International Journal of Clinical Trials
Sapkal N et al. Int J Clin Trials. 2020 Aug;7(3):176-187
http://www.ijclinicaltrials.com
pISSN 2349-3240 | eISSN 2349-3259
DOI: http://dx.doi.org/10.18203/2349-3259.ijct20203104

Original Research Article

Clinical efficacy of different dosage forms containing vitamin D: design and study outcomes of a randomized, comparative clinical trial

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Received: 29 January 2020
Revised: 26 April 2020
Accepted: 27 April 2020

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ABSTRACT

Background: Different dosage forms of vitamin D like tablets, soft gelatin capsules, oral granules, powders, solutions and thin films are available. The objective of the present study was to evaluate and compare the clinical efficacy of three different dosage forms of vitamin D3 namely, orally disintegrating strips, oral granules and oral solution.
Methods: An open label, single centre, prospective, randomized, parallel group, comparative study was conducted for a period of 4 months. The study participants were divided into three groups (A, B, C) and received the respective treatments (orally disintegrating strips, n=20; granules, n=20; oral nano solution, n=10) for the study period. The estimation of blood levels of 25-hydroxy vitamin D [25(OH)D_3] in all the subjects at day 0, 60 and 120 was carried out.
Results: The normalization level of 25(OH)D_3 achieved by the subjects in group A, group B and group C was 100%, 83.3% and 90% respectively after 90 days. Comparison of 25(OH)D_3 level in all three groups showed significant increase at day 60. The levels were maintained at day 90 and 120 even after drastic reduction in dosage in Group A and group C. On day 120, the dose reduction was in the order of group A>group C>group B.
Conclusions: All the three formulations showed increase in the level of 25(OH)D_3. It can be concluded that oral disintegrating strips of 25(OH)D_3 are clinically more efficient than other conventional dosage forms.

Keywords: Vitamin D, Orally disintegrating strips, 25-hydroxy Vitamin D, Ergocalciferol, Cholecalciferol, 25(OH)D_3

INTRODUCTION

Vitamin D includes a group of fat-soluble secosteroids considering mainly two molecules, vitamin D_2 and vitamin D_3. liver converts vitamin D to the prohormone calcidiol, or 25-hydroxy vitamin D [25(OH)D].[1-3] Measurement of 25(OH)D in the circulation is the best diagnostic test for determining a person's Vitamin D deficiency status.[4] Several studies reveal that low plasma levels of the 25(OH)D, are linearly related with bone mineral density and bone fractures, increased risks of vascular and non-vascular mortality.[5,6] The vitamin D deficiency is also linked to many diseases, including several deadly cancers, several autoimmune diseases including type 1 diabetes, type 2 diabetes, multiple sclerosis, rheumatoid arthritis, Crohn's disease, cardiovascular diseases and several infectious diseases.[3,4,7-10]

Vitamin D deficiency is prevalent in many countries including India. There are many factors such as limited sun exposure, pollution in atmosphere, clothing, skin pigmentation, use of sunscreen, dietary patterns and genetics which might be responsible.[11-14] Vitamin D3 is available in dosage range of 200 IU to 3,00,000 IU and can be taken once a day to once a week or once a month depending upon the deficiency status. These supplements are available in various dosage forms like tablets, soft gelatin capsules, granules, oral solutions and orally disintegrating strips (ODSs).

The ODS is a new dosage form that constitutes a flat thin film that rapidly dissolves when kept on the tongue.[15,16] These thin films offer many advantages over the conventional oral dosage forms. These films are not required to be swallowed and hence are an ideal dosage form for paediatric, geriatric and dysphagic patients. Each film constitutes an unit dose and assures accurate, precise dosing every time. The additional features like ease of portability, storage and handling make them convenient for drug administration.

ODSs are used to treat vitamin D3 deficiency since many years, but no published data is available with regard to their clinical efficacy. Accordingly, a comparative study was planned with the main aim to evaluate and compare clinical efficacy and safety of three different dosage forms of vitamin D3 in vitamin D3 deficient subjects. In this study, the three dosage forms used were ODSs, granules and nano solution.

The primary objective of this study was to compare the clinical efficacy of three different forms of Vitamin D supplementation in normalizing $25(OH)D_3$ level. The secondary objectives of the study were to assess the safety of all three vitamin D3 dosage forms; to compare the convenience of dosage form administration and patient compliance to the treatment and to calculate the mean dose intake for all three vitamin D3 dosage forms through the study period.

METHODS

Study design

This study was a phase IV, single centre, open label, prospective, randomized, parallel group, comparative clinical trial to evaluate clinical efficacy and safety of three different dosage forms of vitamin D with standard treatment (calcium tablet) in vitamin D3 deficient subjects. The study duration was 04 months from date of first patient enrolment (April 2018 to July 2018).

This study was performed in compliance with ICH guidelines on good clinical practices (GCPs) including the archiving of essential documents. The study was conducted in accordance with all national and local regulatory requirements, pertaining to the protection of human research subjects. It was also conducted in accordance with the GCP guidelines issued by Central Drugs Standard Control Organization (CDSCO) and Ethical Guidelines for Biomedical Research on Human Subjects, issued by the Indian Council of Medical Research (ICMR). The blood sample analysis was performed at NABL (National Accreditation Board for Testing and Calibration Laboratories) accredited laboratory.

Participants

Inclusion criteria

Inclusion criteria for recruitment of participants included subjects between the age of 18 to 65 years who were able to understand the information given to them and gave written consent and had blood serum level of $25(OH)D_3$ < 30 nmol/l or <12 ng/ml.

Exclusion criteria

Exclusion criteria of participants included creatinine level ≤ 1.9, hyperthyroidism, nephrolithiasis, current use of any dose of glucocorticoids, sarcoidosis, tuberculosis or Paget's disease, vitamin D intoxication and granulomatous diseases. Female volunteers who were pregnant or intended to become pregnant were not enrolled in the study.

Demographic characteristic data

Initially all eligible participants were asked to complete and sign the written informed consent. Demographic data which was considered for the study included date of birth, age, gender, height, weight and body mass index. The information regarding the history of past significant medical disorders, chronic use of medications, allergies, medications consumed during the last three months and personal habits, concomitant illness, concurrent medications and current method for contraception were recorded from subjects. The design of the study is shown in Table 1.

Randomization and treatment

Following successful screening and baseline assessment, the eligible subjects were randomised into three groups. As treatment commenced, subjects randomly received any one of the three products. Cholecalciferol ODSs were received by 20 subjects (Group A), cholecalciferol granules were received by 20 subjects (group B) whereas cholecalciferol nano oral solution were received by 10 subjects (group C) (Table 2). Calcium tablet (500 mg) was given twice daily throughout the study period. Subjects were provided with study-diaries to record their medication information and a reminder was given each week. The three groups and their respective treatments are summarized in Table 2.

Table 1: Study design outline.

Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Day -5 to 1	Day 1	Day 60	Day 90	Day 120
Level of creatinine, calcium and 25(OH)D_3	-	Level of 25(OH)D_3	Level of 25(OH)D_3	Level of calcium and 25(OH)D_3
-	Dose: Cholecalciferol 60000 IU/week + calcium tablets (500 mg)	Dose: Cholecalciferol 60000 IU + calcium tablets (500 mg)		-
Screening	Randomization and dosing	Follow up visit and dose adjustment		End of study

Table 2: Treatment groups.

S. no.	Group	Name of product (dosage form)	Number of subjects (sample size)
1	A	Vitamin D3 ODSs (60000 IU per strip)	20
2	B	Vitamin D3 granules (60000 IU/gm)	20
3	C	Vitamin D3 nano oral solution (60000 IU/5ml)	10

Table 3: Study procedure.

Particulars	Treatment period				
	Visit 1 (day -5 to 1, screening visit)	**Visit 2 randomization visit: day 1 randomization**	**Visit 3 (day 60±3, follow up visit)**	**Visit 4 (day 90±3, follow up visit)**	**Visit 5 (day 120±3, end of visit)**
Informed consent	✓	-	-	-	-
Inclusion and exclusion criteria	✓	-	-	-	-
Social and demographic data	✓	-	-	-	-
Vital signs	✓	-	✓	-	✓
Physical examination	✓	-	✓	-	✓
Medical history	✓	-	-	-	-
Prior and concomitant medication (s)	✓	-	✓	✓	✓
s-Creatinine	✓	-	-	-	-
Total calcium	✓	-	-	-	✓
25(OH)D_3	✓	-	✓	✓	✓
Dosage forms and dosage forms compliance					
Dispensing of vit D dosage forms	-	✓	✓	✓	-
Dose adjustment	-	-	✓	✓	-
Returning of unused dosage forms	-	-	✓	✓	✓
Adverse event recording	✓	✓	✓	✓	✓
Questionnaire	-	-	✓	✓	✓

Vital sign = pulse rate (breath per min), temperature (0F) and blood pressure (systolic and diastolic) (mmHg). Weight was measured on every visit. ✓= activity done on visit.

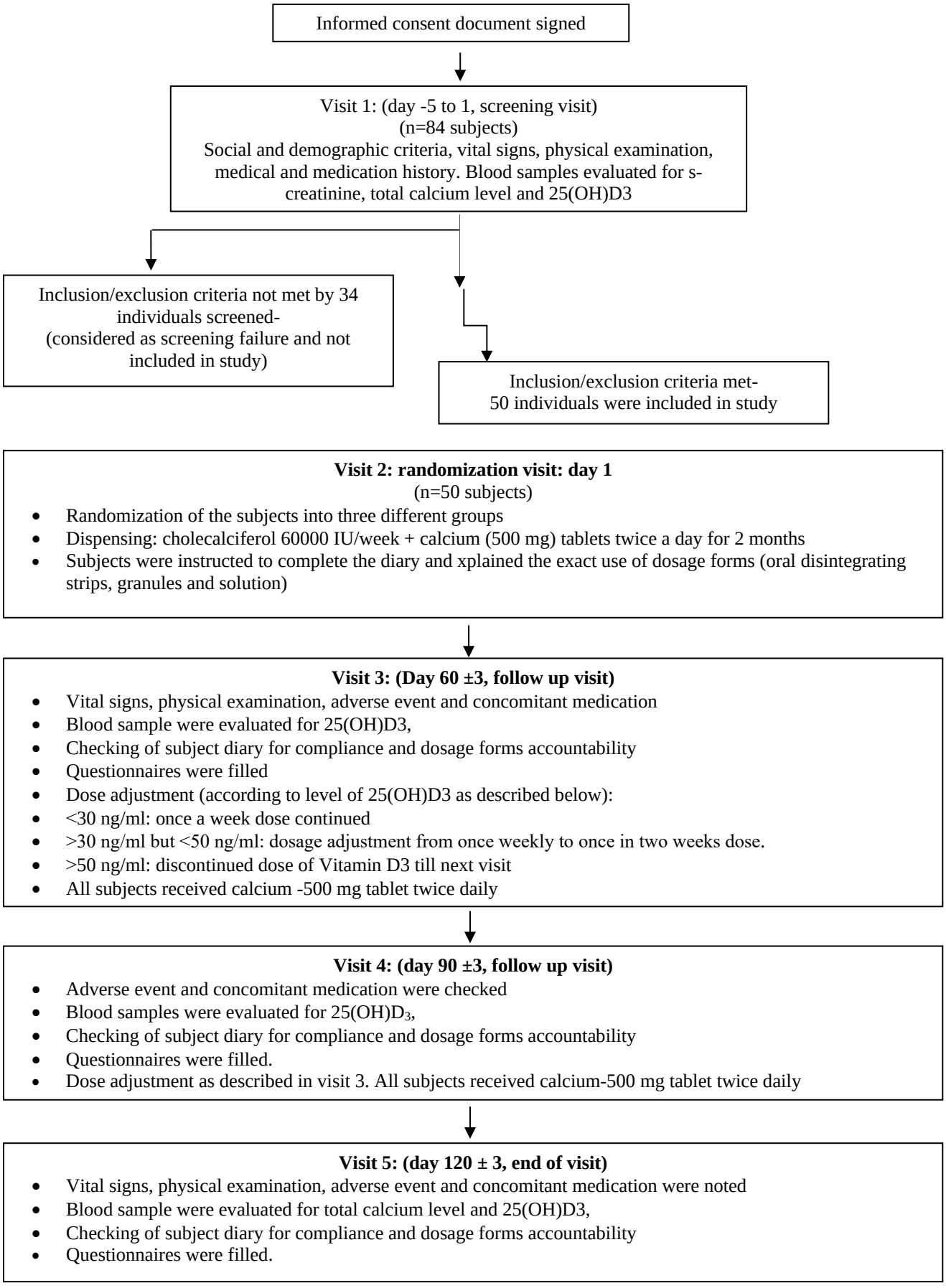

Figure 1: Study flow chart.

Selection of doses in the study

For first two months (for 60 days) dose was fixed for Vitamin D3 products as 60000 IU per week for all subjects. The subjects received any one of the three formulations along with standard treatment of Calcium tablet- 500 mg twice daily.

After two months, the doses of Vitamin D3 formulations were adjusted based on the serum level of $25(OH)D_3$ as $25(OH)D_3$ level <30 ng/ml: 60000 IU continued per week for 1 month; $25(OH)D_3$ level >30 ng/ml but <50 ng/ml: 60000 IU once in 2 weeks for 1 month; $25(OH)D_3$ level >50 ng/ml: discontinuation of vitamin D3 dosage for 1 month

The product was provided for another 1 month as per the dosage adjustment after completion of 2 months of the treatment. On day 90, again the blood level of $25(OH)D_3$ was evaluated and dose was adjusted as deemed necessary for next month same as explained above.

Study procedure

The study procedure is denoted in Table 3.

Visit wise procedure

Visit 1: Screening visit day

Eligibility criteria for the subjects were assessed by the investigators. Vital signs like pulse rate, heart rate, blood pressure (systolic and diastolic) and body temperature were recorded. Physical examination was performed which included the following: assessment of general appearance, respiratory functions, skin, head, eyes, ears, nose, throat, heart, abdomen, reflexes, lymph nodes, and extremities, neurological, mental status. Medical and medication history was collected. The participants' blood samples were evaluated for s-creatinine, total calcium level and $25(OH)D_3$.

Visit 2: Randomization visit: day 1

On randomization visit, subjects were enrolled to receive the formulations. Subjects who met all recruitment criteria were considered as eligible for randomization. Randomized subjects received any of the three test products for 2 months (Table 2). Subjects were randomized to one of the formulations containing Vitamin D3 60000 IU once a week with Calcium tablets twice a day.

Visit 3: Day 60±3: follow up visit

Physical examination including vital signs was performed. Subjects were evaluated for use of any concomitant medications and inter-current illness if any. Adverse event reporting was done, if any. Blood samples were collected for measurement of $25(OH)D_3$. Treatment was adjusted as mentioned above (Section 2.5). Calcium tablets were continued to administer twice a day. Subjects were asked to fill up the preference and acceptability questionnaire.

Visit 4: day 90±3: follow up visit

Adverse event reporting was done, if any. Blood sample was collected to measure the levels of $25(OH)D_3$. Treatment was adjusted as mentioned in Section 2.5. Calcium tablets were continued to administer twice a day.

Visit 5: day 120±3: end of visit

Physical examination including vital signs was performed. Subjects were evaluated for use of any concomitant medications and inter-current illness if any. Adverse event reporting was done, if any. Blood sample was collected for measurement of $25(OH)D_3$ and total calcium level.

The study visits and activities completed are denoted in Figure 1.

Efficacy measurements assessed

End points were assessed against the level of $25(OH)D_3$. Comparison of three treatment options by measuring $25(OH)D_3$ level: It was considered for evaluation of percent of improvement as well as absolute level among all treatment groups from baseline to day 60, 90 and 120. Normalization rate of $25(OH)D_3$: It was evaluated by calculating the rate of improvement till the sufficient level was reached. For evaluation, number of subjects (%) with normal level of $25(OH)D_3$ after treatment was calculated. Mean dose changes among three different groups of Vitamin D3 treatment through day 120: Total mean administered dose per group was calculated based on total dose changes in all treatment groups. The adjustment in dosage to maintain the sufficient level of $25(OH)D_3$ over a period of study duration was also correlated with the percent improvement.

Statistical analysis

For the statistical analysis, quantitative variables were presented as mean±standard deviation. Normality test was used to check the distribution of data for quantitative variables. All the tests were two sided. The level of significance was 0.05. P values of less than 0.05 were considered as statistically significant difference. Categorical variables were presented as absolute number or percentage.

Statistical methods for demographic characteristics data

For comparison between all treatment groups, for quantitative variable, ANOVA or Kruskal-Wallis test was

used. Normality test (Kolmogorov-Smirnov test or Shapiro-Wilks test) was used for quantitative variables.

For categorical variable (e.g. gender), Chi-square test or Fisher exact test was used.

Statistical methods for efficacy measurements

Drug concentration measurement

The 25-Hydroxy metabolite of vitamin D3 was measured for all subjects at day 0, 60, 90, and 120 using the specific ELISA method.

Comparison of three treatment options using 25(OH)D_3 level

Changes from baseline in plasma levels of 25(OH)D_3 were compared between all treatment groups using ANOVA or Kruskal-Wallis test.

Normalization rate of 25(OH)D_3 level at each visit

The number of patients (%) with normal level of 25(OH)D_3 after treatment was compared by Chi-square test or Fisher exact test.

Mean dose changes among three different groups of vitamin D3 treatment through day 120.

Mean dose was compared between all treatment groups using ANOVA or Kruskal-Wallis test.

RESULTS

Screening and enrolment of participants

The study outline designed is demonstrated in Table 1. The study flowchart is given in Figure 1. A total of 84 subjects were screened. Amongst these, 34 (40.48%) subjects were excluded for not meeting exclusion/inclusion criteria. A total of 50 subjects were randomized into the present study. Two subjects were withdrawn before completing the treatment schedule and two subjects lost to follow up on month 2 and 3 respectively. These four subjects were not replaced in accordance with the protocol resulting in 46 subjects being available for analysis. The losses occurred in groups A and B two each. The number of subjects (i.e. 46) who were enrolled into this study, were randomized and were available for analysis as given in Table 4.

Table 4: Analysis.

Number of subjects					
Total screened	**Screening failure**	**Total enrolled**	**Total completed**	**Withdrawn / dropped-out**	**Lost to Follow up**
84	34	50	46	02	02

Table 5: Summary of demographic information.

S. no.	Total Enrolled subjects			Male			Female		
	Age (years)	**Height (cm)**	**Weight (kg)**	**Age (years)**	**Height (cm)**	**Weight (kg)**	**Age (years)**	**Height (cm)**	**Weight (kg)**
Group A – ODS									
N	20	20	20	11	11	11	9	9	9
Mean	43.45	163.7	67.42	43.36	168.91	74.77	43.56	157.33	58.43
SD	12.03	8.1	15.01	13.94	6.36	15.03	10.05	4.72	9.37
Min	23	149	41.5	23	156	58.7	29	149	41.5
Max	59	178	100.3	59	178	100.3	57	164	71
Group B – granules									
N	20	20	20	8	8	8	12	12	12
Mean	42.00	161.10	72.07	41.00	169.88	81.51	42.67	155.25	65.77
SD	10.16	10.02	16.93	9.93	4.55	12.13	10.70	8.17	17.13
Min	24.00	141.00	35.80	27.00	162.00	60.30	24.00	141.00	35.80
Max	59.00	177.00	98.00	53.00	177.00	98.00	59.00	167.00	88.10
Group C – oral nano solution									
N	10	10	10	05	05	05	05	05	05
Mean	38.00	164.70	70.31	39.40	172.60	76.98	36.60	156.80	63.64
SD	11.30	9.32	18.76	12.05	5.37	23.39	11.72	3.27	11.54
Min	21.00	154.00	46.00	21.00	165.00	46.00	25.00	154.00	48.90
Max	52.00	177.00	109.00	52.00	177.00	109.00	52.00	162.00	74.80

Table 6: Level of 25(OH)D_3.

	25(OH)D_3 (ng/ml)			
	Day -5 to 1	**Day 60**	**Day 90**	**Day 120**
Group A – ODS				
N	18	18	18	18
Mean	12.14	51.12	45.37	50.36
SD	4.68	14.77	6.87	8.86
Min	3.00	13.55	32.51	35.05
Max	19.10	70.00	64.09	65.94
Group B – granules				
N	18	18	18	18
Mean	10.83	32.29	34.75	44.25
SD	4.37	8.26	6.24	10.30
Min	3.97	17.05	22.72	25.88
Max	16.80	47.86	43.69	60.58
Group C – oral nano solution				
N	09	09	09	09
Mean	11.79	49.61	41.86	54.24
SD	5.23	12.86	4.47	8.29
Min	5.61	27.52	32.58	44.18
Max	20.04	64.84	48.50	70.00

Efficacy evaluation

Demographic characteristics

Demographic characteristics, age, gender, weight and all other baseline information is summarized in Table 5. The binary and categorical variables are given as numbers (with percentages). The mean (standard deviation) have been presented for continuous normally distributed data.

In general, all the baseline characteristics were well balanced across the groups. All mean values are expressed as mean±SD. Statistical analysis revealed that there were no significant differences in demographic and baseline characteristics between subjects of treatment and control group.

Comparison for vital signs

No clinically significant vitals (pulse rate, blood pressure, respiratory rate, body temperature) changes from baseline to month 4 were observed in any of study subjects. No report of change in vital signs from normal to abnormal was observed during study period.

Efficacy results

Comparison of three treatment options

Comparison between all three treatments; ODS, granules and oral nano solution was done by measuring 25(OH)D_3 level at Day 60, 90 and 120 as compared to base line level. Table 6 summarizes levels of 25(OH)D_3 in all three groups.

Comparison of 25(OH)D_3 level at 2 months (60 Days)

For group A, the mean level of the 25(OH)D_3 was 12.14±4.73 at baseline which then increased to 51.12±14.79 after 2 months of treatment. So, at 60 days, 25(OH)D_3 level was increased to about 404.66±292.81% from baseline.

While for subjects in group B, the mean level of the 25(OH)D_3 for baseline and 2 months were 10.83±4.37 and 32.29±8.29 respectively. So, at 60 days, 25(OH)D_3 level was increased about 243.78±154.99% from the baseline.

For group C, the mean level of the 25(OH)D_3 at baseline was found to be 11.79±5.23 and at 2 months it was 49.61±12.86. So, at 60 days, 25(OH)D_3 level was increased to about 371.66±168.93% from baseline. The mean average dosage consumption was similar for all the subjects.

Comparison of 25(OH)D_3 level at 3 months (90 days)

For group A, the mean level of the 25(OH)D_3 was found to be 45.37±6.87 at day 90 which was still 364.44±312.95% higher from baseline. However, the average dose consumption was reduced drastically to 66666.67 IU between 2 months to 3 months. This reduction in dosage was accounted for 71.43% dose reduction as compared to monthly average dose consumption for first two months.

While for subjects in group B, the mean level of the 25(OH)D_3 at day 90 was found to be 34.75±6.24 which showed increase of 280.23±187.59% from baseline. The

average dose consumption was reduced to 153333.33 IU between 2 months to 3 months. This dosage reduction was only 32.35% as compared to monthly average dose consumption for first two months.

For group C, the mean of the 25(OH)D_3 level at day 90 was 41.86±4.47. So, at 90 days, 25(OH)D_3 level was increased by 316.10±164.34% from baseline. The average dose consumption was reduced drastically to 80000 IU between 2 months to 3 months. This reduction in dosage was of 65.71% as compared to monthly average dose consumption for first two months.

Comparison of 25(OH)D_3 level at 4 months (120 days)

For group A, the mean of the 25(OH)D_3 level was found to be 50.36±8.86 at 4th month. So, at 120 days, 25(OH)D_3 level was increased by 404.89±275.96% from baseline. The average dose consumption was 93333.33 IU between 3 months to 4 months which was accounted for 60% dose reduction as compared to monthly average dose consumption for first two months.

While for subjects in group B, the mean of the 25(OH)D_3 level was found to be 44.25±10.30 at 4th month which was 378.67±215.75% higher from baseline. The average dose consumption got slightly further reduced to 140000.00 IU between 3 months to 4 months. This reduction in dosage was almost similar as 3rd month, at 38.23% as compared to monthly average dose consumption for first two months. This shows that more dose consumption was required to achieve the 'normalization rate'.

For group C, the mean of the 25(OH)D_3 level at 4th month was 54.24±8.29. So, at 120 days, 25(OH)D_3 level had increased to 435.14±206.05% from baseline. The average dose consumption was 120000 IU between 3 months to 4 months. This reduction in dosage was the reason for 48.57% dose reduction as compared to monthly average dose consumption for first two months.

Tabulated interpretation of 25(OH)D_3 level and the percent increase has been captured in Table 7. Figure 2 shows the graphical representation of the increased level of 25(OH)D_3.

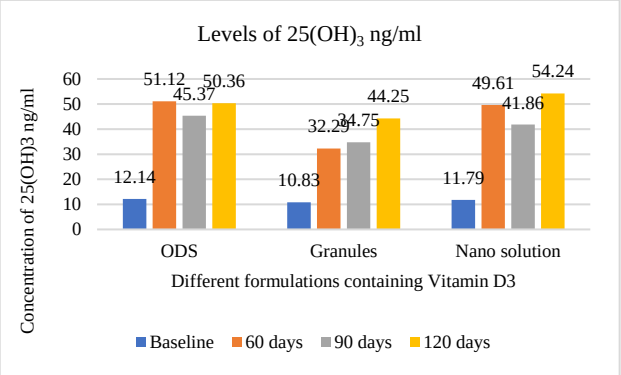

Figure 2: Graphical representation of increased level of 25(OH)$_3$.

Table 8 represents the comparison of 25(OH)D_3 level between the groups along with the statistical data.

Table 7: Improvement of 25(OH)D_3 level (%).

	Serum 25(OH)D_3 (ng/ml)					
	% 25(OH)D_3 increase after day 60 as compared to day 00	% 25(OH)D_3 increase after day 90 as compared to day 00	% 25(OH)D_3 increase after day 90 as compared to day 60	% 25(OH)D_3 increase after day 120 as compared to day 00	% 25(OH)D_3 increase after day 120 as compared to day 60	% 25(OH)D_3 increase after day 120 as compared to day 90
Group A – ODS						
N	18	18	18	18	18	18
Mean	404.66	364.44	-1.23	404.89	12.03	13.53
SD	292.81	312.95	42.79	275.96	66.33	26.93
Min	4.15	137.17	-37.54	106.78	-39.71	-35.83
Max	1247.67	1341.67	140.74	1068.33	255.13	58.43
Group B – granules						
N	18	18	18	18	18	18
Mean	243.78	280.23	10.33	378.67	43.08	29.02
SD	154.99	187.59	14.60	215.75	44.75	29.80
Min	82.56	109.17	-9.74	110.45	-3.94	-25.92
Max	591.02	741.31	35.66	737.78	176.89	104.11
Group C – oral nano solution						
N	09	09	09	09	09	09
Mean	371.66	316.10	-11.51	435.14	14.81	29.41
SD	168.93	164.34	18.53	206.05	27.87	11.08
Min	176.15	140.16	-32.65	202.63	-15.45	13.60
Max	659.25	593.23	18.39	697.49	60.83	47.22

Table 8: Comparison of 25(OH)D_3 level (within group comparison).

Visits	Vitamin D3 60,000 IU formulations					
	Disintegrating strip (n=18)		Granules (n =18)		Oral nano solution (n =9)	
Baseline	12.1 (4.7)		10.8 (4.4)		11.8 (5.2)	
2 months	51.1 (14.8)		32.3 (8.3)		49.6 (12.9)	
3 months	45.4 (6.9)		34.7 (6.2)		41.9 (4.5)	
4 months	50.4 (8.9)		44.3 (10.3)		54.2 (8.3)	
P value* (within group comparison)						
Baseline vs 2 months	<0.001	S	<0.001	S	<0.001	S
Baseline vs 3 months	<0.001	S	<0.001	S	<0.001	S
Baseline vs 4 months	<0.001	S	<0.001	S	<0.001	S
2 months vs 3 months	>0.05	NS	>0.05	NS	>0.05	NS
2 months vs 4 months	>0.05	NS	<0.05	S	>0.05	NS
3 months vs4 months	>0.05	NS	<0.05	S	<0.05	S
P value (between group comparison)**						
Disintegrating strip vs granules						
Baseline, p value (95% CI)	P>0.05; (-1.78 to 4.38)				NS	
2 months, p value (95% CI)	P<0.05; (10.67 to 26.93)				S	
3 months, p value (95% CI)	P<0.05; (6.257 to 15.143)				S	
4 months, p value (95% CI)	P>0.05; (-0.42 to 12.62)				NS	
Disintegrating strip vs oral solution						
Baseline, p value (95% CI)	P>0.05; (-3.79 to 4.39)				NS	
2 months, p value (95% CI)	P>0.05; (-10.46 to 13.46)				NS	
3 months, p value (95% CI)	P>0.05; (-1.74 to 8.74)				NS	
4 months, p value (95% CI)	P>0.05; (-11.13 to 3.53)				NS	
Granules vs oral solution						
Baseline, p value (95% CI)	P>0.05; (-4.93 to 2.93)				NS	
2 months, p value (95% CI)	P<0.01; (-25.71 to -8.89)				NS	
3 months, p value (95% CI)	P<0.05; (-12.02 to -2.39)				NS	
4 months, p value (95% CI)	P>0.05; (-18.06 to -1.74)				NS	

* paired t test; **Mann Whitney test. Values are expressed as Mean (SD).

Table 9: Comparison of normalization rate of 25(OH)D_3 level expressed as number of subjects (%) with normal level of 25(OH)D_3 after treatment.

Normalization reached	Vitamin D_3 60,000 IU formulations			P value#					
	Disintegrating strip (n=18) N (%)	Granules (n=18) N (%)	Oral solution (n=9) N (%)	Disintegrating strip vs granules		Disintegrating strip vs oral solution		Granules vs oral solution	
At 2 months									
Yes	17 (94.4)	11 (61.1)	8 (89)	<0.05	S	>0.05	NS	>0.05	NS
No	1 (5.6)	7 (38.9)	1 (11)						
At 3 months									
Yes	18 (100)	15 (83.3)	9 (100)	>0.05	NS	>0.05	NS	>0.05	NS
No	0	3 (16.7)	0 (0)						
At 4 months									
Yes	18 (100)	16 (88.9)	9 (100)	>0.05	NS	>0.05	NS	>0.05	NS
No	0	2 (11.1)	0 (0)						

#Fisher's exact test. Values are expressed as absolute number (%) of patients in each category.

Normalization rate of 25(OH)D_3

A normalization rate was calculated based on 'percent of the subjects' who showed desired normal serum level of 25(OH)D_3 during the study period through 120 days. It provided the details of the subjects with normal level of 25(OH)D_3 and also the subjects who required dosage adjustment. The level of normalization had been correlated with dosage adjustment. This was evaluated both at 90 days and 120 days.

A normalization rate from baseline to 60 days in group A was 94.4%, in group B it was 61.1% and in group C it was 89.0%. After dosage adjustment, there was 100% normalization rate achieved in group A and group C at 3

months which continued till 4 months. This shows that the subjects in group A and C maintained the levels of $25(OH)D_3$ even after significant dosage reduction at months 3 and 4.

In case of group B, the normalization rate was increased to 83.3% and 88.9% after 3 months and 4 months respectively. This data shows that for achieving normalization rate it required more time also more dose. As the subjects in group B required longer time and longer dose consumption the normalization rate did not achieve till 100%.

Summary of normalization rate of all three group are described in Table 9.

Overall interpretation

During the present study, number of subjects (%) with normal level of $25(OH)D_3$ after treatment with vitamin D3 (60,000 IU) was non-significant in all three groups (A, B, C) as tested using Fisher's exact test. A statistically significant difference using Fisher's exact test was observed only between disintegrating strips (group a) and granules (group B) after 60 days of treatment. A significantly greater number of patients (%) who received ODS (group A) achieved normal level of $25(OH)D_3$ as compared to those who received granules (group B) after 60 days of treatment.

Table 10: Percent reduction in dose consumption compared to baseline.

Group	Treatment	% reduction in dose at 3rd month	% reduction in dose at 4th month
A	ODS	71.43	60.00
B	Granules	32.35	38.23
C	Solution	65.71	48.57

Table 11: Comparison of vitamin D3 dose consumption.

Visits	Vitamin D3 60,000 IU formulations					
	Disintegrating strip (n=18)		**Granules (n=18)**		**Oral solution (n=09)**	
Baseline (dose in IU)	466666.67 (56568.54)		453333.33 (65798.27)		466666.67 (40000.00)	
2 months (dose in IU)	66666.67 (73883.9)		153333.33 (55306.63)		80000 (84852.81)	
3 months (dose in IU)	93333.33 (51335.1)		140000 (46017.9)		120000 (0.00)	
P value* (within group comparison)						
Baseline vs 2 months	<0.0001	S	<0.0001	S	<0.0001	S
Baseline vs 3 months	<0.0001	S	<0.0001	S	NC##	-
2 months vs 3 months	>0.05	NS	>0.05	NS	<0.05	S
P value (between group comparison)**						
Disintegrating strip vs. granules						
Baseline	NC##		-			
2 months	<0.01		S			
3 months	<0.01		S			
Disintegrating strip vs. oral solution						
Baseline	NC##		-			
2 months	>0.05		NS			
3 months	>0.05		NS			
Granules vs. oral solution						
Baseline	NC##		-			
2 months	<0.005		S			
3 months	>0.05		NS			

*paired t test; **Mann Whitney test. Values are expressed as mean (SD). n=Total number of patients in each group; ##NC=Not calculated due null value of SD in both groups. Bold p value (s) indicates significant statistical difference.

Mean dose changes among three different groups of vitamin D3 treatment through day 120 and interpretation of results

The summary of percent reduction in dose consumption is presented in Table 10. Dose consumption in all groups with comparison within different groups is summarised in Table 11.

After taking similar dose for first 2 months, to reach normal level of $25(OH)D_3$ 'required dose consumption' by the subjects was drastically reduced in group A and

group C by 71.43% and 65.71% respectively at the end of 60 days and 60.00% and 48.57% respectively at day 90 as compared to average monthly consumption for first two months.

While for group B it is reduced by 32.35% and 38.23% at the end of 60 days and 90 days from baseline respectively. This shows that subjects in Group B required a greater number of doses to achieve the normalization rate. The dosage reduction was significantly higher in group A at 60 days and 90 days as well as compared to group B and it was significantly higher at day 60 in group C as compared to group B. However, there was no statistical difference observed in dosage changes at days 60 and days 90 between group A and group C. This may be as a result of better absorption of vitamin D3 incorporated in ODS as well as nano solution as compared to granule formulation.

Efficacy conclusions

Comparison of $25(OH)D_3$ level in all three groups showed significant increase at day 60. This increase was significantly different in group A as compared to group B at 60 days and 90 days. However, further changes in levels of $25(OH)D_3$ were not significantly different at 120 days between all 3 treatment groups. The levels were maintained at days 90 and 120, even after drastic reduction in dosage in group A and group C. However, dose reduction in group B was significantly different as compared to the dosage reduction in group A and group C.

Safety evaluation and conclusions

The safety analysis was performed on subjects of group A, group B and group C who received at least a single dose of vitamin D3. Only 2 subjects out of 50 reported the adverse events (AE). The most frequently observed adverse events were fever which was not related to the treatment. The AEs got resolved. Overall, the treatments were found to be safe and well tolerated.

Handling of dropouts or missing data

There were only 4/50 (8%) subjects who dropped out from the study without replacement and with no data after the loss to follow up. All these were lost to follow-up at month 2 and 3. With this low level of missing data, complete case analysis was performed.

DISCUSSION

In a clinical practice, voluntary swallowing of an oral dosage form by the patient is a major difficulty especially with children, elderly, nauseated, bed ridden and mentally challenged individuals. Thus, the physicians and the patients always search for other alternatives in comparison to existing oral dosage forms. A need for better, convenient and non-obstructive dosage form possessing the advantages of conventional dosage forms has been articulated for many years.

The emergence of vitamin D3 deficiency suggests an urgent need for vitamin D3 products which can achieve the immediate increase in the level of $25(OH)D_3$. Different formulations of vitamin D3 show different normalization rate. The currently available vitamin D3 formulations vary in their responses and are inadequate to ensure immediate increase in the blood level of the $25(OH)D_3$. The established products containing vitamin D have some limitations as well in terms of efficacy, dose precision etc. Sometimes cost, taste and method of consumption is not much feasible to the subjects.[15] So, as an approach towards addressing this existing, prevalent and clinically significant problem of vitamin D deficiency, different dosage forms of vitamin D3 were selected and evaluated.

Furthermore, a few studies conducted on various oral dosage forms on absorption rate showed that oral solution has highest absorption rate comparing to the other dosage forms. The availability for absorption decreases in the order: solution >suspension >powder-filled capsule >compressed tablet >coated tablet.[17-19]

The main issue addressed by this trial was to establish comparison of three different forms of vitamin D treatment in normalizing $25(OH)D_3$ level. The results showed that there was significant increase in $25(OH)D_3$ level in all three groups at 2 months. This increase was significantly different in Group A as compared to Group B at month 2 and 3. However, further change in levels of $25(OH)D_3$ was not significantly different at month 4 between all three treatment groups. The levels of $25(OH)D_3$ were maintained at months 3 and 4 even after drastic reduction in dosage in group A and group C. However, dose reduction in group B was significantly different as compared to dosage reduction in group A and C.

The trial revealed that while comparing three different formulations, the dosage i.e. 60000 IU per week resulted in different normalization rates through the period of study. The normalization rate reached to 100% with ODSs (group A) and nano solution (group C) at the end of third month and this was continued till end of fourth month. In case of granules (group B), the normalization rate was 88.9% at the end of 4^{th} month.

From the results it can be observed that the order of 'percent reduction in dose consumption' was group A >group C>group B. This important observation could be a result of variations in dissolution pattern and absorption rate of the vitamin through respective formulations. ODSs and nano solution provide larger surface area when placed on tongue and due to smaller particle size respectively. It means that fewer doses would be adequate to achieve and maintain normal levels of vitamin D. This aspect is important in improving patient compliance as it leads to lesser frequency of dosing. On the basis of present study results, it can be speculated that

the ODS containing vitamin D_3 can show an increased serum concentration of 25(OH)D_3 as compared to other dosage forms. Hereby, it can be stated that this is the first 'open label, single centre, prospective, randomized, parallel group, comparative' clinical trial. It is a most appropriate study of assessment of clinical efficacy of different dosage forms containing vitamin D. The ODS of vitamin D3 were well accepted and patient compliance during the study was good. The improved bioavailability of vitamin D3 along with dose reduction could be attributed to the novel design of ODS. ODS offered many clinical advantages along with reduction in dose over other commercially available, conventional oral dosage forms with vitamin D supplementation. These ODS containing vitamin D3 is a more consumer-friendly and safe, non-invasive dosage form, especially for paediatric and geriatric subjects.

CONCLUSION

From the results of the present study, vitamin D deficiency at baseline reached normal 25(OH)D_3 level after 2 months of treatment with ODSs, even it can reach to higher 25(OH)D_3 level compared to granules and nano solution. Normalization rate could be achieved by the similar dose at first 2 months but still after reduction of the dose, the normalization rate could be continued in case of ODSs. Dose consumption required is minimum up to 4 months to maintain the level of 25(OH)D_3 for the ODSs.

It can be concluded that the oral disintegrating strips containing vitamin D3 could represent as an improved, valid alternative to the current marketed products for the treatment of vitamin D deficiency. Overall impact of this trial is endorsement to ODS as a cost effective and advanced approach for management of vitamin D deficiency.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Sapkal N, Chhaya G, Satya M, Shah D. Clinical efficacy of different dosage forms containing vitamin D: design and study outcomes of a randomized, comparative clinical trial. Int J Clin Trials 2020;7(3):176-87.