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Efficacy of Daily Supplementation of Vitamin D3 Nano Oral Spray vis-à-vis Other Vitamin D3 Formulations in Vitamin D-deficient Indian Adults: RCT

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Abstract: ***Background: This is a randomised controlled trials (RCTs), assessing the effectiveness of a nano vitamin D3 oral mucosal spray against nano and fat-soluble vitamin D3 tablets. Method: Of 324 enrolled participants, 298 completed the 3-month intervention. Subjects were assigned to four groups: Group A received 1000 IU DEKSEL® Nano Oral Spray, Group B received DENCIUM ND3® tablets (1000 IU nano vitamin D3 plus calcium), Group C received TAYO tablets (1000 IU conventional vitamin D3 plus calcium), and Group D received 2000 IU DEKSEL® Nano Oral Spray. High-performance liquid chromatography analysis showed that the actual vitamin D3 content in all formulations exceeded the labeled dose. Serum 25-hydroxyvitamin D [25(OH)D], parathyroid hormone, 1,25-dihydroxyvitamin D, calcium, phosphate, alkaline phosphatase, and urinary calcium/creatinine ratio were assessed before and after supplementation. Results: All groups demonstrated significant increases in serum 25(OH)D concentrations after 3 months (p<0.001), with the greatest increase observed in the higher-dose oral spray group. Although overall intergroup differences were not significant, the rise in serum 25(OH)D per 100 IU of vitamin D3 was significantly greater with the nano oral spray than with the conventional tablet formulation. Conclusion: The study concluded that nano vitamin D3 oral spray is more effective than tablet formulations in improving vitamin D status and may be particularly useful for individuals with malabsorption or difficulty swallowing tablets.***

Keywords: Randomized study; Nano vitamin D3 tablet; Vitamin D3 nano oral spray; conventional vitamin D3; serum 25(OH)D; PTH and $1,25(OH)_2D$

1. Introduction

Vitamin D deficiency is a pandemic affecting all age groups and strata of the Indian population, despite India being a tropical country. This is because vitamin D synthesis induced by sunlight is affected by several host factors, such as clothing and lifestyle, and by environmental factors, such as pollution, latitude, and season (1). High prevalence of rickets in infants and children, osteomalacia in adults, and osteoporosis and fractures in the elderly, as a result of chronic calcium and vitamin D deficiency, have been reported in several studies from India (2-5).

Vitamin D doses and preparations have evolved over time. The majority of commercial preparations available are in the form of tablets, capsules, powders (sachets), liquids (syrups), strips, and chewing gum. Since these are all conventional fat-soluble preparations, they have low solubility in the aqueous fluids of the gastrointestinal tract. Therefore, nano-emulsion formulations of vitamin D3 using nanotechnology have been introduced to the market in recent years. Nano-emulsions help to encapsulate, protect, and deliver fat-soluble bioactive substances efficiently into the system due to stability against phase separation, better bioavailability and absorptive capacity of hydrophobic compounds *in vitro* and *in-vivo* studies (6-9). The liquid preparations of nano vitamin D3 that have been available for the last few years have been shown to achieve higher serum levels of serum 25 Hydroxy D (25(OH)D) as a result of better absorption and bioavailability when compared with powder or capsule preparation of conventional vitamin D3 (10). Recently, there has been considerable interest in the potential use of oral mucosal sprays for vitamin D supplementation. This interest in oral mucosal sprays for vitamin D delivery arises from the potential for achieving higher and faster serum 25(OH)D levels with nano formulation (11,12).

The objectives of the present study were to evaluate, through a randomized controlled trial (RCT): a) the efficacy of supplementing 1000 IU daily of DEKSEL® Nano Oral Spray compared to a similar dose of nano vitamin D3 in tablet form (DENCIUM-ND3®); b) effectiveness of tablet Tablet

DENCIUM-ND3® (1000 IU of Nano vitamin D3 + 500 mg of elemental calcium) vis-a-vis Tablet TAYO (containing 1000 IU of conventional fat-soluble vitamin D3 + elemental calcium 500 mg) as mentioned on the labels of these commercial preparations on serum 25(OH)D, PTH, and $1,25(OH)_2D$ in apparently healthy adult subjects with serum levels of 25(OH)D < 75 nmol/L (30 ng/m); and c) to evaluate the serum 25(OH)D levels achieved with a daily dose of 1000 IU and 2000 IU of DEKSEL® Nano Oral Spray as per the labels.

2. Materials and Methods

The study was a collaborative effort between the Society for Endocrine Health of Elderly, Adolescents and Children (SEHEAC) and Tanvir Hospital in Delhi and Hyderabad, respectively. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/ patients were approved by both the Institutional Ethics Committees of Sur Homeopathic College (No. F. 07(1o9)/SHMC/2018 /2770 dated 4/11/22) and Tanvir Hospital (Ref. No. EC/New/INST/2022/TE/0154). Written informed consent was obtained from all subjects/ patients.

The inclusion criteria encompassed apparently healthy adults with a serum 25(OH)D concentration of <75 nmol/L (<30 ng/ml) using American Endocrine Society criteria which defines vitamin D deficiency, insufficiency and sufficiency as serum levels of <50 nmol/L (<20 ng/ml), <50-75 nmol/L (20-30 ng/ml) and >75 nmol/L (>30 ng/ml) respectively (13).

Exclusion criteria comprised any history of metabolic or bone disease (except osteoporosis) that might interfere with result interpretation, including Paget's disease, rheumatoid arthritis, osteomalacia, osteogenesis imperfecta, osteopetrosis, ankylosing spondylitis, Cushing's syndrome, hyperprolactinemia, and malabsorption syndrome. Subjects undergoing treatment with anti-osteoporotic, anti-tubercular, anti-epileptic drugs, glucocorticoids, calcium and vitamin D supplements that could affect bone mineral metabolism within the past 6 months were also excluded. Additionally, individuals with renal or hepatic insufficiency, autoimmune diseases, fractures in the last 6 months, and a history of hypersensitivity to any investigational agents affecting bone mineral metabolic parameters were excluded. Written informed consent was obtained from all subjects.

2.1 Outcome Measures

2.1.1 Primary endpoint:

Change in serum 25(OH)D levels among different groups at 12 weeks following supplementation.

2.1.2 Secondary endpoints:

- Changes in serum PTH and $1,25(OH)_2D$ levels following 12 weeks of supplementation.
- Changes in serum calcium, phosphate, ALP levels and UCa/CrR between different groups following 12 weeks of supplementation

2.2 Study Design

A multicentric, randomized, open-label, parallel-group study compared DEKSEL® Nano Oral Spray with DENCIUM-ND3® (nano vitamin D3) and TAYO (conventional vitamin D3) tablets, both supposedly containing similar doses of vitamin D3 and calcium, in Indian adults with vitamin D deficiency or insufficiency. While the investigators and subjects were aware of the intervention assignment, the personnel involved in the laboratory analysis were blinded to the intervention protocol. The study was conducted and reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (www.consort-statement.org).

2.2.1 Detailed Study Plan

Candidates, who gave signed informed consent, were screened and included as study subjects based on inclusion and exclusion criteria. A complete clinical examination including anthropometry {height, weight and body mass index (BMI)} was performed at the baseline. Heights and weights were measured by stadiometer to the nearest 3 mm. and digital weighing scale to the nearest 100 grams, respectively. The BMI was calculated by the formula of weight in kg/square of height in meters.

The recruited subjects were randomized into four groups. As per the labels on the vitamin D3 preparations procured from the market, Group A received 1000 IU of nano vitamin D3 oral solution daily through oral spray (a single spray provides 1000 IU of vitamin D3). Group B received Tablet DENCIUM-ND3® (1000 IU of nano vitamin D3 with 500 mg of elemental calcium). Group C received Tablet TAYO daily (1000 IU of conventional vitamin D3 with 500 mg of elemental calcium). Group D received 2000 IU of nano vitamin D3 oral solution through oral spray (a single spray provided 2000 IU of vitamin D3). Among the 298 subjects in the intent-to-treat (ITT) analysis who were allocated different vitamin D preparations for daily supplementation, 160 were compliant, 72 were non-compliant, and 66 dropped out during the treatment period. Each subject in Group B and C was provided strips containing 30 tablets of DENCIUM-ND3® or TAYO for a month and were asked to return the empty strips and collect medicines every month for the next two months. Since each oral spray bottle of 1000 or 2000 IU vitamin D3 contained adequate doses for 3 months, one mucosal spray bottle was given to subjects in Group A and D, and were asked to report back immediately if there was any problem with the spray during this period. However, they were requested to report back every month about compliance. Subjects who missed their daily dose for more than 7 days or more than 3 consecutive days during the last week of supplementation were considered non-compliant. The oral spray bottles, tablets DENCIUM-ND3®, and tablet TAYO were procured from the market. The medicines procured were from the most recent batch with the same manufacturing date.

Analysis of vitamin D3 content of these preparations utilizing HPLC with the ARD/MOA/0-67-00 method, by an accredited laboratory, however, displayed variations from their labeled potency in all the preparations used for the study. Similar disparity in cholecalciferol content was also observed in commercial preparations available in the Indian market (14).

DENCIUM-ND3® tablets had 19.3% (1193 IU Vitamin D3) more vitamin D3 while TAYO tablets had 58.5% (1585 IU) excess than advertised. DEKSEL® oral spray preparations also exhibited higher-than-expected vitamin D content, with 13.9% (1131 IU) vitamin D3 and 12% (2240 IU) vitamin D3 in the 1000 and 2000 IU bottles, respectively. These differences in the content were probably due to different overages added to these preparations. Notably, the vitamin D content in TAYO tablets surpassed the vitamin D content in DENCIUM-ND3® tablets by approximately 395 IU and the DEKSEL® Nano Oral Spray preparations by 454 in 1000 IU and by 465 IU in 2000 IU preparation.

Serum calcium, inorganic phosphate, ALP, 25(OH)D, PTH, 1,25$(OH)_2$D, and random UCa/CrR were measured at baseline and following supplementation. Serum Total calcium reference range (RR) 8.6-10 mg/dL (2.14 - 2.49 mmol/L), inorganic phosphate (RR 0.87-1.45 mmol/L (2.7-4.5 mg/dL), and alkaline phosphatase (RR 42-128 U/L) were measured by an autoanalyzer (Cobas 8000, Roche Diagnostics, Germany) using Cobas 701/702 kits. Serum 25(OH)D (RR 27.5–107.25 nmol/L) and PTH (15-65 pg/mL or 1.59-6.89 pmol/L) were measured by electrochemiluminescence assay (ECLIA, Cobas 8000, Roche Diagnostics, Germany) with a coefficient of variation (CV) of 6-8%. 1,25$(OH)_2$D (RR 19.9-79 pg/mL) was measured by chemiluminescence assay (CLIA, DiaSorin LIAISON) with an intra-assay CV varying from 2-5% and an inter-assay CV varying from 3.6-6.6%. Serum levels of more than 65 pg/ml (6.89 pmol/L) was defined as secondary hyperparathyroidism. For UCa/CrR, measurements of both calcium and creatinine were taken in mg/dL using the Cobas-C 111 (Roche Diagnostics).

Five milliliters (or 1 teaspoon) of blood were collected on ice in the fasting state between 8 and 9 AM. The blood was then immediately centrifuged and transported to the laboratory. The serum was divided into three aliquots, which were used to investigate serum biochemistry and hormone levels for all subjects. The serum samples were sent to the laboratory on dry ice and evaluated on the same day.

2.2.2 Study Size

Since no intervention studies have been published comparing the effectiveness of a daily vitamin D3 oral spray with nano vitamin D3 and conventional fat soluble vitamin D3 in tablet forms, we opted to conduct a pilot study. Our goal was to enroll as many adult subjects as possible within a specified 3-month period, focusing on individuals with serum 25(OHD) levels below 75 nmol/L. Out of 349 subjects initially assessed for the study, 324 were ultimately randomized and allocated intervention after applying exclusion criteria. However, 26 subjects did not collect their medication, resulting in a final analysis group of 298 subjects based on modified intention-to-treat (MITT) (Figure 1).

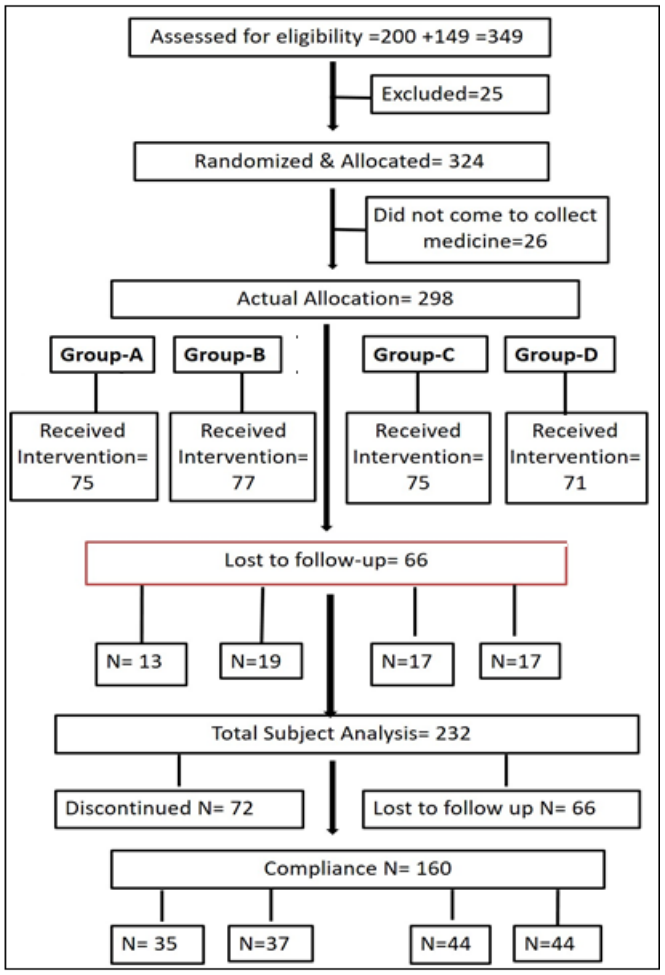

Figure 1: Flowchart of the study progress

2.3 Statistical Analysis

Data entry and management were performed using Microsoft Excel. Statistical analysis was conducted with Stata 15.0 (Stata Corp LP, Texas, USA). Data are presented as number (percentage), mean ± standard deviation (SD), or median (interquartile range (P25-P75)), as appropriate. Baseline categorical variables were compared between groups using the chi-square test. Continuous variables with normal distribution were compared using an unpaired t-test, while the Wilcoxon rank-sum test was used for non-normally distributed variables. Both ITT and PP analysis were performed for primary outcomes. For secondary outcomes, only PP analysis was conducted. Primary outcomes, including serum 25(OH)D and 1,25$(OH)_2$D, were assessed among the groups using a one-way analysis of variance (ANOVA), followed by Bonferroni post-hoc analysis. This involved pairwise comparisons of groups A and B, B and C, A and C, and A and D. The results were presented as the difference in means, accompanied by a 95% confidence interval. Since PTH did not exhibit a normal distribution, the Kruskal-Wallis test was employed to compare it among the groups, and the results were expressed as median (P25–P75). For secondary outcomes, a one-way ANOVA or the Kruskal-Wallis test was utilized to compare them among the groups. To account for baseline imbalances in characteristics such as age and gender, which were statistically significant, a linear mixed model was used to estimate the adjusted differences in means between the groups. A two-sided p-value of less than 0.05 was considered statistically significant.

3. Results

The baseline anthropometric, biochemical, and hormonal parameters of the subjects are shown in Table 1. The mean age and BMI were 39.56±14.78 years and 25.65±4.19 kg/m², respectively. We did not evaluate the results in men and women separately because there was no clinically significant difference in baseline serum 25(OH)D and biochemical parameters between genders. Additionally, no appreciable differences in the baseline parameters were observed among the comparable groups (A, B and C) receiving supplementation. Among the 298 subjects finally recruited for the study, 43 (14.3%) were obese, 120 (40.2%) were overweight, and 135 (45.3%) were normal weight. However, their distribution did not differ significantly between the groups.

Table 1: Baseline subject characteristics, including anthropometrics and biochemical parameters

Baseline Characteristics	Vitamin D supplementation groups				P-value
	Group A* (Mean ±SD)	Group B† (Mean ±SD)	Group C‡ (Mean ±SD)	Group D§ (Mean ±SD)	
Age (Year)	37.6±14.5	36.1±15.2	40.6±16.1	44.2±11.8	0.004
Gender (%)					
Male	41.3	32.5	52.7	54.2	0.023
Female	58.7	67.5	47.3	45.8	
BMI (Kg/m^2)	25.3±4.2	25.4±4.6	26.0±3.7	25.9±4.2	0.679
Serum 25(OH)D (nmol/L)	30.70±17.97	33.95±15.97	31.95±15.97	30.95±14.73	0.5697
Serum PTH (pmol/L)					
Median	7.8	7.3	7.7	8.7	0.5027
P25 – P75	4.74 – 12.14	5.16 – 10.6	5.40 – 12.95	5.47 – 12.0	
Total Ca (mmol/L)	2.35±0.17	2.4±0.07	2.37±0.10	2.37±0.10	0.1006
Serum Phosphates (mmol/L)	1.10±016	1.16±0.16	1.10±0.16	1.07±0.23	0.1063
Serum ALP (U/I)	93.1±30.4	90.1±31.1	93.8±23.3	95.0±22.9	0.734
UCa/CrR					
Median	0.05	0.05	0.05	0.06	0.5293
P25 – P75	0.03 – 0.08	0.03 – 0.09	0.03 – 0.07	0.03 – 0.09	
Serum 125(OH)D (pg/ml)	114.0±48.1	103.3±28.0	100.0±28.9	110.4±33.4	0.0969

* Group A: Nano Vitamin D3 Oral Spray, 1000 IU
† Group B: Tablet Nano Vitamin D31000 IU + Calcium 500 mg
‡ Group C: Tablet Conventional Vitamin D31000 IU + Calcium 500 mg
§ Group D: Nano Vitamin D3 Oral Spray, 2000 IU
BMI, Body Mass Index; 25(OH)D, 25-hydroxyvitamin D; PTH, Parathyroid Hormone; Ca, Calcium; ALP, Alkaline Phosphate;1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; UCa/CrR, Urinary Calcium/Creatinine Ratio

Of all the study participants, 251 (84.2%) were deficient (<50 nmol/L), and 47 (15.8%) were insufficient (50-75 nmol/L) according to the American Endocrine Society classification. Alternately, 159 (53.4%) were classified as vitamin D deficient (<30 nmol/L), 92 (30.9%) were deemed insufficient (30-50 nmol/L), and 47 (15.8%) were categorized as vitamin D sufficient (>50 nmol/L) as per Institute of Medicine (IOM) classification (15).

3.1 Serum 25(OH)D

No significant differences were observed in baseline serum 25(OH)D values between the groups. The mean baseline serum 25(OH)D in 298 subjects undergoing MITT analysis increased significantly from 31.90±16.17 nmol/L to 48.55±24.01 nmol/L (p <0.001) following three months of daily supplementation. This post-supplement rise in serum 25(OH)D was also significant within each of the four groups (p <0.001 in all groups). A similar trend was noted in 160 subjects of PP analysis group, but the overall increase from 29.45±15.43 nmol/L to 59.73± 24.61 nmol/L (p <0.001) was significantly greater than that observed in MITT subjects. The post-supplementation rise within all individual groups was also significant and noticeably higher in the PPA group compared to that observed in individual groups in the MITT population. These results remained consistent even after adjusting for age and gender, with centres as a random effect in both MITT and PPA (Table 2 & 3).

Table 2: Effect of Vitamin D Supplementation on Serum Vitamin D, PTH and 1,25(OH)$_2$D: modified intention-to-treat analysis.

Outcome measures	Vitamin D supplementation groups				P value
	Group A* (N=75)	Group B† (N=77)	Group C‡ (N=74)	Group D§ (n=72)	
Serum 25(OH)D (nmol/L)					
Baseline					
Mean	30.70	33.94	31.94	30.95	0.5697
SD	17.97	15.97	15.97	14.72	
Post-supplementation					
Mean	44.67	44.68	45.92	59.15	0.0002
SD	20.88	21.71	18.47	30.45	
P-value (paired)	<0.001	<0.001	<0.001	<0.001	
Mean increase	13.97±21.14	10.73±18.86	13.97±18.39	28.20±35.69	0.0013

Serum PTH (pmol/L)					
Baseline					
Median	7.76	7.33	7.74	8.73	0.5027
P25 – P75	4.74 – 12.14	5.16 – 10.61	5.40 – 12.96	5.47 – 12.01	
Post-supplementation					
Median	5.81	5.26	4.83	5.75	0.2132
P25 – P75	4.04 – 8.10	3.55 – 7.38	3.86 – 6.62	4.45 – 8.42	
P-value (paired)	<0.001	<0.001	<0.001	<0.001	
Median decrease	0.78	0.69	1.91	0.89	0.553
Serum 1,25(OH)$_2$D (pmol/L)					
Baseline					
Mean	273.6	247	240	264.96	0.0969
SD	115.44	67.2	69.36	80.16	
Post-supplementation					
Mean	305.28	285.12	292.56	309.36	0.6785
SD	107.76	179.04	98.64	103.2	
P-value (paired)	0.028	>0.05	<0.001	<0.001	
Mean increase	31.68±129.6	36±201.5	52.56±99.2	44.4±97.0	0.266

* Group A: Nano Viatmin D3 Oral Spray 1131 IU
† Group B: Tablet Nano Vitamin D31193 IU + Calcium 500 mg
‡ Group C: Tablet Conventional Vitamin D31585 IU + Calcium 500 mg
§ Group D: Nano Vitamin D3 Oral Spray, 2240 IU
25(OH)D, 25-hydroxyvitamin D; PTH, Parathyroid Hormone; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D

Table 3: Effect of Vitamin D Supplementation on Serum Vitamin D, PTH and 1,25(OH)$_2$D: Per-protocol Analysis

Outcome measures	Vitamin D supplementation groups				P value
	Group A* (N=35)	Group B† (N=37)	Group C‡ (N=44)	Group D§ (n=44)	
Serum 25(OH)D (nmol/L)					
Baseline					
Mean	26.71	32.20	31.70	27.21	0.2517
SD	15.72	14.72	16.72	13.98	
Post-supplementation					
Mean	55.91	55.66	55.16	70.64	0.0065
SD	21.96	21.72	16.22	32.20	
P-value (paired)	*<0.001*	<0.001	<0.001	<0.001	
Mean increase	29.15±21.74	23.46±19.51	23.46±17.42	43.43±31.63	<0.001
Rise in serum 25 (OH) D per 100 IU Vitamin D3 supplementation	2.58±1.93	1.98±1.46	1.49±1.23	1.94±1.41	A Vs B-0.487 A Vs C-0.016 B Vs C-0.891
Serum PTH (pmol/L)					
Baseline					
Median	7.26	6.17	7.34	7.50	0.7189
P25 – P75	4.19 – 10.82	3.91 – 10.60	5.00 – 11.30	4.82 – 11.88	
Post-supplementation					
Median	5.07	4.34	4.73	5.50	0.0373
P25 – P75	3.87 – 6.89	3.16 – 5.77	3.30 – 5.89	4.49 – 6.60	
P- value (paired)	*0.003*	<0.001	<0.001	<0.001	
Median decrease	1.06	1.52	2.73	1.74	0.711
Serum 125(OH)D (pmol/ml)					
Baseline					
Mean	270.48	260.16	244.9	273.5	0.3895
SD	120.96	58.8	70.8	84.1	
Post-supplementation					
Mean	302.16	289.68	292.9	315.0	0.7183
SD	85.2	119.04	105.7	96.5	
P-value (paired)	*>0.05*	>0.05	0.003	0.011	
Mean increase	30.3±123	30.3±123.36	47.76±99.21	41.04±108.28	0.9035

* Group A: Nano Vitamin D3 Oral Spray 1131IU
† Group B: Tablet Nano Vitamin D3 1193 IU + Calcium 500 mg
‡ Group C: Tablet Conventional Vitamin D3 1585 IU + Calcium 500 mg
§ Group D: Nano Vitamin D3 Oral Spray, 2240 IU
25(OH)D, 25-hydroxyvitamin D; PTH, Parathyroid Hormone; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D

However, in contrast, no significant difference was noted in serum 25(OH)D levels between the groups A and B, B and C, and A and C in PPA group as well as MITT subjects following daily supplementation with different vitamin D3 preparations

(Table 2). This could probably be due to different concentrations of vitamin D3 in the preparations supplemented to groups A, B and C. In order to make the groups truly comparable, we calculated the rise in serum 25(OH)D per 100 IU of vitamin D3 supplementation per day for 3 months in each of the group. This re-evaluation showed that, though, the rise in Group A was higher than in groups B and C (2.58±1.93, 1.98±1.46, 1.49±1.23 nmol/L) but the significance was only observed between Group A and C (2.58±1.93 vs 1.49±1.23 nmol/L, p<0.016). Likewise, there was an appreciably higher rise in Group B than in Group C but it did not achieve statistical significance (Table 3). No case of hypercalcemia was detected. The significantly higher post-supplementation serum 25(OH)D levels in Group D compared to Group A (p = 0.046) is in all probability due to higher daily dose of 2240 IU in Group D compared to1131 IU in Group A.

The percentage of subjects with vitamin D levels <50 nmol/L at baseline in groups A, B and C together decreased significantly from 85.3% to 41.4% following daily supplementation for 3 months (P=0.001). However, the percentage of subjects reaching levels (>50 nmol/L) was not significantly different between the groups A and B, A and C and B and C receiving different preparations of vitamin D3. Similarly, the percentage of subjects who achieved serum levels of >50 nmol/L increased significantly from 9.1% to 75% in Group D compared to Group A where the increase was from 8.57% to 54.29% (65.91% vs. 45.72%, p < 0.001). In the multi-linear regression, the rise in serum 25(OH)D was significantly affected by age (β = -0.131, p = 0.007) and baseline serum 25(OH)D levels (β = -0.367, p = 0.001), but not by BMI (β = -0.102, p = 0.555).

3.2 PTH Status

There were no appreciable differences observed in serum PTH values between groups at baseline. The overall median baseline serum PTH having an interquartile range (IQR) of 7.72 (5.16–11.85) pmol/L and 7.29 (4.60–11.29) pmol/L in the MITT and PPA groups significantly decreased to 5.3 (3.95–7.64) pmol/L and 5.05 (3.67–7.0) pmol/L respectively (p = 0.050 and 0.032) following supplementation with vitamin D3. The decline within groups in both MITT (p < 0.001 for Groups A, B, C and D) and PPA (p = 0.003 for Group A and p < 0.001 for Groups B, C, and D) following supplementation was also significant, as detailed in Table 2. However, the decline in serum PTH levels was no different between Groups A and B, B and C, and A and C following supplementation with different preparations of vitamin D3 in both the MITT and PPA groups.

The overall decrease in the prevalence of secondary hyperparathyroidism was significant, decreasing from 59.4% to 19.9% in the MITT analysis and from 52.9% to 18.1% in compliant PPA (P<0.001) after supplementation. Although this reduction in the prevalence of secondary hyperparathyroidism persisted within groups A, B, and C, it did not reach statistical significance when comparisons were made between the groups (A and B, B and C, and A and C).

3.3 Serum 1,25(OH)2D

Like 25(OH)D and PTH, no significant differences were noted in the baseline serum values of $1,25(OH)_2D$ between the groups. The overall mean baseline values of serum $1,25(OH)_2D$ in the MIIT analysis and PPA group were 259.3±87.1 and 263.4±59.3 pmol/L, respectively, and increased significantly to 300.9±180.8 (p=0.02) and 301.8±102.3 (p<0.001). However, when pre- and post-supplementation serum 1,25(OH)2D values were compared within each group, significance was noted in only Group A (p=0.02), Group C (p<0.001) and Group D (p=0.005) in the MITT analysis, and only in Group C (p=0.003) and Group D (p=0.011) in PPA. Inter-group analysis (MITT and PPA) revealed no significant difference in the mean increase in serum $1,25(OH)_2D$ post-supplementation.

3.4 Serum Total Calcium, Phosphate, ALP and UCa/CrR

No significant differences were found in the baseline or post-supplementation values of serum calcium, phosphate, ALP, and UCa/Cr ratios across all groups. Except for changes in serum ALP in groups C and B, and the UCa/Cr ratio in Group B after supplementation, no significant changes were observed in other biochemical parameters in any group post-supplementation (Table 4).

Table 4: Effect of Vitamin D Supplementation on other biochemical parameters

Other Outcomes	Vitamin D supplementation groups				P-value
	Group A*	Group B†	Group C‡	Group D§	
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
Total Calcium (mmol/l)					
Baseline	2.37 ±0.10	2.40 ±0.07	2.40 ±0.10	2.37 ±0.10	0.5091
Post-supplementation	2.40 ±0.10	2.40 ±0.10	2.40 ±0.12	2.40 ±0.10	0.7382
P-value	> 0.05	> 0.05	> 0.05	> 0.05	
Serum Phosphate (mmol/l)					
Baseline	3.4±0.4	3.5±0.5	3.5±0.5	3.3±0.7	0.1732
Post-supplementation	3.6±0.4	3.6±0.6	3.6±0.5	3.4±0.6	0.2219
P-value	0.006	> 0.05	> 0.05	> 0.05	
Serum ALP (U/l)					
Baseline	1.10±0.13	1.13±0.16	1.13±0.16	1.07±0.23	0.7479
Post-supplementation	1.16±0.13	1.16±0.19	1.16±0.16	1.10±0.19	0.4485
P-value	> 0.05	> 0.05	0.001	> 0.05	

UCa/CrR					
Baseline					
Median	0.05	0.05	0.05	0.05	0.4864
P25 – P75	0.02 – 0.08	0.03 – 0.09	0.02 – 0.07	0.02 – 0.09	
Post-supplementation					0.0777
Median	0.05		0.05	0.07	
Range P25 – P75	0.02 – 0.09			0.02 – 0.12	
P-value	> 0.05	<0.001	> 0.05	> 0.05	

Adverse Reaction: One lady on DEKSEL® Nano Oral Spray supplementation developed a burning sensation in the oral cavity and was excluded from the study. There was no case of hypercalcemia and the median UCa/CrR was normal. There were no symptoms and signs of renal stones such as pain abdomen, nausea, vomiting etc. in any of the subjects during the trial though we did not undertake ultrasound of lower abdomen for detection of renal stones at baseline and post supplementation.

4. Discussion

Commercially available conventional fat-soluble vitamin D3 supplements come in diverse forms, including tablets, capsules, sachets, and liquid formulations. This allows individuals, who find one form difficult to take, to choose a more suitable option. Lately, there have been liquid nano vitamin D3 formulations utilizing nanotechnology to disperse the vitamin within water-soluble micellar spheres, facilitating their delivery to the absorptive surface of the gastro-intestinal tract, ultimately leading to higher bioavailability (10). The newly introduced DEKSEL® Nano Oral Spray vitamin D3 formulation administers vitamin D3 in nano droplet form directly into the mouth, facilitating absorption through the oral and sublingual mucous membranes. This form is often deemed convenient for individuals with malabsorption issues, difficulty swallowing pills, or a preference against traditional supplement forms. Nevertheless, the efficacy and bioavailability of these newer alternative vitamin D formulations may vary among individuals and therefore, further research is essential to comprehensively assess their advantages over the traditional pill or capsule formulations.

4.1 Serum 25(OH)D

Since there are multiple factors influencing the rise in serum 25(OH)D levels such as dose per unit weight, age, baseline levels, and supplementation dose and duration, finding comparable studies on vitamin D supplementation in literature remains a challenge. In the absence of human trials with these newer nano formulations of vitamin D3 in oral/ mucosal spray and tablet forms, and conflicting reports with existing studies on conventional vitamin D3 oral/ mucosal spray, oral liquid and capsule preparations (12,16-18), we initiated the first open label RCT to evaluate the efficacy and safety of these newer nano preparations vis-a-vis conventional fat soluble vitamin D3 tablet preparation in men and women with vitamin D deficiency/ insufficiency.

The significant overall rise in serum 25(OH)D in the present study, ranging between 23.54 to 29.18 nmol/L in the compliant (PPA) group, following daily supplementation for 3 months with different preparations of vitamin D3 namely nano vitamin D3 oral spray, nano vitamin D3 tablet and conventional vitamin D3 tablet, is in agreement with the reported rise of 15-25 nmol/L shown by other workers following supplementation with 1000 IU of vitamin D3 (19). However, the overall rise in serum 25(OH)D not being significantly different among the groups A, B and C is possibly due to supplementation of different strengths of vitamin D3 (1139, 1193 and 1585 IU) among these groups against the labeled strength of 1000 IU in the three vitamin D3 preparations. Since the rise in serum 25(OH)D is primarily dependent on the supplementation dose, group A, B and C are not truly comparable.

Significant higher rise in serum 25(OH)D per 100 IU of daily nano vitamin D3 oral spray supplementation than that achieved with conventional fat soluble vitamin D3 tablet preparation (2.58±1.93 vs 1.49±1.23 nmol/L, P<0.016) is consistent with the observation of Unsur *et al* from Turkey who while evaluating oral vitamin D3 spray and oral drops supplementation also showed higher levels being achieved in healthy infants supplemented with mucosal spray (17). Likewise, Nandgaye *et al* comparing the relative bioavailability of vitamin D3 nano solution with vitamin D tablet and capsule, reported oral solution of vitamin D3 to have achieved higher Cmax and Area Under the Curve (AUC) when compared with tablet and capsule formulation (20). In contrast, Todd *et al* in their randomized, open label, crossover study comparing the efficacy of vitamin D3 liquid capsules and oral spray solution showed absence of variation in the effectiveness between the two groups (18). Similar results have also been reported by Nalbantoglu *et al* showing buccal spray and oral drops being equally effective in children and adolescents (16).

The rise in serum 25(OH)D of 2.58±1.93, 1.98± 1.4 and 1.49±1.23 nmol/L per 100 IU daily vitamin D3 supplementation among the compliant subjects in group A, B and C was also consistent with the general rule of approximate rise of 2.5 nmol/L (1.75-to 2.75) with 100 IU of vitamin D3 supplementation (21).

One may argue, justifiably, that the significant increase in serum 25(OH)D following supplementation in Group A than in groups B and C could be due interference by calcium present in tablet preparations. However, existing literature do not suggest that calcium intake alters either intestinal absorption of vitamin D or hepatic hydroxylation (22, 23). On the contrary, Berlin and Bjorkhem showed that supplementing 2000 mg of calcium per day for 6 weeks raised the serum 25(OH)D levels by 30% (24).

Similarly, though supplementation with the DENCIUM-ND3 tablet preparation also achieved appreciably higher levels of serum 25(OH)D when compared with TAYO conventional vitamin D3 tablet, it did not achieve statistical significance. These observations in the present study only reiterate our earlier observations of achieving higher levels of serum

25(OH)D with nano liquid preparations *vis-a-vis* conventional fat soluble vitamin D3 preparations in children and adults (10).

The rise in serum 25(OH)D levels with nano oral spray was notably higher with daily supplementation of 2240 IU of vitamin D3 compared to daily supplementation with 1131 IU of oral spray (43.51 vs. 29.18 nmol/L; p=0.042), as anticipated. This rise of 43.65 nmol/L (27.25–70.75 nmol/L) with daily 2240 IU in the present study was similar to a rise from (35.25±8.53.4-72±13.5 nmol/L) with daily supplementation of 2000 IU for 75 days as shown by Niet *et al* (25) and a rise of 34.5 nmol/L on supplementation with 2400 IU vitamin D3 for 16 weeks reported by Shieh *et al* (26). In contrast, Kimmee *et al* and Ganaie *et al* observed a significantly greater rise of 94.35 and 87 nmol/L respectively in serum levels of 25(OH)D with daily supplementation of 2000 IU of Vitamin D3 for 12 weeks (27, 28).

4.2 Serum PTH

High prevalence of secondary hyperparathyroidism (52.9%) observed in vitamin D deficient / insufficient subjects is consistent with several other studies showing secondary hyperparathyroidism ranging from 10.3% to 59% in healthy Syrian adults (29) and 18.2% in mild vitamin D deficiency to 61.1% in severe vitamin D deficiency in healthy Libyans (30). The significant decline in serum PTH levels and percentage decline from 52.9% to 18.1% in secondary hyperthyroidism post supplementation in all individual groups reflect resolution of secondary hyperparathyroidism and is consistent with earlier reports on the relationship between vitamin D and PTH (31,32). However, absence of this difference in decline in serum PTH levels and secondary hyperparathyroidism between different groups (A, B and C) following supplementation can be explained by the fact that PTH is a functional marker of serum 25(OH)D levels, and the post-supplementation serum 25(OH)D values were not significantly different between the groups.

4.3 Serum $1,25(OH)_2D$

The median baseline values of serum $1,25(OH)_2D$ in MIIT and PPA group showing a significant rise following supplementation with vitamin D3 has also been reported by other workers (33). We in our recent publication have also reported a significant rise in $1,25(OH)_2D$ following supplementation with 25(OH)D (34). As synthesis of $1,25(OH)_2D$ is substrate (25(OH)D) dependent, significantly higher overall rise in serum $1,25(OH)_2D$ in group C and D could be explained by higher supplementation of vitamin D3 doses (1593 and 2240 IU in groups C and D than 1131 & 1193 IU in groups A and B) in PPA group.

Absence of correlation between the change in serum 25(OH)D and change in $1,25(OH)_2D$ following vitamin D3 supplementation in the overall study population is consistent with what has been reported by Saleh Lanja *et al* in 2017 on the impact of a single oral dose of 100000 IU of vitamin D3 on the profile of 25(OH)D and its metabolites $24,25(OH)_2D$ and $1,25(OH)_2D$ (35). This is probably due to the fact that several factors like serum calcium, phosphate, calcium intake, 25(OH)D substrate, PTH and kidney functions control and maintain the serum levels of $1,25(OH)_2D$ within a narrow range for body's physiological functions.

4.4 Serum Biochemistry

The serum levels of calcium, phosphates, and alkaline phosphatase remained within normal limits in all groups after supplementation. There were no significant differences observed between the groups post-supplementation. The absence of hypercalcemia development in any of the subjects after three months of supplementation underscores the safety of the new vitamin D3 formulations.

4.5 Limitations of the Present Study

1) The study was of a short duration of 3 months. 2) Daily sun exposure and dietary evaluation of calcium and vitamin D intake were not carried out. 3) Bone formation and resorption makers could not be done due to lack of funds. 4) The three groups A, B and C were not truly comparable as the vitamin D3 content of the three different preparations varied significantly on analysis and group B and C received additional 500 mg of calcium along with vitamin D3 unlike group A which received only vitamin D3. Though calcium is not known to affect the absorption of vitamin D3 from GIT as per literature, future studies should ensure that all preparations being supplemented had equal amount of vitamin D3 and calcium before starting the trial for comparing efficacy.

4.6 Strengths of the study

1) This is the first randomized trial comparing the efficacy of nano oral spray with nano tablet and conventional fat-soluble vitamin D3 tablet preparations. 2) Pre- and post-evaluation of serum $1,25(OH)_2D$ levels was undertaken for the first time in vitamin D deficient adults being supplemented with different vitamin D3 preparations. 3) Subjects were asked not to change their lifestyle as it could have bearing on the results.

5. Conclusion

Although supplementation with all the three vitamin D3 preparations resulted in a significant rise in serum 25(OH)D levels, nano oral spray of vitamin D3 appeared to be more efficacious in achieving higher levels of serum 25(OH)D per 100 IU of daily vitamin D3 supplementation for 3 months than nano vitamin D3 tablet and conventional fat soluble vitamin D3 tablet. Absence of difference in the overall rise of serum 25(OH)D between the three preparations, possibly, due to difference in vitamin D3 content of these preparations, and calls for better quality assurance of vitamin D3 preparations.

Nano oral spray could be used as an alternative to tablet forms of vitamin D3 preparations in subjects with inability to swallow tablets or those with malabsorption. Comparative studies in future should ensure equal vitamin D3 content in both nano and conventional vitamin D3 preparations.

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Conflict of Interest: The authors declare that they do not have any conflict of Interest.

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Contributions
RKM designed and supervised the study in Delhi, and wrote the manuscript. Meeta conducted and supervised the study in the Hyderabad Centre and helped in manuscript preparation. KM carried out a detailed statistical analysis. SM helped in the analysis of data and preparation of the manuscript. VD helped in the collection of data and supervision of the study in Delhi. AN helped in the recruitment of subjects, distribution and supervision of supplementation of vitamin D preparations. DA carried out the laboratory investigations. SR helped in recruitment of subjects and supervision of study in Delhi.

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