Does Vitamin D3 Have an Impact on Clinical and Biochemical Parameters Related to Third Molar Surgery

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Abstract: The purpose of this study was to evaluate the clinical effect on the biochemical inflammatory markers of a single oral high dose of cholecalciferol in vitamin D-deficient patients undergoing the surgical removal of lower third molars.

A randomized, split-mouth, single-blind study was conducted on 25 vitamin D-deficient patients ranging between 18 and 40 years of age requiring lower third molars extraction and referred at the Oral Surgery Unit of the School of Dentistry of the University of Messina.

All patients, with vitamin D3 blood levels $\leqq 30$ ng/mL, underwent bilateral surgical removal. The first extraction (control group) being conducted with the administration of a placebo, the second one (test group) being conducted with the preliminary administration of 300,000 IU of cholecalciferol 4 days before the procedure.

At each surgery, clinical indexes, such as pain, edema and any functional limitation have been recorded. Clinical and biochemical parameters were registered 4 days before, immediately after, 3 and 7 days after the surgical procedure. The data obtained were processed using paired *t*-test. The clinical outcome parameters showed a slight to moderate improvement between the control and the vitamin-D treatment group, with statistical significance being obtained regarding the edema at defined time points. Interleukin-1-beta, interleukin-6, and tumor necrosis factor-alpha values were significantly lower ($P < 0.01$) for the test group after the surgery. The increase of vitamin D serum levels showed an impact on the outcome of the third molar surgery, eliciting a reduced inflammatory response and leading to a more favorable clinical course.

Key Words: Edema, third molar surgery, vitamin D3

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Vitamin D3 is a well-known prohormone and an essential nutrient[1,2] strictly related to the bone metabolism; it is acquired by the diet intake or synthesized in skin through ultraviolet irradiation of 7-dehydrocholesterol. This form is biologically inert and must be metabolized to 25-hydroxyvitamin D3 in the liver and then to 1-alpha, 25-dihydroxyvitamin D3 in the kidney before becoming active.[3,4]

The hormonal form of vitamin D3 provides many functions: it affects the intestinal absorption of calcium and phosphate, the calcium mobilization in bone through the stimulation of RANK-L, the calcium resorption in the kidney. Moreover, it is involved in immune system regulation, dampening the T-cell immunity and inflammatory response[5]; in fact, different studies discussed how vitamin D actually takes part in the immune system cells metabolism as a modulator, reducing the prostaglandinis secretion and enhancing the immune competence.[6–9] Additional roles of vitamin D has been investigated in several fields of medicine and dentistry, showing beneficial effects in clinical outcomes, such as pain control in dysmenorrea and muscoskeletal pain.[10]

In vitamin D status determination, only the metabolite 25(OH)D, as the major circulating form of vitamin D, is hugely recommended.[11] Recent data reported the potency (and the safety) of a single, large, oral 300,000 IU dose of cholecalciferol, in enhancing serum 25(OH)D concentration with concomitant decrease in parathyroid hormone secretion.[12]

Purpose of this study was to investigate the effects of vitamin D serum concentration on lower third molar surgery, usually followed by swelling, pain and limited masticatory function.[13–22] When impacted third molars are removed, the postop is characterized by difficulties on the mouth opening, pain, reduced masticatory capability, and swelling of variable degree. The clinical outcome and the influence on the biochemical inflammatory markers of a single-loading oral dose of cholecalciferol in vitamin D-deficient patients undergoing surgical removal of impacted lower third molars were analyzed.

MATERIALS AND METHODS

During an 8 months period (October 2013–May 2014), a randomized, split-mouth, and single-blind study was conducted at the University of Messina.

A group of 72 patients, aged between 18 and 40 years old, without smoking habit and in apparent good general health conditions, referred at the Unit of Oral Surgery, were examined because requiring impacted lower third molars extractions. All impacted teeth were in Pell & Gregory class II B and II C. The indications of the extractions were pericoronal tissue infections, decayed teeth, and orthodontic reasons.

All were referred to the unit of bone metabolic disease, to determine their vitamin D status.

TABLE 1. Vitamin D Status of Each Patient Recorded in the Study

Case Number°	Initials	Age	Sex	25(OH)D Serum Levels (ng/mL)
1	AE	21	♀	29.2
2	CA	22	♀	21.6
3	CA	32	♀	20.8
4	CC	25	♂	22.5
5	CF	25	♂	23.6
6	CG	22	♀	14
7	CG	38	♀	27.3
8	DPD	27	♂	21.2
9	FE	18	♀	20.9
10	GD	26	♀	14.1
11	GT	42	♀	20.4
12	HE	35	♀	15.4
13	ID	21	♀	17.1
14	LTV	21	♀	25.4
15	MD	19	♂	15.7
16	MD	18	♂	22
17	MF	27	♂	12.3
18	MF	26	♀	27.5
19	MR	26	♀	12.7
20	NN	26	♀	21.1
21	RMG	40	♂	25
22	SF	29	♀	22.9
23	SF	27	♂	27.5
24	SV	21	♀	32.5
25	SV	22	♂	13
	Mean	26.24		21.028

Among them, 25 patients, showing vitamin D3 blood levels ≦30 ng/mL prior the surgical procedures were recruited in a treatment protocol including:

- Two surgical lower third molar extractions;
- Single dose treatment of hypovitaminosis D, accordingly a therapeutic scheme adopted at our bone metabolic diseases center, before the second surgical procedure (Table 1).

Patients were informed that their data would be used for statistical analysis and gave their informed consent to the treatment. No ethical committee approval was sought to start this study, as it was not required by national law or by ordinance of the local inspective authority. The prospective study was performed in accordance with the principles stated in the Declaration of Helsinki and the Good Clinical Practice Guidelines, informed consent was obtained from all participants. Patients underwent bilateral surgical impacted teeth removal in a recorded sequence and the first impacted tooth being chosen randomly:

- The first step, control, being conducted with the preliminary 4 days before administration of a placebo (a biscuit wet with saline solution); the second step, performed after a 6-month time interval, test, being conducted with the preliminary 4 days before administration of a biscuit wet with a single dose solution of cholecalciferol 300,000 IU (Dibase® 300.000, Abiogen Pharma s.p.a., Pisa, Italy).

Each step was conducted accordingly to the following scheme:

- T0 clinical parameters assessment (cheek edema, mouth opening), blood samples collection for biochemical analyses, administration of placebo/vitamin D;
- T1 (4 days after T0) surgical procedure; blood samples collection for biochemical analyses;
- T2 (7 days after T0), clinical parameters assessment (cheek edema, mouth opening), blood samples collection for biochemical analyses (at the time of the expected maximum 25(OH)D serum concentration);
- T3 (10 days after T0) suture removal, clinical parameters assessment (cheek edema, mouth opening).

A single oral surgeon performed all the surgeries. Infiltration alveolar nerve block was performed before surgery.

A full-thickness 4-cornered mucoperiostal flap was reflected. After the flap elevation, bone removal with a drill and cold sterile saline irrigation was performed when needed.

Once sufficient access was obtained, the tooth sectioning was executed with a drill and the fragments removed by the use of levers. The socket was then curetted and irrigated with cold sterile saline (Fig. 1A).

The wound was then closed with a simple interrupted stitch with a 5.0 size Nylon suture (Ethilon®, Ethicon Inc., Somerville, NJ) removed 7 days later.

Clinical Parameters Assessment

Clinical parameters were collected during the trial, as listed below.

Pain Intensity

Assessed by the patient using a visual analog scale (VAS)-based scoring as follows: no pain (VAS 0), score = 0; low pain (VAS 1–3), score = 1; medium pain (VAS 4–7), score = 2; intense pain (VAS 8–10), score = 3.

Schedule: every 12 hours for 7 days after the surgical procedure.

Cheek Edema

It is determined by measuring the tragus–labial commissure and tragus–pogonion distances with a tape measure (mm scale).

Schedule: T0, T2, T3 (Fig. 1B-C).

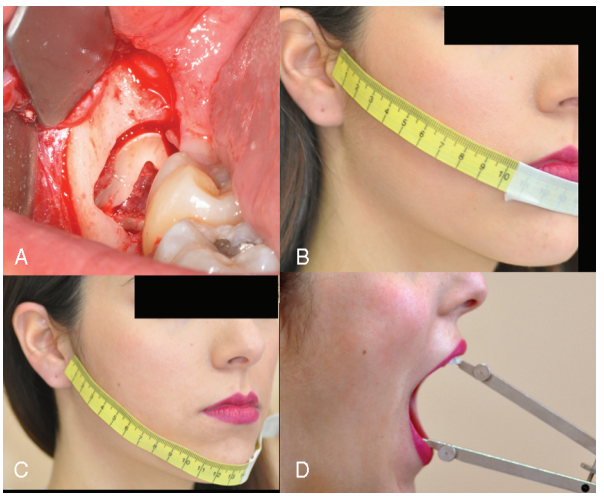

FIGURE 1. A: Sample of the surgery of an inferior impacted third molar. A full thickness flap and the following osteotomy seem to be related with the patients post op discomfort. B, The measurement from the anterior ear to the labials angle for the evaluation of the post op edema. C, The measurement from the anterior ear to the centre of the chin is another parameter for the edema postop evaluation. D, Open mouth evaluation or contraction is another element for evaluation mouth function after the surgery.

Trismus and Maximum Mouth Opening

Determined by measuring the maximum interincisal distance with a caliper (mm scale);

Schedule: T0, T2, T3 (Fig. 1D).

Biochemical Parameters Assessment

After collection, the blood samples were processed by the Central Laboratory of Clinical Pathology at the School of Medicine, University of Messina for determining the following values:

Vitamin D levels: estimated by measuring the 25(OH)D serum level by the use of high-performance liquid chromatography, and expressed as ng/mL.

Schedule: T0, T2.

Inflammatory indexes: time of ertrihocyte sedimentation, C-reactive protein, Alpha-1-glycoprotein, interleukin (IL)-1-beta, IL-6, tumor necrosis factor (TNF)-alpha.

Schedule: T0, T1, T2.

Statistical Analysis

Patients' clinical and biochemical measures, according to times from surgery and vitamin D load were reported as mean ± SD, and differences were assessed via percentage variation. Comparisons between groups at baseline and between baseline and follow-up values at each time point were performed by paired *t*-test. Significance was set at a *P* value of 0.05. MedCalc v15 (MedCalc Software bvba, Ostend, Belgium) was used for statistical calculations.

RESULTS

Vitamin D levels of all participants compared at T0 and T2 points of control and test steps are reported in Table 2. Basal values of

TABLE 2. Control and Test Group Presence of Vitamin D

	25(OH)D Serum Levels (ng/mL) After Placebo Administration		25(OH)D Serum Levels (ng/mL) After Cholecalciferol Administration	
Case N°	T0	T2	T0	T2
1	29.2	26.8	21.0	80.2
2	21.6	19.4	19.0	117.3
3	20.8	18.7	21.0	98.0
4	22.5	22.3	25.6	74.3
5	23.6	16.5	14.3	96.0
6	14.0	15.4	14.5	86.8
7	27.3	21.9	22.5	64.1
8	21.2	19.7	27.4	76.8
9	20.9	18.1	24.1	104.5
10	14.1	12.6	18.0	61.4
11	20.4	19.1	25.5	81.0
12	15.4	15.2	16.0	104.7
13	17.1	21.4	22.7	127.0
14	25.4	20.9	29.2	80.2
15	15.7	21.2	25.1	47.9
16	22.0	24.0	12.8	78.8
17	12.3	14.5	15.2	72.3
18	27.5	33.1	29.8	80.0
19	12.7	20.3	20.0	96.2
20	21.1	30.8	21.5	64.7
21	25.0	19.9	32.5	99.0
22	22.9	26.4	31.8	110.2
23	27.5	25.1	55.3	131.6
24	32.5	29.9	58.5	85.9
25	13.0	15.1	29.0	65.0
Mean	21.0	21.1	25.3	87.4

TABLE 3. Clinical Parameters Recorded for Each Patient

	Clinical Parameters: Control Group								
	T0			T2			T3		
	T-P	T-L	MO	T-P	T-L	MO	T-P	T-L	MO
1	13.2	10.4	4.8	14	11	3.7	13.8	10.9	4
2	13.4	10.5	5	14.6	11	2.5	14	10.4	3.3
3	15	11.5	3.5	14.7	11.6	3.3	14.4	11.4	4
4	15	11.5	5.3	15.9	11.8	4.5	15.7	11.5	5
5	13	10.5	5.2	13.3	10.5	3	13.1	10.5	3.5
6	14.5	11	4.6	15.1	11.4	3	14.8	11.2	4
7	13.7	10.5	6	15.5	11.3	2.9	14.5	11.2	4.8
8	14.3	11.1	4.5	15	11.7	2.7	14.7	11.3	3.7
9	13.2	10.2	5	14	11	3.6	13.5	10.4	4.8
10	13.2	10.8	4.5	14.4	11.1	1.5	13.7	11	3.2
11	13	10.4	5	14	11	3.9	13.4	10.7	4
12	15	11.7	6	16	13.3	4	15.6	12.5	5
13	12.5	11	4.5	12.8	11.1	3	12.6	11	4
14	14	11.5	4.5	15	11.9	4	14.6	11.7	4.4
15	15.3	11.7	5.7	16	12	3.5	16.5	13	4
16	14.5	11	5	15.5	11.5	4.4	14.9	11.3	5
17	14.1	10.9	4.7	15	11.7	2.5	14.7	11.6	3.5
18	15.2	12.2	4.3	15.3	12.2	2.5	15.2	12.2	2.8
19	13	10.2	5.4	13	11	4.5	13	10.5	5
20	13.9	11.5	4.4	14.9	11.7	4	14.5	11.6	4.5
21	15	11.5	5.5	15.8	12	2.5	15.9	12	3
22	13.8	11	5	15	11.4	4	15	11.2	4.5
23	14.1	11	4.9	15	11.9	3	15	11.5	3.5
24	15.5	13	4.6	16.3	13	2.5	15.7	13.3	3
25	13	11	5	13.5	11.5	4	13.2	11.1	4.8
Mean	14.02	11.10	4.92	14.78	11.58	3.32	14.48	11.40	4.05

	Clinical Parameters: Test Group								
	T0			T2			T3		
	T-P	T-L	MO	T-P	T-L	MO	T-P	T-L	MO
1	13.3	10.6	4.8	14.3	11	3.7	13.8	10.7	4.2
2	13.5	10.5	5	14.5	10.8	2.8	13.8	10.6	3.5
3	14.8	11.3	3.5	15	11.7	2.9	15	11.8	3.5
4	15	11.5	5.3	16	12	4.2	15.8	11.7	4.7
5	13.1	10.6	5.4	13.5	10.5	2.5	13.3	10.5	2.9
6	15	11.2	4.5	14.8	11.7	2.7	14.5	11.4	3.5
7	13.8	10.5	6	15.4	11.2	3	14.3	11	5
8	14	11.1	4.5	14.7	12	2.4	14.2	11.3	3.4
9	13.2	10.1	5	13.8	10.7	4	13.3	10.2	5
10	13.2	10.5	4.5	14	11	3	13.3	10.8	4
11	13.2	10.6	5	13	10.4	3.1	13	10.4	4.5
12	14.5	11.7	6	15.4	11.9	4.6	15	11.9	5
13	13	11	4.5	13	11.3	2.5	12.8	11	3
14	14	11.5	4.7	14.8	11.7	4.5	14,4	11.5	4.7
15	15.2	11.7	5.7	16	12.5	4.5	15.7	12.2	5
16	14.7	11.1	5	15.8	11.7	4.2	15	11.3	4.9
17	14.1	11	4.7	14	11	4	14	11	4.5
18	15.2	12.2	4.3	15.8	12.5	2.8	15.3	12.2	3.3
19	13.1	10.1	5.4	13.9	11	4	13.5	10.6	4.5
20	13.6	11.6	4.6	14.8	11.6	4.1	14.3	11.5	4.4
21	15.1	11.5	5.5	15.6	11.9	3.7	16	11.9	4.5
22	13.9	11.1	5	15	11.2	4.6	14.7	11.1	4.8
23	14	11	4.9	15	11.7	2.8	14.7	11.4	3.7
24	15.3	12.9	4.3	16.2	13.4	2.7	15.5	13.2	3.1
25	13	10.9	5	13	11	4.4	13	11	5
Mean	14.03	11.11	4.92	14.69	11.50	3.51	14.33	11.29	4.18

	Clinical Parameters Δ%: Control Group					
	T2-T0			T3-T0		
	T-P	T-L	MO	T-P	T-L	MO
1	6.1	5.8	−22.9	4.5	4.8	−16.7
2	9.0	4.8	−50.0	4.5	−1.0	−34.0
3	−2.0	0.9	−5.7	−4.0	−0.9	14.3

TABLE 3. *(Continued)*

	Clinical Parameters Δ%: Control Group					
	T2-T0			T3-T0		
	T-P	T-L	MO	T-P	T-L	MO
4	6.0	2.6	−15.1	4.7	0.0	−5.7
5	2.3	0.0	−42.3	0.8	0.0	−32.7
6	4.1	3.6	−34.8	2.1	1.8	−13.0
7	13.1	7.6	−51.7	5.8	6.7	−20.0
8	4.9	5.4	−40.0	2.8	1.8	−17.8
9	6.1	7.8	−28.0	2.3	2.0	−4.0
10	9.1	2.8	−66.7	3.8	1.9	−28.9
11	7.7	5.8	−22.0	3.1	2.9	−20.0
12	6.7	13.7	−33.3	4.0	6.8	−16.7
13	2.4	0.9	−33.3	0.8	0.0	−11.1
14	7.1	3.5	−11.1	4.3	1.7	−2.2
15	4.6	2.6	−38.6	7.8	11.1	−29.8
16	6.9	4.5	−12.0	2.8	2.7	0.0
17	6.4	7.3	−46.8	4.3	6.4	−25.5
18	0.7	0.0	−41.9	0.0	0.0	−34.9
19	0.0	7.8	−16.7	0.0	2.9	−7.4
20	7.2	1.7	−9.1	4.3	0.9	2.3
21	5.3	4.3	−54.5	6.0	4.3	−45.5
22	8.7	3.6	−20.0	8.7	1.8	−10.0
23	6.4	8.2	−38.8	6.4	4.5	−28.6
24	5.2	0.0	−45.7	1.3	2.3	−34.8
25	3.8	4.5	−20.0	1.5	0.9	−4.0
Mean	5.51	4.39	−32.04	3.30	2.66	−17.07
	Clinical Parameters Δ%: Test Group					
	T2-T0			T3-T0		
	T-P	T-L	MO	T-P	T-L	MO
1	7.5	3.8	−22.9	3.8	0.9	−12.5
2	7.4	2.9	−44.0	2.2	1.0	−30.0
3	1.4	3.5	−17.1	1.4	4.4	0.0
4	6.7	4.3	−20.8	5.3	1.7	−11.3
5	3.1	−0.9	−53.7	1.5	−0.9	−46.3
6	−1.3	4.5	−40.0	−3.3	1.8	−22.2
7	11.6	6.7	−50.0	3.6	4.8	−16.7
8	5.0	8.1	−46.7	1.4	1.8	−24.4
9	4.5	5.9	−20.0	0.8	1.0	0.0
10	6.1	4.8	−33.3	0.8	2.9	−11.1
11	−1.5	−1.9	−38.0	−1.5	−1.9	−10.0
12	6.2	1.7	−23.3	3.4	1.7	−16.7
13	0.0	2.7	−44.4	−1.5	0.0	−33.3
14	5.7	1.7	−4.3	2.9	0.0	0.0
15	5.3	6.8	−21.1	3.3	4.3	−12.3
16	7.5	5.4	−16.0	2.0	1.8	−2.0
17	−0.7	0.0	−14.9	−0.7	0.0	−4.3
18	3.9	2.5	−34.9	0.7	0.0	−23.3
19	6.1	8.9	−25.9	3.1	5.0	−16.7
20	8.8	0.0	−10.9	5.1	−0.9	−4.3
21	3.3	3.5	−32.7	6.0	3.5	−18.2
22	7.9	0.9	−8.0	5.8	0.0	−4.0
23	7.1	6.4	−42.9	5.0	3.6	−24.5
24	5.9	3.9	−37.2	1.3	2.3	−27.9
25	0.0	0.9	−12.0	0.0	0.9	0.0
Mean	4.70	3.48	−28.60	2.09	1.59	−14.88
	Clinical Parameters Δ% Analysis					
	T2-T0			T3-T0		
	T-P	T-L	MO	T-P	T-L	MO
p	0.1151	0.1193	0.0994	0.0105	0.0306	0.1800

Legend
T-P: tragus-pogonion.
T-L: tragus-labial commissure.
MO: mouth opening at incised edges (at max opening).
Measurements are expressed in cm.

25(OH)D did not differ between the whole group of 25 subjects. The administration of vitamin D (test step) was safe in all patients. Every patient recovered from the hypovitaminosis D condition with a significant change in 25(OH)D levels as observed at T2 ($P < 0.001$). Particularly, average values at T0 consisted of 25.29 ± 5.28 ng/mL, and reached 87.4 ± 20.9 ng/mL at T2. The highest 25(OH)D level achieved at T2 of the second step was 110.2 ng/mL. After the surgical procedures, there were no adverse events and every patient showed an improvement in the oral clinical conditions that justified the oral procedure. The clinical outcome parameters and percentage variations between baseline values and following times are represented in Table 3. In the test group, a slight to moderate (but not significant) improvement of clinical parameters is observed at T2. Moreover, statistically significance was obtained comparing T3 percentage variations between control and test groups for T-P and T-L measurements ($P < 0.05$). Interleukin-1-beta, IL-6, and TNF-alpha values are significantly lower ($P < 0.01$) for the test group at T2 and T3 time points (Table 4). Pain assessment by the adopted score system showed no significant difference in values for both groups at all times (Table 5). No particular correlation was shown between the surgical outcome and independent variables, such as sex and age (under/over 25 years old).

DISCUSSION

Low serum levels of 25(OH)D determine the pathologic phenomenon of hypovitaminosis D that is a quite prevalent condition among the general population, especially during the cold seasons[23–28]; it can lead to secondary hyperparathyroidism and other metabolic disorders thus compromising the bone mineralization and slowing down the bone healing.[29–33]

In the worst cases, the hypovitaminosis D causes skeletal consequences such as rickets, in the young, and osteomalachia in the adult.[34–37] Moreover, it seems to be related to nonskeletal manifestations, as enhanced risk of chronic disease and cancer.[38–40]

To date, there is no common agreement regarding the serum level of 25(OH)D to be considered sufficient; the Institute of Medicine of the National Academies (IOM) proposes a 20 ng/mL threshold, whereas several studies and expert guidelines suggest values higher than 30 ng/mL[41,42] Similarly, there are no clear clinical recommendation regarding the frequency of administration and the ideal dose of vitamin D in the treatment of the hypovitaminosis D condition, and multiple treatment regimens were proposed for both the young and the adult.[43–45]

The hypovitaminosis D is a condition frequently observed in both young and adult healthy Italians, particularly among those living in the southern part of the country.

In our clinical trial, low levels of 25(OH)D may be justified by the period of sampling (October–May), probably because of reduced sun exposure, causing a low endogenous synthesis of vitamin D as already highlighted by other studies.[46]

To our knowledge, this is the first study investigating the effect of a single high dose of vitamin D in outcome of third molar surgery.

Vitamin D insufficiency has been treated accordingly to an administration scheme already in use at our center, and the single high oral dose of cholecalciferol has improved the serum 25(OH)D levels via an inactive and harmless calcitriol precursor in a safe way.

The role of vitamin D has been previously investigated in dentistry. Several researches have been performed on the control of postoperative edema in oral surgery. The vitamin D impact on the outcomes of periodontal surgery procedures has already been studied, suggesting a key role in the postsurgical healing.[47] This beneficial effect may be related to its effect in the conditioning of the inflammatory response, lowering the inflammatory cytokines secretion, and improving the healing processes.

TABLE 4. Biochemical Parameters Recorded for Each Patient

	Biochemical Parameters: Control Group												
	Vit D3 ng/mL		PCR mg/dL		TNF-ALPHA pg/mL			IL-1 BETA pg/mL			IL-6 pg/mL		
	T0	T2	T0	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
1	29.2	26.8	0.2	0.1	1.5	1.4	1	0.3	0.6	1.3	0.9	0.7	0.9
2	21.6	19.4	0.1	0.1	1.2	1	1	0.5	0.4	1	1.3	1.7	0.9
3	20.8	18.7	0.2	0.9	1	2.1	1.5	0.6	0.9	0.5	1	1.1	0.5
4	22.5	22.3	0.1	1	1.3	0.9	1.2	0.9	0.4	0.3	1	0.7	0.6
5	23.6	16.5	0.1	0.9	2	1.9	1	0.5	0.4	1.2	1.2	1.8	1.4
6	14	15.4	0.4	1.4	1.5	1.8	1.2	0.2	0.8	0.8	0.9	0.9	0.9
7	27.3	21.9	0.1	0.1	1.4	1.2	1.3	1.5	0.4	1.8	0.8	0.9	1.2
8	21.2	19.7	0.3	1.2	1	1.7	1.5	0.3	0.9	1.2	0.7	1.9	1.7
9	20.9	18.1	0.1	1.5	1.9	1	1.2	0.5	0.7	1.3	0.9	0.9	4.2
10	14.1	12.6	0.1	0.2	1	1	1.6	0.1	0.3	0.6	0.7	0.2	0.4
11	20.4	19.1	0.1	1.2	1.2	1.8	1	1	1	1.3	0.4	0.5	0.3
12	15.4	15.2	0.2	1.5	1	1.7	1.5	0.5	1	1.2	0.7	1	1.5
13	17.1	21.4	0.2	1.1	0.9	1.2	1.3	0.8	4	0.4	0.5	1.3	0.5
14	25.4	20.85	1.4	1.1	0.9	1.2	1.2	0.4	0.5	1	0.1	0.3	0.9
15	15.7	21.2	0.1	1	0.7	1.5	1	0.5	1	1.2	0.2	0.8	1
16	22	24	0.2	1.4	1	1.8	1.5	0.8	1.1	1.3	0.1	0.3	1
17	12.3	14.5	0.1	0.5	0.6	1.1	1.6	1.1	1	0.6	0.6	0.1	0.6
18	27.5	33.1	0.1	1.1	0.8	1	1.2	0.4	1.1	0.4	0.2	0.8	0.6
19	12.7	20.3	0.1	0.2	0.7	1.3	1.2	0.4	0.4	0.5	0.3	0.2	0.2
20	21.1	30.8	0.3	0.2	1.6	1	1.1	1.6	0.8	0.9	0.2	0.6	0.8
21	25	19.9	0.1	0.1	0.8	1	1	0.6	0.8	0.8	0.7	0.9	0.6
22	22.9	26.4	0.1	0.4	1.1	0.6	1.2	0.7	0.7	0.5	0.3	0.3	0.1
23	27.5	25.1	0.1	1.2	2	1.3	1	0.9	1	0.5	0.8	0.1	0.2
24	32.5	29.9	0.8	0.7	1.4	1.4	1.2	1	0.8	0.9	0.9	0.6	0.8
25	13	15.1	0.5	1	1.3	1.6	1.6	1	1.1	1.2	0.7	0.9	1.2
Mean	21.03	21.13	0.24	0.80	1.19	1.34	1.24	0.68	0.88	0.91	0.64	0.78	0.92
	Biochemical Parameters: Test Group												
	Vit D3 ng/mL		PCR mg/dL		TNF-ALPHA pg/mL			IL-1 BETA pg/mL			IL-6 pg/mL		
	T0	T2	T0	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
1	21	80.2	0.1	1.1	1	1.6	1.2	0.6	0.1	0.1	0.6	0.1	0.1
2	19	117.3	0.1	0.7	1.2	1	1.2	0.3	0.2	0.2	0.3	0.2	0.2
3	21	98	0.1	0.1	2	1.4	1	0.3	0.3	0.3	0.3	0.3	0.3
4	25.6	74.3	0.1	0.8	1.6	0.6	1.2	0.3	0.6	0.5	0.3	0.6	0.5
5	14.3	96	0.2	1.1	0.8	1.9	0.3	0.4	0.5	0.2	0.4	0.5	0.2
6	14.5	86.8	0.2	1.1	1.6	1.1	1.6	0.5	0.2	0.4	0.5	0.2	0.4
7	22.5	64.1	0.3	1.5	1.2	1.2	0.6	0.1	0.7	0.7	0.1	0.7	0.7
8	27.4	76.8	0.2	0.6	1	1.1	0.9	1	0.7	0.1	0.6	0.7	0.1
9	24.1	104.5	0.1	1.1	1.6	0.9	1.8	0.3	0.2	0.6	0.3	0.2	0.6
10	18	61.4	0.1	0.2	1.4	0.9	1.2	0.3	0.1	0.1	0.3	0.1	0.1
11	25.5	81	0.5	0.5	1.1	1.3	1.6	0.2	0.8	0.4	0.2	0.8	0.4
12	16	104.7	0.3	0.5	1.1	1.3	0.2	1	0.9	0.1	0.5	0.6	0.2
13	22.7	127	0.1	0.4	1.3	1.2	1.2	1	0.8	0.2	0.8	0.6	0.1
14	29.2	80.2	0.1	0.5	0.9	1	0.8	0.9	0.9	0.2	0.3	0.5	0.2
15	25.1	47.9	0.1	1.1	1.5	1	1	0.9	0.6	0.8	0.9	0.6	0.8
16	12.8	78.8	0.1	0.7	0.8	1.1	0.9	0.8	0.9	0.1	0.3	0.6	0.3
17	15.2	72.3	0.1	0.6	0.6	0.8	0.9	0.1	0.2	0.3	0.1	0.2	0.3
18	29.8	80	0.1	0.4	0.9	0.9	0.8	0.5	0.7	0.3	0.5	0.7	0.9
19	20	96.2	0.1	0.1	1.9	1.5	1	0.3	0.2	0.1	0.3	0.2	0.1
20	22	64.7	0.4	0.3	1.1	1	1.1	0.7	0.7	0.8	0.7	0.7	0.8
21	32.50	99	0.1	0.3	0.9	1	0.4	1	0.9	0.1	0.9	0.7	0.3
22	31.83	110.2	0.1	0.2	1.1	0.9	1	0.5	0.6	0.5	0.5	0.6	0.5
23	55.30	132	0.2	1.0	1.3	1.2	1.1	0.4	0.5	0.5	0.4	0.5	0.5
24	58.50	85.9	0.2	1.1	1.3	1.3	1.3	0.6	0.6	0.6	0.6	0.6	0.6
25	29	65	0.3	1	1.1	1.2	0.3	0.9	0.9	0.1	0.6	0.6	0.1
Mean	25.29	87.36	0.17	0.68	1.21	1.14	0.98	0.56	0.55	0.33	0.45	0.48	0.37
	Biochemical Parameters Δ%: Control Group												
	Vit D3 ng/mL		PCR mg/dL		TNF-ALPHA pg/mL			IL-1 BETA pg/mL			IL-6 pg/mL		
	T2-T0		T2-T0		T1-T0		T2-T0	T1-T0		T2-T0	T1-T0		T2-T0
1	−8		−50		−7		−33	100		333	−22		0
2	−10		0		−17		−17	−20		100	31		−31
3	−10		350		110		50	50		−17	10		−50
4	−1		900		−31		−8	−56		−67	−30		−40

TABLE 4. *(Continued)*

	Biochemical Parameters Δ%: Control Group							
	Vit D3 ng/mL	PCR mg/dL	TNF-ALPHA pg/mL		IL-1 BETA pg/mL		IL-6 pg/mL	
	T2-T0	T2-T0	T1-T0	T2-T0	T1-T0	T2-T0	T1-T0	T2-T0
5	−30	800	−5	−50	−20	140	50	17
6	10	250	20	−20	300	300	0	0
7	−20	0	−14	−7	−73	20	13	50
8	−7	300	70	50	200	300	171	143
9	−13	1400	−47	−37	40	160	0	367
10	−11	100	0	60	200	500	−71	−43
11	−6	1100	50	−17	0	30	25	−25
12	−1	650	70	50	100	140	43	114
13	25	450	33	44	400	−50	160	0
14	−18	−21	33	33	25	150	200	800
15	35	900	114	43	100	140	300	400
16	9	600	80	50	38	63	200	900
17	18	400	83	167	−9	−45	−83	0
18	20	1000	25	50	175	0	300	200
19	60	100	86	71	0	25	−33	−33
20	46	−33	−38	−31	−50	−44	200	300
21	−20	0	25	25	33	33	29	−14
22	15	300	−45	9	0	−29	0	−67
23	−9	1100	−35	−50	11	−44	−88	−75
24	−8	−13	0	−14	−20	−10	−33	−11
25	16	100	23	23	10	20	29	71
Mean	3.27	427.31	23.37	17.68	61.36	85.94	55.94	118.92
	Biochemical Parameters Δ%: Test Group							
	Vit D3 ng/mL	PCR mg/dL	TNF-ALPHA pg/mL		IL-1 BETA pg/mL		IL-6 pg/mL	
	T2-T0	T2-T0	T1-T0	T2-T0	T1-T0	T2-T0	T1-T0	T2-T0
1	282	1000	60	20	−83	−83	−83	−83
2	517	600	−17	0	−33	−33	−33	−33
3	367	0	−30	−50	0	0	0	0
4	190	700	−63	−25	100	67	100	67
5	571	450	138	−63	25	−50	25	−50
6	499	450	−31	0	−60	−20	−60	−20
7	185	400	0	−50	600	600	600	600
8	180	200	10	−10	−30	−90	17	−83
9	334	1000	−44	13	−33	100	−33	100
10	241	100	−36	−14	−67	−67	−67	−67
11	218	0	18	45	300	100	300	100
12	554	67	18	−82	−10	−90	20	−60
13	459	300	−8	−8	−20	−80	−25	−88
14	175	400	11	−11	0	−78	67	−33
15	91	1000	−33	−33	−33	−11	−33	−11
16	516	600	38	13	13	−88	100	0
17	376	500	33	50	100	200	100	200
18	168	300	0	−11	40	−40	40	80
19	381	0	−21	−47	−33	−67	−33	−67
20	201	−25	−9	0	0	14	0	14
21	205	200	11	−56	−10	−90	−22	−67
22	246	100	−18	−9	20	0	20	0
23	138	400	−8	−15	25	25	25	25
24	47	450	0	0	0	0	0	0
25	124	233	9	−73	0	−89	0	−83
Mean	290.58	377.00	1.16	−16.66	32.37	5.23	40.91	17.63

	Biochemical Parameters Analysis							
p	VitD	PCR	TNF-ALPHA (T1)	TNF-ALPHA (T2)	IL-1-BETA (T1)	IL-1-BETA (T2)	IL-6 (T1)	IL-6 (T2)
Flat Values	0.0000	0.1454	0.0009	0.0080	0.0098	0.0000	0.0046	0.0013
Δ%	0.0000	0.2956	0.0391	0.0034	0.2513	0.0455	0.3423	0.0499

Our collected data, after the statistical processing, highlighted the impact of the vitamin D balance on different parameters, both clinical and biochemical. It has been well documented how vitamin D3 treatment for 14 days enhanced not only mineralization but also expression of osteocyte markers, including dentin matrix protein-1 and fibroblast growth factor-23, in iPSop cells. Treatment with 1,25(OH)2 vitamin D3 could promote osteogenic differentiation in iPSop cells and could accelerate the expression of osteocyte marker genes. The active metabolite of vitamin D, 1α,25-dihydroxyvitamin D (1α,25(OH)2D3) or calcitriol, is an important regulator of mineral and bone metabolism. Calcitriol regulates proliferation, differentiation, and function of many cell

TABLE 5. Pain Evaluation During the Considered Time Intervals

	Average VAS Values													
	Day 1 (8.00 PM)	Day 2 (8.00 AM)	Day 2 (8.00 PM)	Day 3 (8.00 AM)	Day 3 (8.00 PM)	Day 4 (8.00 AM)	Day 4 (8.00 PM)	Day 5 (8.00 AM)	Day 5 (8.00 PM)	Day 6 (8.00 AM)	Day 6 (8.00 PM)	Day 7 (8.00 AM)	Day 7 (8.00 PM)	Day 8 (8.00 AM)
Control	2.24	1.86	1.76	1.67	1.67	1.19	1.24	1.05	1.05	0.95	0.95	0.81	0.62	0.57
Test	2.38	2.14	1.95	1.9	1.67	1.24	1.24	0.95	0.95	0.81	0.81	0.57	0.57	0.48

types, both normal and malignant. Biologic activation of vitamin D, a 2-step process, starts with carbon-25-hydroxylation to calcidiol (25-hydroxyvitamin D, 25(OH)D) primarily by the cytochrome enzymes CYP2R1 and CYP27A1, and subsequent carbon-1α-hydroxylation by CYP27B1/1α-hydroxylase. The CYP24A1/24-hydroxylase regulates and inactivates 1α, 25(OH)2D or 25(OH)D in kidney, skin, and bone cells. In vitro, 1α,25(OH)2D stimulates bone formation and matrix mineralization but also stimulates bone resorption under different circumstances.

The clinical results of this study clearly evidence how the patients with high level of vitamin D3 underwent better healing after the third molar surgery. The healing was uneventful and the postoperative discomfort was comfortable.

Although the pain score system, however, did not show a significant improvement after the administration of cholecalciferol, it is possible to notice how test group values are lower than the control group ones after T2, the time point where the 25(OH)D serum levels peak is registered.

Moreover, the vitamin D concentration seems to have an impact on the inflammatory response of the patient, reducing specific indexes, such as TNF-alpha, IL-1-Beta, and IL-6. This effect results in a reduced swelling after the recover from the hypovitaminosis D condition. Because the intensity of the inflammatory response is inversely related to the tissue healing speed, and vitamin D levels are related to the bone mineralization process, it is possible to suppose that the correction of the hypovitaminosis D may improve the tissue healing after minor surgical procedures.

CONCLUSIONS

The hypovitaminosis D, a condition that can lead to bone mineralization and metabolic disorders, is frequently observed during cold seasons, even in insulated areas such as Southern Italy.

Impacted lower third molars have a high prevalence among the general population, and often require surgical treatment, followed by pain, swelling, and functional limitations.

Our processed data have demonstrated that the administration of a single oral dose of 300,000 IU of cholecalciferol is useful in rapidly and safely enhancing 25(OH)D levels in young and adult people with vitamin D deficiency and has shown an impact on the clinical outcome of the third molar surgery. It may be concluded that the recovery from the impacted third molars surgery is influenced by various endogenous and exogenous elements, one of them being the 25(OH)D serum levels.

Moreover, higher serum level of vitamin D seems to elicit a less pronounced inflammatory response, auspiciously leading to a better and faster healing process.

Further investigations may focus on role of vitamin D in the healing process of different oral surgical procedures likewise in cases of dental implant placement.

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