Original Research Article

Pharmacokinetic and pharmacodynamic evaluation of a single dose of new, first in world, high dose aqueous formulation versus conventional oil based preparation of cholecalciferol

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ABSTRACT

Background: Vitamin D deficiency (VDD) is a common condition in India with prevalence in general population varying from 50-94%. VDD has been associated with increased susceptibility to a wide range of viral infections including COVID-19. It is associated with worse outcomes and greater morbidity and mortality in medical as well as surgical ICUs. The objective of the study was to evaluate the pharmacokinetic (PK)/pharmacodynamic (PD) of aqueous cholecalciferol injection in comparison with conventional oil-based injection in VDD healthy adult subjects.
Methods: 24 eligible vitamin D deficient healthy adult males, fulfilling the inclusion/exclusion criteria, were enrolled in the study. Subjects received a single dose of either test (T) [Aqueous cholecalciferol injection 600K IU/2 mL] or reference (R) [Cholecalciferol 600K IU oil-based injection] intramuscularly. Various PK parameters (C_{max}, AUC0-t, AUC0-∞ and T_{max}) and PD parameters (concentration of cholecalciferol and 25(OH)D) were measured along with safety analysis.
Results: A significantly higher concentration of cholecalciferol and 25(OH)D were observed with test product compared to reference (p<0.01) from 1-hour post-administration till end of the study. A statistically significant difference between test and reference product was observed for the calculated C_{max} and T_{max} for cholecalciferol and 25(OH)D (p<0.0001). All 12 (100%) subject in test arm achieved the normal level of 25(OH)D by 72 hr post-administration as compared to none in reference arm (p=0.0017). There were no serious adverse events (SAEs) or deaths reported during the study.
Conclusions: This first in world, aqueous formulation of cholecalciferol injection was found to be superior in various PK/PD parameters as compared to conventional oil based injection, which resulted in rapid and sustained rise in serum 25(OH)D levels.

Keywords: Cholecalciferol, VDD, Aqueous formulation, PKs, PDs

INTRODUCTION

Vitamin D is an endogenous lipophilic vitamin, 90% of which is synthesized below the skin under sunlight exposure.[1] In India, VDD is observed in population of all segments. Serum level of 25(OH)D <30 ng/ml is considered as insufficiency and <20 ng/ml is considered as deficiency.[2] Presently in India, the prevalence of VDD in community is observed to be ranging from 50-94%.[1]

Vitamin D3 (cholecalciferol), synthesized under the skin or supplied via diet, is an inactive form which gets activated by hydroxylation subsequently in liver and kidney to 25(OH)D [calcifediol] and 1,25(OH)2D [calcitriol].[3] 25(OH)D is normally measured in serum for the assessment of vitamin D.[4]

Vitamin D contributes to a variety of physiological functions in the body. It plays a pivotal role in calcium homeostasis, innate and adaptive immunity, functioning of muscles, PTH and insulin. It also contributes to the endothelial functions and renin angiotensin system.[5]

VDD increases the susceptibility to a variety of viral infections including COVID-19.[6-8] VDD is associated with worse outcomes, greater illness, morbidity and mortality in medical/surgical ICUs. Vitamin D has immunomodulatory, anti-inflammatory, anti-infective properties which is beneficial for the patient of pneumonia, acute respiratory failure, COVID-19 and lung transplantation. Vitamin D also works through nuclear factor kappa β and mitogen activated protein kinase inhibition to exert the anti-inflammatory action, which has its role in the management of sepsis.[9] Also in sepsis, it works by modulating immune response and suppression of hyper-inflammatory response.[10,11]

In critical illness, disrupted metabolism, fluid imbalance, decreased binding protein production, increased vascular permeability results in extravasation and renal wasting of vitamin D causing its rapid fall. Thus, normalization of vitamin D level is important in critically ill patients.[12] High level of 25(OH)D is shown to be associated with less organ dysfunction, shorter hospital length of stay and decreased inflammatory parameters in critically ill patients.[13] Use of calcifediol in critical patients results in reduction of ICU admission and mortality.[14-16] Oral high dose vitamin D given to critical patients of COVID-19 for this purpose, has shown limited benefits.[17,18] Only half of the patients treated with cholecalciferol achieve serum 25(OH)D level >30 ng/ml, which might be the reason for limited benefits.[17]

Compared to oral, intramuscular application of cholecalciferol results in more rise in 25(OH)D levels.[19-21] However, these oil based injections have to be injected deep intramuscularly which are painful.[22] Also, it results in slow release of drug[20] resulting in slower correction of vitamin D over 15 days.[22]

A new aqueous, oil/alcohol free formulation of cholecalciferol is developed by Cadila pharmaceutical limited to overcome the limitations of traditional oil based formulation. This is the first of its kind formulation in the world. The objective of the present study was to evaluate the PK/PD of the aqueous cholecalciferol injection 600K IU/2 mL in comparison with conventional oil-based injection in the vitamin D deficient, but otherwise healthy, adult human subjects under the fed condition.

METHODS

The present study was a randomized, open-label, two-treatment, single dose, parallel, comparative, PK/PD study. It was conducted at the department of clinical pharmacology, R and D centre, Cadila pharmaceuticals Ltd, Dholka, Gujarat during January 2022 to October 2022.

The study was approved by the institutional ethics committee (IBIOME-IEC, protocol no. 21-006, 28/09/21). The study was registered on the clinical trial registry of India (CTRI) with registration number CTRI/2021/10/037407. 24 eligible vitamin D deficient but otherwise healthy adult male volunteers, who fulfilled the inclusion/exclusion criteria, were enrolled in the study. Volunteers of 18-65 years of age and BMI with in normal limits (18.5-30 kg/m^2), willing to provide consent, otherwise healthy but vitamin D level <30 ng/ml and not taking any vitamin D supplements were included in the study. Normal ECG findings and vital signs as well as normal routine blood reports were also mandatory for the inclusion. Exclusion criteria were known hypersensitivity to cholecalciferol, non-willingness to provide consent, any known medical disorder, abnormal cardiovascular parameters at the day of recruitment, HIV or hepatitis B/C positive, abnormal blood parameters, recent consumption of alcohol or substances known to interfere with the drug or pregnancy.

The enrolled subjects were kept on fast (overnight) for at least 10 hours prior to dosing. Subjects received a single dose of either Test (T) [Aqueous cholecalciferol injection 600K IU/2 mL of Cadila pharmaceuticals limited, India] or reference (R) [Arachitol (Cholecalciferol) 600K IU oil-based injection, Abbott India limited] formulations intramuscularly into the deltoid muscle of the arm slowly at scheduled dosing time (over one-two minutes) in sitting position at ambient room-temperature after breakfast.

Methods of assigning subjects to treatment groups

The order of receiving the test (T) and reference (R) products for each subject during each period of the study was determined according to the randomization schedule, utilizing a two-treatment, single period, parallel design study. The randomization was generated using SAS® software (SAS Institute Inc., CARY, USA) version 9.4 prior to initiation of the study.

Prior and concomitant therapy

Subjects who were enrolled into the study were asked not to take any prescribed medications beginning two weeks prior and OTC medications beginning one week prior to first dosing of study at the time of screening. Subjects were also instructed to avoid all active vitamin D compounds and vitamin D-supplemented food during the conduct of the study.

Efficacy and safety measurements assessed

PD measures were considered as number and proportion of subjects achieving >30 ng/mL concentration of active metabolite [25(OH)D] with test (T) and reference (R) product. PK measurements were C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and T_{max} calculated for cholecalciferol and its metabolites as descriptive statistical analysis. Adverse events were monitored throughout the study by the clinical research physician clinical laboratory assessment was performed at the time of screening (haematology, biochemistry, serology and urinalysis) and at end of study (haematology and biochemistry) for all subjects as per the protocol.

Sample collection and processing

A total of fourteen (14) blood samples (6.0 mL each) were collected during the trial per subject. Pre-dose {-24.00, -18.00, -12.00 and 00.00 hour (within 05 minutes prior to dosing)} as well as post-dose (01.00, 02.00, 03.00, 06.00, 09.00, 12.00, 24.00 hours, day 3, 7 and 14 post-dose) blood samples were collected at the predefined time points.

After collection of blood samples, study-personnel centrifuge the samples at 3000 rpm for 10 minutes at 4ºC as soon as possible but not more than 60 minutes of the actual-time of sample collection. After centrifugation the obtained serum samples were divided into two aliquots and stored in two different pre-labelled (label mentioning study no., subject no., period no., sampling time-point) amber color RIA vials (Aliquot 01 of 02 and Aliquot 02 of 02). The labelled amber color RIA vials were than stored at –20±5°C or colder till withdrawn for analysis. After the completion of the clinical phase of the study the serum samples were transferred to the in-house Bioanalytical laboratory for bio-analysis by maintaining the temperature and integrity of the samples.

Statistical analysis

Statistical analysis was performed on PK parameters using statistical analysis software® ver 9.4. mean serum-concentrations at different time points estimated in both arms for cholecalciferol and its metabolite. Descriptive of PK parameters (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) and (T_{max}) were estimated. Data are presented as mean ± SD.

RESULTS

Demography results

The present study was conducted in 24 VDD, but otherwise healthy, adult, male human subjects under fed condition. These subjects were randomized as per the randomization schedule into the study to receive either test product (n=12) or reference product (n=12). The demographic characteristics were comparable with no significant difference between both the study arms for

age, weight, height and body mass index (BMI). None of the subject was discontinued from the study (Table 1).

Table 1: Baseline characteristics of the volunteers with test and reference vitamin D3 injection.

Vriables	Treatment groups		P value
	Test arm, (n=12)	Reference arm, (n=12)	
Gender, n (%)			
Male	12 (100)	12 (100)	-
Female	0	0	
Age (In years)			
Mean (SD)	41 (13.60)	39.42 (13.06)	
Median	36.00	41.50	0.7739
Range	22-64	21-62	
Weight (kg)			
Mean (SD)	61.75 (10.44)	61.15 (7.63)	
Median	57.15	59.30	0.8755
Range	50.20-86.60	51.20-77.30	
Height (cm)			
Mean (SD)	166.54 (7.48)	166.58 (7.27)	
Median	169	165.50	0.9891
Range	151-176	156-181	
BMI (kg/m^2)			
Mean (SD)	22.21 (2.93)	22.02 (2.07)	
Median	21.91	22.32	0.8581
Range	18.85-28.60	18.92-24.65	

Cholecalciferol

PK evaluation

The comparative baseline corrected concentration profile of cholecalciferol for the test (n=12) and reference (n=12) product for each time-point post-dosing was presented in Table 2 by summarizing concentration as arithmetic mean, standard deviation (SD) corresponding p value and ratio of concentration between two arms.
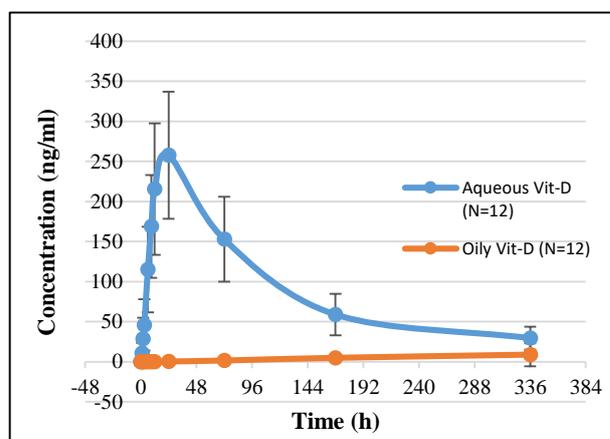

Figure 1: Cholecalciferol: concentration versus time graph.

It is evident from the concentration data that, for all time points from 1 hr onwards post-administration, significantly higher concentration of cholecalciferol was observed with test product compared to reference product ($p<0.01$) till end of the study.

Additionally, calculated PK parameters for up to 14 days after administration was compared between test and reference product. The comparative data was summarised in Table 3.

Mean calculated C_{max} for test product was 258.767 ng/mL which was achieved at mean T_{max} of 23 hr. Compared to that, reference product achieved mean C_{max} of 8.678 at mean T_{max} of 345.10 hr. A statically significant difference between test and reference product was observed for the calculated C_{max} as well as for T_{max} for cholecalciferol ($p<0.0001$). Similarly, statistically highly significant difference ($p<0.0001$) was observed for calculated mean AUC_{0-t} for test product (31774.516 ng.hr/ml) compared to reference product (1479.178 ng.hr/ml). C_{max} for test arm (258.77 ng/ml) is 29 fold higher than the reference product. The corresponding T_{max} was achieved significantly earlier (by 23 hr) with test arm compared to the reference arm (by 345 hr) ($p<0.0001$). AUC_{0-t} of test arm (31775.54) is 21 fold higher than the reference arm (1597.80).

Calcifediol (25(OH)D; active metabolite)

The comparative base-line corrected concentration profile of 25 (OH)D for the test (n=12) and reference (n=12) product for each time-point post-dosing is presented in Table 4 by summarizing concentration as arithmetic mean, standard deviation (SD) corresponding p value and ratio of concentration between two arms.

Table 2: Cholecalciferol: comparative concentration (ng/mL) profile of test and reference product.

Time point (hr)	Aqueous vit D (n=12)	Oily vit D (n=12)	P value	Ratio
0	00.00±0.00	0.00±0.00	0	NE
1	10.456±13.32	0.000±0.00	0.02	NE
2	28.391±26.53	0.000±0.00	0.0035	NE
3	45.808±32.15	0.000±0.00	0.0004	NE
6	115.042±53.32	0.000±0.00	<0.0001	NE
9	168.877±64.13	0.000±0.00	<0.0001	NE
12	215.546±82.03	0.000±0.00	<0.0001	NE
24	257.482±79.26	0.205±0.71	<0.0001	1257.03
72	152.799±52.94	1.392±1.84	<0.0001	109.76
168	58.857±25.91	4.763±3.71	<0.0001	12.36
336	29.371±14.46	8.678±4.67	0.0004	3.38

Note: Data presented as calculated mean±SD for baseline corrected concentrations. NE=Non-evaluable.

Table 3: Cholecalciferol: comparative calculated PK parameters of test and reference product (untransformed).

Variables	Aqueous vit D (n=12)	Oily vit D (n=12)	P value	Ratio
C_{max} (ng/ml)	258.767±78.09	8.678±4.67	<0.0001	29.82
AUC_{0-t} (ng*hr/ml)	31774.51±10844.11	1479.17±981.10	<0.0001	21.48
$AUC_{0-\infty}$ (ng*hr/ml)	36779.785±13647.95	NE	-	NE
T_{max} (hr)	23.000±3.46	345.128±28.29	<0.0001	0.07

Note: Data presented as calculated mean ± SD. NE=Non-evaluable.

Table 4: 25 (OH) D: Comparative concentration (ng/mL) profile of test and reference product.

| Time point (hr)	Aqueous vit D, (n=12)	Oily vit D, (n=12)	P value	Ratio
0 hr	0.00±0.00	0.00±0.00	NA	-
1 hr	0.218±0.32	0.106±0.19	0.315	2.06
2 hr	0.764±0.5	0.14±0.25	0.0013	5.46
3 hr	1.418±0.69	0.108±0.17	<0.0001	13.13
6 hr	3.176±1.18	0.027±0.07	<0.0001	117.63
9 hr	5.461±1.51	0.05±0.08	<0.0001	109.22
12 hr	8.51±2.52	0.203±0.45	<0.0001	41.92
24 hr	19.116±5.27	0.548±0.44	<0.0001	34.88
72 hr	50.565±14.55	3.099±1.08	<0.0001	16.32
168 hr	60.157±18.25	6.729±1.84	<0.0001	8.94
336 hr	62.112±16.74	16.162±4.24	<0.0001	3.84
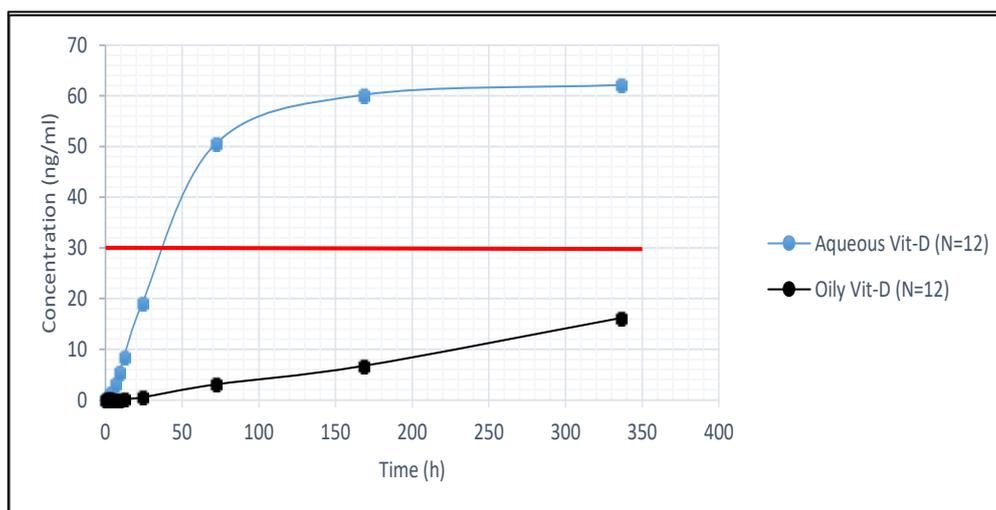
Figure 2: 25 (OH) D: concentration versus time graph.

At 1 hr and 2 hr, the serum 25 (OH) D was 2 fold and 5 fold higher in test arm (0.218 and 0.764 ng/mL, respectively) compared to reference arm (0.106 and 0.139 ng/mL, respectively), respectively.

At 3 hr, 13 fold higher serum 25 (OH) D was observed in test arm (1.418 ng/ml) compared to reference arm (0.108 ng/ml) (p<0.0001). This was increased at 6 hr by 117 fold higher serum 25 (OH) in test arm (3.176 ng/ml) compared to reference arm (0.027 ng/ml) (p<0.0001).

This trend was continued at 9 hr by 109 fold higher serum 25(OH)D in test arm (5.461 ng/ml) compared to reference arm (0.050 ng/ml) (p<0.0001). At 12 hr and 24 hr, the serum 25(OH)D was 41 fold and 34 fold higher in test arm (8.510 and 19.116 ng/ml, respectively) compared to reference arm (0.203 and 0.548 ng/ml, respectively), respectively.

The serum 25(OH)D at 72 hr (day 3) in test arm (50.565 ng/ml) was 16 fold higher compared to reference arm (3.099 ng/ml) (p<0.0001). At 168 hrs (day 7), the serum 25(OH)D in test arm (60.157 ng/ml) was 8 fold higher compared to reference arm (6.729 ng/ml) (p<0.0001).

By 336 hr (day 14), the serum 25(OH)D was 3 fold higher in test arm (62.112 ng/ml) compared to reference arm (16.162 ng/ml) (p=0.0004). It is evident from this comparison of concentration data that from 2 hr onwards post-administration, a significantly higher concentration of 25(OH)D was observed with test product compared to reference product (p<0.01) till end of the study.

Additionally, calculated PK parameters for upto 14 days after administration was compared between test and reference product. The comparative data was summarised in Table 5.

Table 5: 25(OH)D: Comparative calculated PK parameters of test and reference product (untransformed).

Parameters	Aqueous vit D, (n=12)	Oily vit D, (n=12)	P value	Ratio
C_{max} (ng/ml)	62.965±17.51	16.162±4.23	<0.0001	3.90
AUC_{0-t} (ng*hr/ml)	17495.912±4979.10	2572.716±609.88	<0.0001	6.80
T_{max} (hr)	295.031±75.73	345.128±28.29	0.0498	0.85

Note: Data presented as calculated mean ± SD. NE = Non-evaluable.

For the defined study duration of 14 days post-administration, mean calculated C_{max} for test product was 62.965 ng/mL achieved at mean calculated T_{max} of 295.031 hr compared to that reference product achieved mean C_{max} of 16.162 hr at mean T_{max} of 345.128 hr. A statically significant difference between test and reference product was observed for the calculated C_{max} 25 (OH) D (p<0.0001). Similarly, statistically significant difference (p<0.0001) was observed for calculated mean AUC_{0-t} for test product (17495.912 ng.hr/ml) compared to reference product (2572.716 ng.hr/ml). While $AUC_{0-\infty}$ was not evaluable for both the study arms.

The calculated C_{max} for the present study duration for test arm (62.97 ng/ml) is 4 fold higher than the reference product (16.16 ng/ml). The corresponding T_{max} was earlier with test arm (by 295.03 hr) compared to the reference arm (by 345.13 hr) (p=0.0498). Similarly,

AUC_{0-t} of test arm (17495.98 ng/ml) is 6 fold higher than the reference arm (2572.75 ng/ml).

PD analysis

Serum concentration for 25(OH)D of more than 30 ng/ml is considered as normal level for the study. The number

and percentage of subjects achieving this normal level of 25 (OH) D in both treatment arms during the study was presented in Figure 3.

At 6 hr post-administration, 1 (8.3%) subject in test arm achieved the normal level of 25 (OH) D and none of the subject in reference arm achieved this level (p=0.3070). Further, at 9 hr post dose, 2 (16.7%) subjects in test arm opposed to none in reference arm achieved the normal

level of active metabolite. (p=0.1369) Similarly, 3 (25.0%) and 7 (58.3%) subjects achieved normal level of active metabolite at 12 hr and 24 hr, respectively. While none of the subjects in reference arm reported normal level at the same time points (p=0.0641). All 12 (100%) subject in test arm achieved the normal level of 25 (OH) D by 72 hr post-administration as compared to none in reference arm (p=0.0017).
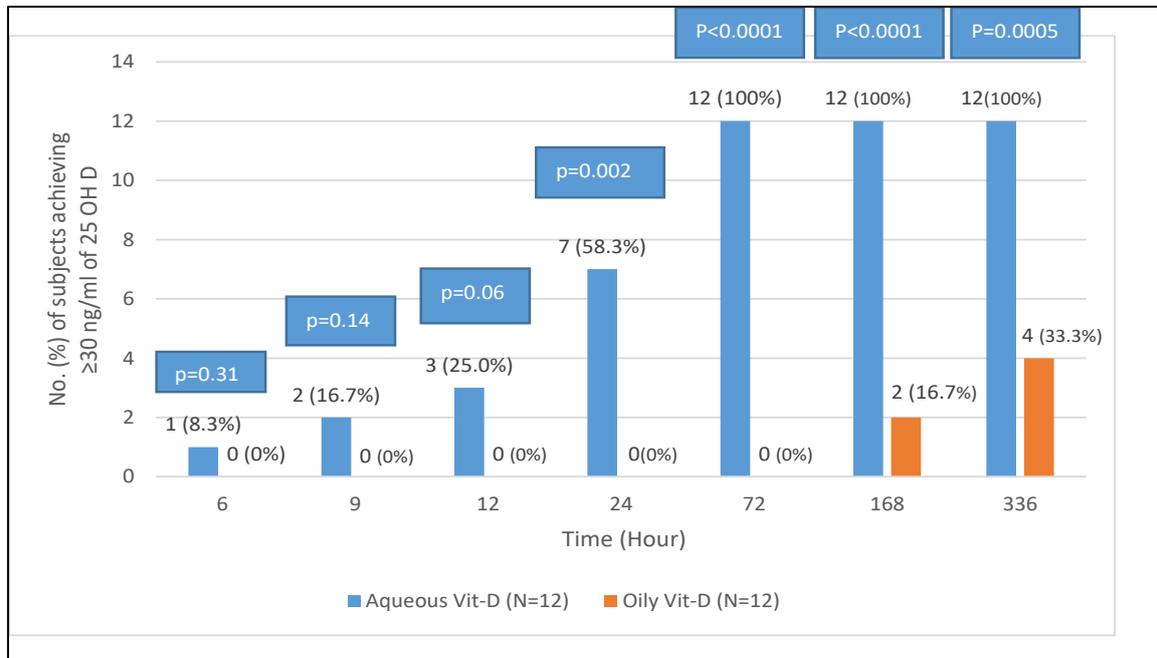

Figure 3: Number (%) of subjects achieving normal 25(OH) D (>30 ng/ml).

By the end of the study, all 12 (100%) subjects in test arm achieved more than 30 ng/mL level of 25 (OH) D, while only 4 (33%) subjects in the reference arm achieved the same (p=0.0005).

Safety

There were no serious adverse events (SAEs) or deaths reported during the study. Two mild AEs (1 incidence each of dyspepsia and elevate triglyceride) were observed during the study by 2 subjects. These includes 1 incidence each of dyspepsia and elevate triglyceride, which were resolved and not related to the study treatment. None of the subjects was discontinued from the study due to AE. All the values of pre-study and post-study laboratory measurements for each individual subject were found to be either normal or clinically not significant as per clinical research physician.

DISCUSSION

Deficiency of vitamin D is prevalent in common population and for the correction of the same several treatment approaches with oral as well as injectable preparations have been suggested. While oral supplementation is sufficient in many cases, for rapid

correction of VDD injectable formulation is preferred. Particularly in cases of acute as well as critical illnesses, rapid correction of vitamin D levels has shown promising results.

High levels of serum calcifediol (25(OH)D) has shown to be beneficial in critical illness in terms of decreased organ dysfunction, reduced hospital length of stay, reduction in ICU admission as well as mortality. It has been shown to reduce inflammatory parameters also.[16-18] Presently available parenteral formulation of cholecalciferol does not result in a quick rise of serum 25(OH)D.[20,22] Oil based intramuscular injection of the drugs does not always assure complete or rapid bioavailability.[23] This might interfere with the beneficial outcomes of the vitamin D. The data of the present study showed that single application of the new formulation of cholecalciferol results in immediate rise of 25(OH)D in the serum. This rise was 2 fold higher at 1 hr, 5 fold higher at 2 hrs and 13 fold higher at 3 hrs compared to oil based preparation. Subsequently serum 25 (OH) D in test arm was significantly higher by 117 fold at 6 hrs, 109 fold at 9 hrs and 41 fold at 12 hr compared to oil based preparation (p<0.0001). Also, this maximum rise in 25(OH)D was achieved faster with the new formulation.

The rapid and more pronounced response obtained with the new aqueous formulation can be attributed to the aqueous base of the formulation. It is a known fact that absorption of an aqueous formulation after intramuscular administration is rapid and the absorption process can reasonably be described as a pseudo-first order process.[24] Oil based formulations remain protracted *in-situ* resulting in a slow release and resultant slow rise in the 25(OH)D levels which was also evident in the present study in the reference arm results.[23]

Increase of serum 25(OH)D above 30-40 ng/mL from the third day of treatment in COVID-19 patients has shown to provide significant mortality benefit.[14] In the present study it was shown that mark of 30 ng/ml of 25(OH)D was achieved in the aqueous formulation within 48 hours of injection. It is noteworthy that the same level was never reached with the oil based preparation during the 14 days of the study period. At 72 hours, the level of 25(OH)D in 100% of the population receiving aqueous formulation was above 30 ng/ml. Whereas in the oil-based formulation none of the subjects achieved 25(OH)D levels of at least 30 ng/ml. This observation of the oil based preparation was well in agreement with the results shown by previous workers.[22,23]

Studies using oral calcifediol have shown that rapidly achieving rise in serum 25(OH)D has a significant role in the management of critical illness.[14] Looking in to unavailability of calcifediol in India, this new aqueous formulation would provide a promising option in the critical care as well as respiratory segment.

Some authors have pointed out association of vitamin D injections and resultant hypervitaminosis D.[25,26] However, it was observed that the hypervitaminosis D incidences were purely because of the inadvertent usage of vitamin D. In some case of vitamin D toxicity, high doses (600k IU) were administered at frequent intervals (daily to weekly).[25] The mean cumulative doses used in one of the case series was 3,967,500 IU over the mean period of 7.4 weeks.[25] Such incidences were not observed in any of the subjects in the present study. Also, oil based injectable formulations are known to be painful for the patient and resultantly it might interfere with the patient compliance.[22] Aqueous formulation is painless on injection which shall help in increasing the patient compliance.

The study had some limitation. Considering the nature of this study being a PKPD study, the sample size taken was limited. Outcomes of the present study were quite promising, going forwards it will be interesting to see the results in larger population in the real-world settings. Also, the study duration was 14 days. During the study period, the maximum rise in 25(OH)D achieved in the comparator arm was below 30 ng/mL. This is attributable to the inherent properties of oil-based injections in terms of slow diffusion from the deposition site. Had the duration of study was longer, it might have crossed the sufficiency level mark.

This was a PKPD study, which evaluated various respective parameter of the new formulation and compared the same with the conventional formulation. Considering the promising results of the study and its smaller sample size, it will be interesting to see the results in a study with large sample size in the real-world settings.

CONCLUSION

This, first in world, aqueous formulation of cholecalciferol injection is found to be superior in various PKs and PDs parameters as compared to conventional oil-based injection. It results in rapid and sustained rise in serum 25(OH)D levels. This new formulation would be of appropriate use in the management of acute and critical illnesses and has promising role in improving clinical outcomes.

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