure these metabolites in the circulation. Serum 25(OH)D is the barometer for vitamin D status. Serum $1,25(OH)_2D$ provides no information about vitamin D status and is often normal or even elevated due to secondary hyperparathyroidism associated with vitamin D deficiency. Most experts agree that 25(OH)D of < 20 ng/ml is considered to be vitamin D deficiency whereas a 25(OH)D of 21-29 ng/ml is considered to be insufficient. The goal should be to maintain both children and adults at a level > 30 ng/ml to take full advantage of all the health benefits that vitamin D provides.

Historical Perspective

The association of sunlight and vitamin D for bone health began with the industrialization of northern Europe. The lack of adequate sun exposure resulted in an epidemic of children with severe growth retardation and bony deformities that was commonly known as rickets.[1] In 1919, Huldschinsky et al[2]reported that exposure to ultraviolet radiation cured rickets. This was followed by Hess and Unger in 1921[3]who observed that exposure to sunlight cured rickets.

In the 1930's, it was appreciated that ultraviolet irradiation of yeast extract was effective in producing an antirachitic substance known as vitamin D. This vitamin D was structurally identified and called vitamin D_2. Vitamin D_3 was identified by the irradiation of 7-dehydocholesterol. Because vitamin D_2 was inexpensive to produce, vitamin D_2 was used widely for the fortification of foods including milk and bread in the United States and Europe. When 7-dehydrocholesterol was easily extracted from lanolin from sheep's wool, vitamin D_3 was inexpensively made and was used in food fortification and for supplements.

In the early 1950's, there was an outbreak of hypercalcemia thought due to the over fortification of milk with vitamin D, and as a result, most European countries forbid the fortification of milk and other dairy

products with vitamin D. In the United States, milk and orange juice are fortified with vitamin D_3 whereas a majority of multivitamin supplements and pharmaceutical preparations contain vitamin D_2.[1, 4]

The appreciation that vitamin D (D represents either D_2 or D_3) required a hepatic hydroxylation on carbon 25 to produce 25-hydroxyvitamin D [25(OH)D] (Fig 1) led to the development of a binding protein assay using the vitamin D binding protein (DBP) to measure circulating levels of 25(OH)D in the circulation.[5-7]The identification of 1,25-hydroxyvitamin D as being the biologically active form of vitamin D led to the development of a binding protein assay using the vitamin D receptor as the binder to measure circulating levels of $1,25(OH)_2D$.[8-10]

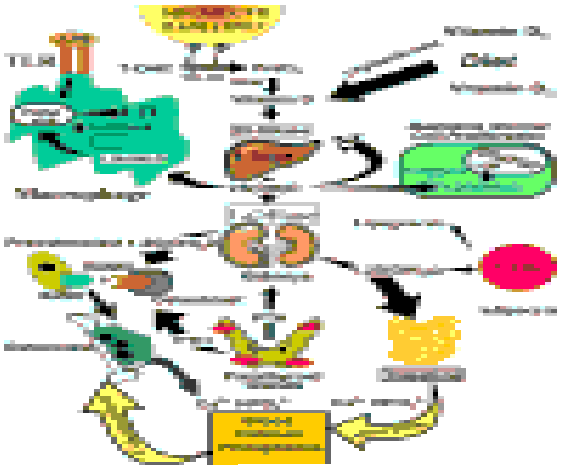

Figure 1

The metabolism and biologic function of vitamin D. During exposure to sunlight, 7-dihydrocholesterol (7-DHC) is photolyzed to previtamin D_3 ($preD_3$). Body heat converts $preD_3$ to vitamin D_3. Vitamin D_2 and vitamin D_3 in the diet and vitamin D made in the **...**

25(OH)D Assays

The first assays for 25(OH)D used the competitive protein binding format with the vitamin D binding protein (DBP) as the binder. The advantage of this assay was that DBP recognized $25(OH)D_2$ equally as well as $25(OH)D_3$. The major limitation of this assay was that the assay measured 25(OH)D in a serum sample that contained other vitamin D metabolites including 24,25-dihydroxyvitamin D [$24,25(OH)_2D$], 25,26-dihydroxyvitamin D and the 25,26-dihydroxyvitamin D -26, 23-lactone.[11] However, typically these more polar metabolites of 25(OH)D circulate at less than 10-15% of the total concentration of 25(OH)D and were usually insignificant.

In 1985, a radioimmunoassay (RIA) was developed for 25(OH)D.[11, 12] This assay (Diasorin[@]) recognized $25(OH)D_2$ equally as well as $25(OH)D_3$.[11, 12] However, like the DBP competitive protein binding assay, the RIA for 25(OH)D also recognized $24,25(OH)_2D$ and other polar metabolites to the same extent. Thus, both the DBP and the RIA assays typically overestimated 25(OH)D levels by approximately 10-20%. However, since the coefficient of variations (CV) for the intra and interassay variations were 8-15%, this was within the CV for the assay. More recently IDS developed an RIA which has a 100% specificity for $25(OH)D_3$ and only 75% specificity for $25(OH)D_2$.

To remove interfering vitamin D metabolites, simple preparative chromatography was developed to separate 25(OH)D from more polar metabolites that interfered with the assay. In the mid-1970's, high performance liquid chromatography (HPLC) was applied to the 25(OH)D assay.[11, 13] This assay included a lipid extraction of the serum followed by preparative chromatography and the 25(OH)D fraction was applied to HPLC and the UV absorption of 25(OH)D was used to measure its concentration. HPLC was considered to be the gold standard but was a very cumbersome assay, and, thus, was not routinely used by reference laboratories for clinical samples.

The advances in liquid chromatography tandem mass spectroscopy (LC-MS) was applied for the direct measurement of 25(OH)D in human serum. This assay quantitatively measured both $25(OH)D_2$ and $25(OH)D_3$.[14, 15]

1,25-Dihydroxyvitamin D Assays

Once $1,25(OH)_2D$ was identified, an assay using the chicken intestinal vitamin D receptor was developed as a competitive protein binding assay to measure circulating levels of 1, 25(OH)D.[8] It was observed

that the bovine thymus was an excellent source for the VDR and competitive binding protein assay for 1, $25(OH)_2D$ was developed using bovine VDR as the binding protein.[9, 11]
Radioimmunoassays were later developed to measure 1,25(OH)D. The Diasorin assay was reported to measure $1,25(OH)D_3$ equally as well as $1,25(OH)D_2$. However, the IDS assay recognized $1,25(OH)_2D_3$more effectively than $1,25(OH)_2D_2$.

Determination of Vitamin D Status

25(OH)D is the only vitamin D metabolite that is used to determine whether a patient is vitamin D deficient, sufficient or intoxicated.[1, 4, 16, 17] 25(OH)D is the major circulating form of vitamin D that has a half life of approximately 2-3 weeks. 25(OH)D is a summation of both vitamin D intake and vitamin D that is produced from sun exposure.[1, 4]

Although $1,25(OH)D_3$ is the biologically active form of vitamin D, and, thus, would be thought to be the ideal measure for vitamin D status, it is not. There are several reasons for this. The circulating half life of circulating 1,25(OH)D is only 4-6 hours. Circulating levels of 1,25(OH)D are a thousand fold less than 25(OH)D. As a patient becomes vitamin D deficient, there is a decrease in intestinal calcium absorption which lowers ionized calcium transiently. This signal is recognized by the calcium sensor in the parathyroid glands to increase the production and secretion of parathyroid hormone (PTH).[18] PTH regulates calcium metabolism by increasing tubular reabsorption of calcium in the kidney, increasing mobilization of calcium from the skeleton and by increasing the renal production of 1,25(OH)D.[1, 4, 16] Thus, as a patient becomes vitamin D insufficient and deficient, the increase in PTH levels result in normal or elevated levels of 1,25(OH)D. This makes the $1,25(OH)_2D$ assay useless as a measure of vitamin D status.

1,25(OH)D assay, however, has been effectively used to help in the diagnosis of several inherited and acquired disorders in calcium metabolism as they relate to alteration in the renal or extra renal production of 1,25(OH)D.[4, 16, 19]

Definition of Vitamin D Insufficiency and Deficiency

There is no absolute consensus as to what a normal range for 25(OH)D should be. Part of the difficulty is how a normal range is determined, i.e., typically it is done by obtaining blood from several hundred volunteers and deeming them to be normal and to perform the measurement of the analyte and do a distribution with a mean ± 2SD as the normal range. However, since it is now recognized that 30-50% of both the European and US population are vitamin D insufficient or deficient, the previously reported normal ranges of 10-55 ng/ml are totally inadequate.[1, 4, 16, 20-23] Chapuy et al[24] reported that a dot plot of serum 25(OH)D levels as a function of PTH levels provided an insight as to what the serum 25(OH)D levels should be to be considered sufficient. They observed that the PTH levels began to plateau at their nadir when 25(OH)D levels were between 30-40 ng/ml. A similar observation was made by Thomas et al and Holick et al.[14] (Fig 2)

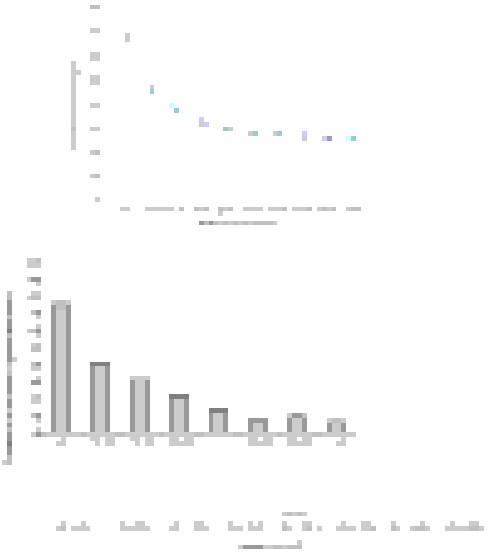

Fig. 2

A, Mean (±SE) serum PTH (picograms per milliliter) by serum 25(OH)D subgroups. Subject PTH concentrations (picograms per milliliter) relative to serum 25(OH)D concentrations sorted by subgroups delineated by predefined cutoffs for analyses of **...**

Malabanan et al[26] did provocative testing by giving healthy adults who had a 25(OH)D of between 11 and 25 ng/ml, 50,000 IU of vitamin D once a week for 8 weeks. At the end of 8 weeks, it was observed that 25(OH)D levels increased on average by more than 100%. An analysis of the change in PTH levels for each of the subjects revealed that on average, the mean decrease on PTH levels declined by 55% in subjects who had 25(OH)D between 11-15 ng/ml and declined by 35% for those with 25(OH)D levels of between 16-19 ng/ml. Those subjects who had 25(OH)D > 20 ng/ml had no significant change in their PTH level. (Fig 3) Thus, based on the provocative testing, it was suggested that vitamin D deficiency should be defined as 25(OH)D above 20 ng/ml. Heaney et al [27] measured the efficiency of intestinal calcium absorption in women who had on average a 25(OH)D of 20 ng/ml and then in the same women who received $25(OH)D_3$ to raise their blood level on average to 32 ng/ml. They reported a 45-65% increase in the efficiency of intestinal calcium transport when women were able to achieve a 25(OH)D of > 32 ng/ml.

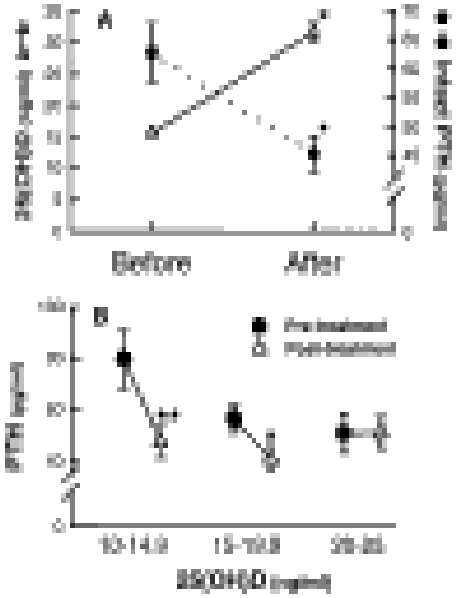

Figure 3

(A) Serum levels of 25(OH)D (-Δ-) and PTH (-o-) before and after therapy with 50,000 IU of vitamin D_2 and calcium supplementation once a week for 8 weeks. **(B)** Serum levels of PTH levels in patients who had serum 25(OH)D levels of between 10 and **...**

With all of this information collectively, most experts now agree that vitamin D deficiency should be defined as a 25(OH)D of < 20 ng/ml. Vitamin D insufficiency is now recognized as a 25(OH)D of 21-29 ng/ml. The preferred level for 25(OH)D is now recommended by many experts to be > 30 ng/ml.[1, 4, 28]

The upper limit of normal has also been questioned.[4, 29, 30] The upper limit being 55 ng/ml seemed to be inadequate especially since lifeguards who are exposed to a lot of sunlight typically have reported levels of 100-125 ng/ml.[4, 16, 29] There has never been a reported case of vitamin D intoxication from sun exposure, and lifeguards have not been reported to be vitamin D intoxicated. Based on the literature, it appears that vitamin D intoxication does not occur until blood levels are above 150-200 ng/ml.[31, 32] Vitamin D intoxication is defined as a 25(OH)D > 150 ng/ml that is associated with hypercalcemia, hypercalciuria and often hyperphosphatemia.

Based on all of this information, many of the reference laboratories are now using a normative range for 25(OH)D to be 20-100ng/ml. However, several reference laboratories are also recognizing the recommendation by some experts that a preferred level of > 30 ng/ml is most desirable.

At least two of the reference laboratories are now using LC-MS routinely to measure 25(OH)D. Since they are able to quantitatively measure $25(OH)D_2$ and $25(OH)D_3$, they report them out as individual levels. They also report the total 25(OH)D which is summation of $25(OH)D_2$ and $25(OH)D_3$. Physicians only need to be aware of the total 25(OH)D level.

Inclusion and Exclusion Criteria

Inclusion criteria

Aged 18-60 y

25-Hydroxyvitamin D level <50 nmol/L

Chronic recurrent musculoskeletal pain (>3 episodes of >1 month pain in 2 years) or long-standing pain (>3 months)

Nonspecifi c pain (no obvious cause such as arthritis, lumbar disc herniation, trauma; patients with fi bromyalgia, depression, and low back pain could be included)

Exclusion criteria
Pregnancy
Signs of rickets
Use of vitamin D in the last 4 months
Erythrocyte sedimentation rate >30 mm/h
Use of statins, cyclosporins, or oral steroids
Hypercalcemia (calcium level >2.55 mmol/L), sarcoidosis, tuberculosis
Creatinine level >150 mmol/L

II. Methods

Participants & recruitment

Eighty nine patients were recruited for participation among patients coming to orthopaedic OPD with nonspecific musculoskeletal pain aged between 18 and 50 years who visited for frequent, recurrent musculoskeletal pain or pain lasting more than 3 months without an obvious cause (eg, trauma, arthritis, or sciatica). Participants were given a questionnaire and had an assessment of physical performance, height and weight and gave a blood sample. They were tested for vitamin D deficiency, which we defined arbitrarily as a level of 25-hydroxyvitamin D (25-OHD) of less than 50 nmol/L. Patients who were deficient and met inclusion and exclusion criteria were invited to participate in the study by interpreters who were trained at introducing the study and explaining the questionnaires. Ethical approval was obtained and all participants gave written informed consent. Then patients were randomised into the study and the control groups. The study group would receive Vitamin D supplementation. The control group on the other hand would receive a placebo instead of the supplementation, which would be similar in appearance as that of the actual counterpart.

Baseline assessment

The baseline questionnaire included questions about lifestyle, including smoking and frequency of alcohol consumption and outdoor exercise [21]. Participants were asked how many days a week they consumed alcohol (response set = never, <1, 2–3, 3–4, 5–6 and > 7 days per week). Smoking status was assessed by were asking whether participants had ever smoked at least 100 cigarettes or been a regular pipe or cigar smoker. Those answering yes to any of the questions were considered as ever smokers. Outdoor exercise was assessed by asking how long participants typically spent outdoors walking or cycling each day (response set = <30 minutes, 30–60 minutes and >60 minutes).

Participants were asked to self-report in the questionnaire a range of 16 comorbid conditions which included whether they had ever been diagnosed with cancer, told by a doctor they had a stroke and if they were currently receiving treatment for epilepsy, hypertension, chronic bronchitis, asthma, peptic ulcers, diabetes and heart, liver, kidney, prostate, adrenal, pituitary, thyroid or testicular disease. They were also asked if they had ever fractured a bone after the age of 25. The interpreters conducted interviews about their pain levels in 4 areas of the body on questionnaires using a visual analogue scale (VAS; range, 0-100) and localized pain by marking a mannequin. Participants completed a 50 foot walking test and also a sit to stand test with the time taken to complete each task recorded in seconds [25, 26].

Assessment of pain

At baseline and follow up participants rated their pain levels in 4 areas of the body on questionnaires using a visual analogue scale (VAS; range, 0-100). They were asked to shade where they experienced pain on a 4 view body mannequin, Fig. 1. To assess chronicity they were asked 'Thinking about this ache or pain, have you been aware of it for more than 3 months?' Pain was then coded to number of pain sites using a 29-region coding frame [27]. Three groups were defined: Joint pain was pain in joints only, including pain in the upper arm at the side of a painful shoulder. Proximal pain was pain in the proximal legs and arms, both left and right (lower axial pain could replace pain in proximal legs, and upper axial pain was considered equivalent to pain in the proximal arms). Widespread pain was axial pain plus pain on both sides of the body affecting at least 1 arm or leg.[33].

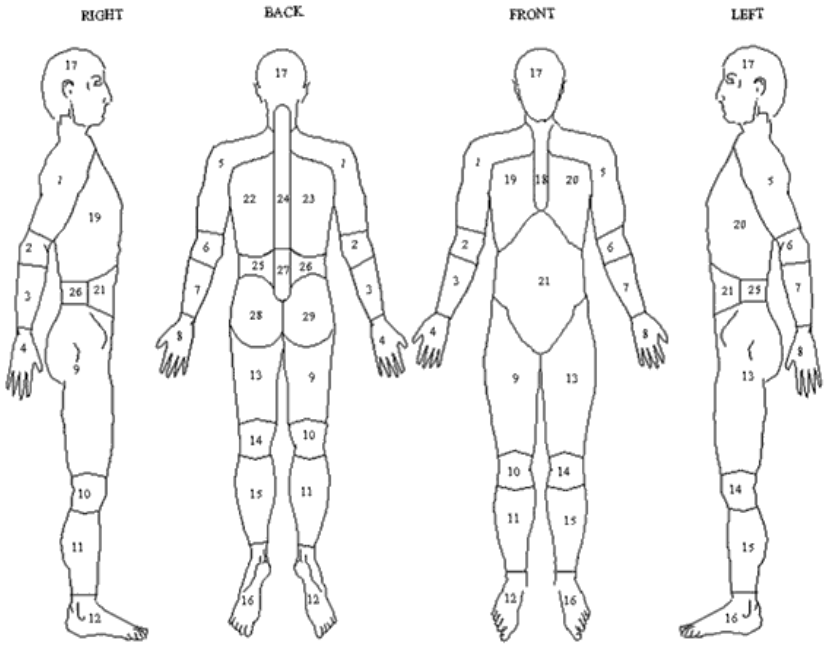

Fig. 1

Four view body mannequin, with imposed 29 region coding frame, on which participants were asked to shade the site of their pain

]. For subjective assessment of the disability , patients were asked to fill up the SF36 questionnaire. Average pain free period, tireless walking distance were other markers for improvement.

Biochemical assays

At baseline, serum levels of 25-(OH) D, as the recommended marker of vitamin D status [31], were determined by radioimmunoassay. Intra-and inter-assay coefficients of variation (CV) for 25-(OH) D were 11 % and 8 %, respectively. The detection limit of the radioimmunoassay kit for 25-(OH) D was 1.5 ng/ml. We also assessed the serum levels of 1,25-dihydroxyvitamin D (1,25-(OH) $_2$D) as this is the biologically active form of vitamin D. Serum 1, 25-(OH) $_2$D levels were measured using high performance liquid chromatography-tandem mass spectrometry as described by Casetta et al. [32] and modified by Vanderschueren et al. [33] with results expressed as pg/mL. The limit of quantification was <6.25 pg/mL. The inter-day imprecision of pooled serum at high and low serum 1,25-(OH) $_2$D concentrations was assessed. For serum with a low concentration (mean 7.16 pg/mL) the CV was 10.1 % and 5.9 % for serum with a high concentration (mean 55.8 pg/mL).

III. Statistical analysis

Descriptive statistics were used to describe the characteristics of the study sample. Depending on their distribution parametric (t-tests) and nonparametric (wilcoxon rank sum) tests to determine the significance of any differences in any of the continuous variables between those who developed new CWP at follow up and those who were pain free at both time points. χ^2 tests were used to test differences in categorical data. 25-(OH) D and 1,25-(OH) $_2$D levels were categorised into quintiles. We used logistic regression to determine the association between the new occurrence of CWP and 25-(OH) D and 1,25-(OH) $_2$D with those in the highest quintile as the reference category. Adjustments were made for factors which significantly differed between those with new CWP at follow up and those who remained pain free throughout. This included age and centre and subsequently physical performance (time to sit from standing and time to walk 50 feet), number of comorbidities, BMI and the presence of depression (determined by BDI ≥10). We also assessed the relationship between vitamin D deficiency and CWP with 25-(OH) <20 ng/mL considered to represent deficiency [13]. We looked also at the number of painful sites (out of 29) at baseline and follow up. Because of the large number of participants with no painful sites (N = 89) zero-inflated negative binomial regression was performed to assess the relationship between vitamin D and the numbers of pain sites reported at follow up. Adjustments were made for age and centre and subsequently for physical performance (time to sit from standing and time to walk 50 feet), number of comorbidities, BMI and the presence of depression (determined by BDI ≥10). To limit the analysis to change in number of pain sites over the follow up period we also adjusted for the number of pain sites reported at baseline. Results of the logistic regression were presented as Odds Ratios (OR) and for zero-

inflated negative binomial regression as Incidence Rate Ratio (IRR); both with 95 % confidence intervals. Statistical analysis was performed using Stata SE 11.2 (Stata Corp, College Station, Texas, USA).

IV. Results & Observations

Out of 89 patients , 4o were males and 49 were females. The mean age was 39(±15) years . the Duration of symptoms ranged from 3 months to 30months 9.4 (±5.5) months . The mean VAS score was 5.4((±2.3) at their index visit. Sixty eight patients were vitamin D deficient as per their serum 25 hydroxy vitamin D estimation , 15 were found to have insufficient levels while the rest 6 patients had adequate serum levels. After random allocation of the patients into the study and control groups , the attributes and cahractaristics did not differ between the two groups.

Table : pre-intervention characteristics of the study cohort

Attributes	Overall	Study group (Vit D group)	Control group (Placebo group)
Number of cases	89	43	45
Age (yrs)	39 (±15)	41 (±11)	39 (±9)
Male : Female:	40:49	20:23	20:25
Duration of pain (months)	9.4	9.6	9.2
VAS before study	5.4	5.3	5.4

The pain at 6 months, 12 months and at the end of the study , at 18 months were assessed in the two groups . Pain scores were lower in the vitamin D supplementation group at all the three time frames of assessement than their control group (Table) . The difference was statisitically significant. Also the mean pain free period in the vitamin D group was much longer (4.3 months) than in the control group (1.6 months) . The efficiency of the ptients in terms of performance increased in vitamin D groups as 88% patients in the group could perform tireless 1 km walk , against only 56 % in the control group. Subjective assessment revealed that 93 % of patients experienced significant improvement in the sense of well being against only 38% in the control group despite undergoing similar analgesic medication protocol and physical therapy. There were no symptoms of vitamin D toxicity in the treated patients nor were there any adverse effects of the treatment.

Table : comparison of outcomes of two groups

Attributes	Vit D group	Plaebo group	Significance (P value)
Pain improvement at 6 months	3.4	2.1	<0.001
Pain improvement at 12 months	3.6	2	<0.001
Pain improvement at 12 months	3.6	2.1	<0.001
Pain free period (months)	4.3	1.6	<0.001
Improvement in Quality of life (SF36)	37 points	14 points	<0.001
Distance of pain free walking	1.3 km	0.6 km	<0.001

V. Discussion

As rightly said, 1,25 dihydroxy colecalciferol has been regarded as a new age hormone than a mere vitamin. Contrary to popular belief , its role in the human metabolism is much more complex and diverse than calcium homeostasis. VitaminD has its contribution in immunity, cell signal pathways, fertility and pain perception in complex ways yet poorly understood. Long standing vitamin d deficiency has been projected to be a significant contributor of chronic non specific pain. Chronic musculo-skeletal pain is a distressing condition , which has the potential to derange the quality of life of the sufferer. The situation is very stressful as the no clinical diagnosis is often reached despite repeated clinical assessment and laboratory tests. In this randomised control study , the cohort of patients undergoing vitamin D supplementation along with the other conventional therapeutic modalities of pain showed an overall better outcome than the control group. The decrease in the duration of pain ($p<0.001$) , the severity of pain ($p<0.001$) and the efficiency in the ambulation ($p<0.001$) and improvements in the endurance ($p<0.001$) reflect vital effects of vitamin D on these clinical parameters. Also the subjective improvement , assessed in the study with the help of SF36, revealed significant improvement in the quality of life of the patient who recovered from a vitamin D deficiency state. Out of the 3 pateints who had sufficient level of vitamin D levels in their serum were also found to benefit from supplementation. It is notworty , however that levels of all three patients were on the low normal side, besides the patients being clinically suffering from musculoskeletal pain. On the other hand , the other 3 patients who were in the control group could only marginall improve with the conventional therapy. There was no adverse effect seen in the study and no signs of toxicity symptoms. This reasserts the high safety profile of vitamin D supplementation .

VI. Conclusion

Patients with undiagnosed non-specific musculoskeletal pain should undergo serum vitamin D estimation as a routine protocol. If found having insufficient levels in blood, they should be treated with Vitamin D supplementation. This study provides an evidence that vitamin D deficiency can be a cause of non-specific musculoskeletal pan and vitamin D supplementation is helpful in relieving symptoms in such patients.

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