Drops Supplementation for Treating Vitamin D Deficiency in Healthy Children and Adolescents: The Important Role of Sun Exposure

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Abstract: *Background*: High prevalence of Vit D deficiency had been worldwide reported and supplementation was recommended in deficient cases with rapid therapeutic response. As there is no clear evidence to what really is required during childhood in order to prevent adult diseases, this study aimed to evaluate the effectiveness of supplementation therapy in healthy children with vit D deficiency. *Methods*: Thirty six children, mean age 10.9±3.4 years old, with Vit D deficiency were included in the study and were randomly divided in two groups: Those who received supplementation therapy with 2000 IU Vit D3 drops daily for three months and those who had no intervention. Socioeconomic status, sun protection and diet habits, outdoors physical activity and fracture history were evaluated through a questionnaire and blood samples before and after intervention were collected to assess 25(OH)Vit D levels and bone mineral status. *Results*: There was no significant difference between the two groups in relation to socioeconomic and family characteristic, outdoors physical activity and dietary intake. Sunscreen use was limited during summer vacation in 85% of the cases but the amount of use was significantly negatively correlated to baseline levels of Vit D during last year. The 25(OH)Vit D levels at baseline were 14.72±3.63 ng/ml (6.7 - 19.7 ng/ml) whereas a significant increase 25.21±7.08 ng/ml (8-42.2 ng/ml) was observed on re-evaluation (p=0.0001). No difference was found between treated children and non-treated (p=0.13). Skin type and sunscreen habits were not related to therapeutic response rate in both groups. *Conclusion*: This randomized clinical trial revealed a significant improvement in Vit D levels both in treated and non treated groups but no significant difference was established between the groups. The supplementation of vit D in healthy children and adolescents with Vit D deficiency could not be suggested. Sun protection may interfere with Vit D levels. Re-evaluation and re discussion of sun health benefits on Vit D synthesis and national sun protection strategies may be needed.

Keywords: Sun exposure, supplementation therapy, children, vitamin D deficiency.

INTRODUCTION

During the last decade, a lot of discussion has been held on the need for vitamin D (VitD) supplementation in children who breastfeed or consume less than 400 IU of vitamin D daily through diet [1,2]. Until the end of the last century, one tea-spoon of cod liver oil or 10 minutes sun exposure daily were considered adequate to prevent VitD deficiency contrary to recent thoughts and disagreements about which values are considered sufficient in children and adolescents. Even though a recent report indicated that Vit D status of pregnant women pertain to offspring skeletal growth in early adulthood [3], no clear evidence to what really is required during childhood in order to prevent adult diseases have been established.

The Vit D status of an individual is maintained through the combination of dietary intake and the formation of Vit D in the skin under the influence of ultraviolet light. Vit D content of the diet is generally limited unless foods, such as milk or margarine, are Vit D fortified. On the other hand, sun exposure has been considered responsible for skin cancer and sunshine avoiding behaviour has been introduced in children's life. Taking as a threshold the Recommended Dietary Allowance reference (RDA: 50 nmol/L), worldwide studies have confirmed an increased incidence in Vit D insufficiency which occurs quite commonly among healthy European infants, children and adolescents reaching up to 42 % [4-7].

Thus, in order to achieve recommended levels and prevent deficiency, an Estimated Average Requirement (EAR) of Vit D of 400 IU/d (10 mg/d) and a RDA of 600 IU/d (15 mg/d) are proposed for toddlers and children older than 1 year of age by the Institute of Medicine (IOM) in accordance with the American Academy of Pediatrics (AAP) [1,2]. In cases with Vit D deficiency various doses have been suggested by different researchers with similar outcomes [5]. Whether short term low daily or higher weekly scheme of

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administration was chosen, the treatment outcome in young children with deficiency was equivalent [8].

On the other hand, the therapeutic response either in the form of Vit D supplements or by ultraviolet radiation of the skin during sun exposure was found to be rapid in symptomatic cases. However, the necessity and effect of treating healthy individuals was not clarified. In this study, the authors conducted an interventional randomized study to evaluate the effectiveness of vitamin D supplementation in healthy children and adolescence with Vit D deficiency in a geographical area (Attiki, Greece) characterized by excessive sunlight though the year. The main hypothesis was that Vit D supplementation was beneficial in correcting vitamin deficiency even in healthy cases.

PATIENTS AND METHODS

During January 2012 until November 2013, healthy children with greek nationality age 5-18 years old visiting the outpatient Pediatric Unit of KAT General Hospital for routine schedule vaccination were asked to participated to the study. Parents were asked to a) complete a questionnaire and b) give permission with itheir children to be blood tested for Vitamin D levels and bone mineral status.

A questionnaire was designed to collect information about socioeconomic status of the family, perinatal and postnatal medical history of the child and core family, breast feeding, fracture history, physical exercise and use of sunscreen habits. A weekly food frequency questionnaire with some additional question on yearly customary diet habits (i.e. breakfast, fruits, vegetable, milk products, egg, fish) was also completed. Greek children who supported the inclusion criteria were clinically evaluated by the same paediatrician. They had their height and weight measured by a telescopic height measuring instrument and an electronic weight scale respectively. The BMI was calculated using the weight divided by height squared (kg/m^2) and z value scores were calculated according to WHO charts for children 5-19 years old. In addition, all the children were classified into one of the five maturation stages described by Tanner and Whitehouse and skin type was defined according to Fitzpatrick classification [9,10].

Initial evaluation was performed in 230 children and baseline measurements were obtained. The measurement 25(OH)Vit D eferences reported that the active metabolite (1,25-(OH)VitD) concentrations were found low, normal or high in children with rickets since the serum half-life of 1,25(OH)Vit D is short and regulated by PTH [1,5,11]. Vit D status was classified into four groups according to the international guidelines: sufficiency or optimal levels ≥ 30ng/ml (75 nmol/l); insufficiency levels 20-30 ng/ml (50-75 nmol/l); deficiency levels 10-19.9 ng/ml (27.5-49.99 nmol/l) and severe deficiency levels <10ng/ml (< 27.5 nmol/l). According to this classification, 88 cases (38.2%) were found to have Vit D deficiency. Thirty six cases whose first evaluation was done during autumn and winter were selected for this study. Four declined to participate. Thirty-six were randomly divided to two groups: first group included 18 cases that were recommended to receive supplementation therapy 2000 IU of D3 Vit D drops for three months during winter and spring. The second group included 18 cases that had no intervention. Re-evaluated visits were scheduled after summer vacation (next autumn) in an attempt to minimise seasonal variation and bias due to sun exposure (Figure **1**). In addition, parents were asked to refer to any changes in sunscreen habits or outdoor physical activity. The levels of total serum 25(OH)VitD, calcium, inorganic phosphase, total and bone specific alkaline phosphatase, parathormone (PTH), total cholesterol and triglycerides were measured. A rough indicator of allergy profile through total IgE was also included.

The study was performed in accordance to the ethical guidelines of the Declaration of Helsinki and was approved by the Hospital ethical Committee. Children's parents or guardians were informed about the aims and design of the study and provided their consent.

Specimen Collection and Biochemical Analyses

Blood samples were collected after a 12-hour overnight fast by venepuncture at the hospital between 08.00 and 10.00 morning hours during the year 2012 – 2013, were centrifuged at 3600 rpm for 15 minutes, were aliquoted and stored at -20^{o}C until tested. Total 25(OH)VitD serum and bone-specific alkaline phosphatase levels were measured by a commercial manual ELISA assay (IDS-immunodiagnostic systems, Boldon, UK) and results were reported in ng/mL and μg/L respectively. The analytical sensitivity of this assay was 2ng/mL. The Coefficients of Variation (CV) for intra-assay and inter-assay analyses were less than 6.7% and 8.7% respectively. Total calcium serum and inorganic phosphate levels were measured by colorimetric assays on Abbott-Architect ci8200

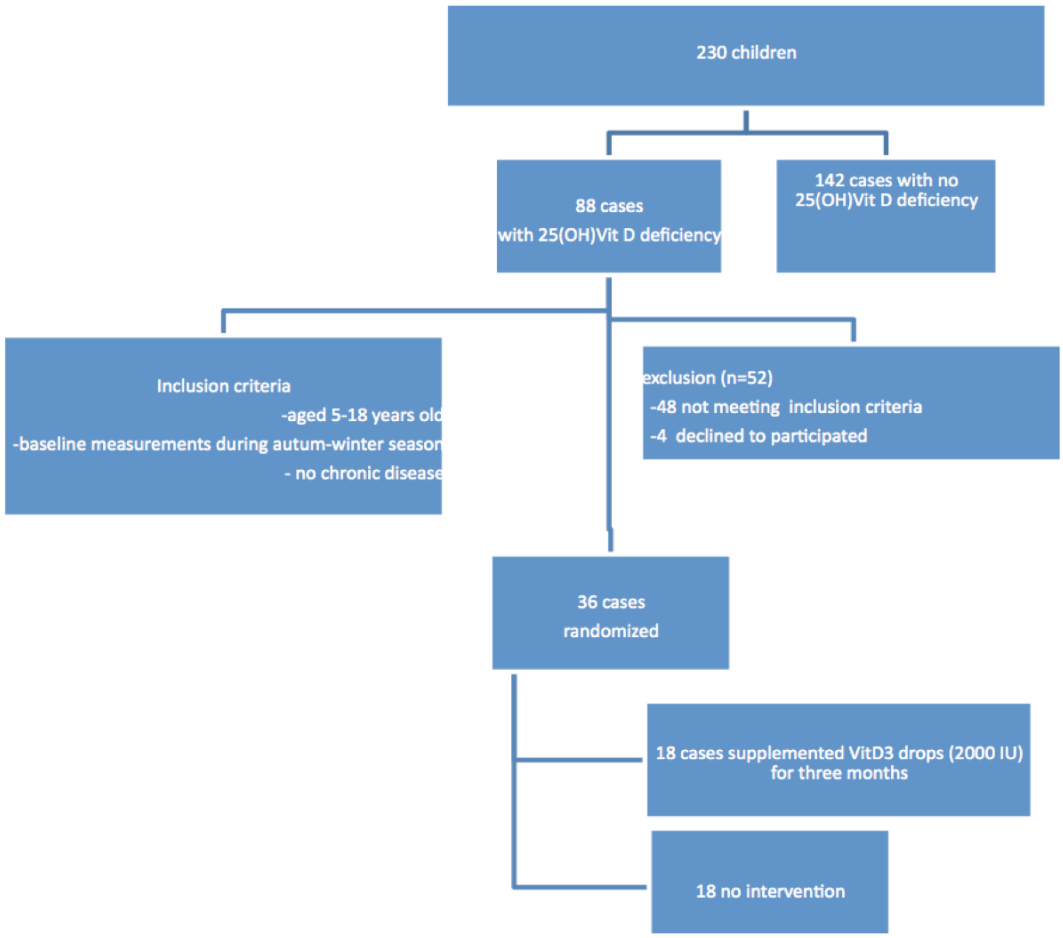

Figure 1: The cases included in the protocol of the study.

Automated Analyzer (Abbott Laboratories, Chicago Il, USA), with intra- and inter-assay CVs of 0.6 and 1.2%, and 1.4 and 1.9% respectively. Serum levels of total cholesterol and triglycerides were measured by an enzymatic assay on Abbott-Architect ci8200 Automated Analyzer (Abbott Laboratories, Chicago IL, USA), with intra- and inter-assay CVs of 0.8 and 1.5%. Serum levels of apolipoprotein A1 and B were measured by immunoturbidimetric assays Abbott-Architect ci8200 automated analyzer (Abbott laboratories, Chicago IL, USA) with intra- and inter-assay CVs of 0.6 and 1.2%, and 1.4 and 1.9% respectively. 1.9 and 3.2% and 4.4 and 6.3% respectively. Plasma levels of PTH were determined with an Electrochemiluminescence Immunoassay (ECLIA) on Cobas e411 (Roche, Manheim, Germany). The sensitivity of this assay was 1.2 pg/ml, and the intra- and inter-assay CVs were 4 and 4.3%, respectively.

Determining vitamin D serum concentrations is not straightforward: one study reported substantial interassay differences of commercially available 25(OH)VitD tests and another study reported seasonal inter-individual differences up to 40% [12,13]. Thus, blood samples were measured by the same biochemical laboratory and on re-evaluation, critical changes were considered only those that were > 40%.

Lifestyle Determinant Factors

Parental and maternal smoking habits and smoking at home were described with using a yes/no question. Breast feeding history and duration in months of exclusive breast feeding were also reported. As sun exposure has a cumulative effect in vit D synthesis, physical activity per week was given in detailed description (type, age since started and hours per week) emphasizing outside activity hours. Lastly, sunscreen cream use since birth was reported. Mothers were asked to recall first use, days per year, season of and years of regular use, thus a cumulative dose in days was calculated based on the child's age. In addition, days of sunscreen use during last year were selected separately.

Attika Climate

Sun exposure during the study period was recorded by the Hellenic National Meteorological Service [14].

Greece is situated in the most southeaster part of Europe and the climate is typical of the Mediterranean climate: mild and rainy winters, relatively warm and dry summers and, generally, extended periods of sunshine throughout most of the year. The coldest months are January and February with mean minimum temperature in Athens (and the area of Attiki) ranging between 5-10 degrees Celsius. Rainfall even in the winter, does not last a lot of days and the sky does not remain cloudy for several consecutive days, as in other regions of the world. Bad winter weather days are often interrupted, during January and the first fortnight of February, with sunny days, well known as 'Alkion days' from ancient times. The warmest period is the last ten-day period of July and the first one of August, when the mean maximum temperature lies in the range of 29.0 and 35.0 degrees Celsius.

Statistical Analysis

The prevalence of cases with deficiency was defined as the ratio of cases divided by the number of participated children. Comparisons between the children subgroups prevalence on categorical characteristics like socioeconomic status of the family, gender, skin type, Tanner classification, breast feeding, fracture history, smoking, diet habits and physical activity were performed using the Pearson's chi-square test whereas continuous variables (months of exclusive breast feeding, BMI, days of sunscreen use, years of physical activity were compared with the independent sample Student's t- test standardized by the Levene's Test for Equality of Variances. Paired sample t-test was chosen to evaluate the differences in the same case between the metabolic changes at baseline and re-evaluation of blood samples stratified with supplemented drops. Significant difference was defined if $p<0.05$. SPSS 18.0 statistical programme (SPSS Hellas, Athens, Greece) was used to analyse the data.

RESULTS

Thirty six children aged 5 -18 years old (mean age 10.9±3.4) with 25(OH)Vit D levels less than 20ng/ml were included in the study and divided into two group. There was no significant difference between the two groups in relation to socioeconomic and family characteristic (Table **1**). Cases' physical activity, outdoor physical activity and sunscreen use were also similar. Changes in physical activity and sun protection

Table 1: Comparison of the Socioeconomic, Lifestyle and Clinical Characteristics of the Treated and Non Treated Group

	Treated n=18	**Non-Treated n=18**	***p***	**ALL n=36**
Age (years)	10.2±3.16	11.7±3.3	0.17	10.9±3.4
Male (%)	10(55.5)	9(50)	1	19(52.8)
Tanner III-VI	7(38.8)	11(61.1)	0.40	18(50)
Parents with academic education, (%)	10(55.5)	10(55.5)	1	11(30.5)
Maternal smoking, %	6(33.3)	6(33.3)	1	12(33.3)
Paternal smoking, %	5(27.7)	4(28.8)	1	9(25)
Smoking at home	3(16.7)	3(16.7)	1	6(16.7)
Breast feeding %	14(77.8)	14(77.8)	1	28(77.8)
Exclusive Breastfeeding (mean months)	4.70±4.55	5.33±7.3	0.80	5.0±5.9
BMI (mean)	18.2±3.04	18.8±4.09	0.63	18.5±3.52
Physical activity >3 days/ week (%)	13(72.2)	10(55.5)	0.18	23(63.9)
Outside physical activity >3 days/week	9(50)	4(22.2)	0.16	11(30.5)
Y Years of physical activity >3/week	3.46±2.79	3.10±3.11	0.77	3.30±2.93
Sunscreens use: mean days since birth mean days/last year >3 months/last year	414±290 48±42 4(22.2)	310±205 27±19 -	0.28 0.06 0.10	362±287 38±33 4(11)
Skin type II III	14(77.8) 4(22.2)	12(66.6) 6(33.3)	0.71	26 (72.2) 14 (27.8)
Fracture history, %	3(16.7)	1(5.6)	0.60	4(11.1)
Eating dairy product daily, % Eating fish ≥1/week, % Eating fruits daily, % Eating vegetables daily, %	11(61.1) 15(83.3) 5(27.7) 8(44.4)	10(55.5) 13(72.2) 4(22.2) 5(27.8)	1 0.71 1 0.48	21(58.3) 28(77.7) 12(33.3) 13(36.1)

were not reported during the study but a moderate difference of sunscreen use was established during last year between supplementation group and group with no intervention and it was a significant negative correlation to levels of Vit D (p=0.02). None of the case used sun protection during October-April. Daily application of sunscreen use with high SPF>30 from May to Sept last year was reported in 4 children that was included in treated group. Significant dissimilarity was not found in skin type. No difference was detected on reported fracture history and diet habits. Moreover no significant difference was detected in 25(OH)VitD low levels in relation to breastfeeding (p=0.6). Obesity was not the case of these children (BMI mean 18.50±3.52, range 10.22-26.63). Levels of IGE were higher than normal but with no significant difference between the groups (Table **2**). Ca, AP, BSAP, cholesterol and triglyceride concentrations were similar in both groups. The P was significantly higher in the group that received Vit D supplement. Hypercalcemia was not observed in any of the subjects even after drops supplementation (Figure **2**). Levels 25(OH)vitD at baseline ranged from 6.7 to 19.7 (mean±SD: 14.72±3.63) and were significantly lower in subjects who received supplementation (13.42±3.77 vs. 16.03±3.07, p=0.03). On re-evaluation, levels ranged from 12.1 to 42 (mean ±SD: 25.21±7.08). A significant increase in 25(OH)VITD levels was observed in both groups compared to baseline levels (p=0.0001, Figure **3**) but there was no significant different levels between the two groups (23.42±6.66 vs. 27,01 ± 7.22, p=0.13). The increased rate was calculated to 74,5% in the supplemented group versus 68.5% in no intervened group (p=0.53). Twenty four cases (66.6%) had a critical increase of 25(OH)VitD levels (>40% from baseline, Table **2**). With evaluating the response rate in relation to breast feeding, no significant difference was detected (p no drops/drops = 0.11 / 0.79). No difference in the response rate in relation to skin type was detected (p no drops/ drops =0.99/0.19) either. Four cases had severe deficiency with levels of 25(OH)VITD <10ng/ml). Three of them randomly received supplemented drops but one did not experienced immense improvement. The number is too small to be used for any statistical analysis.

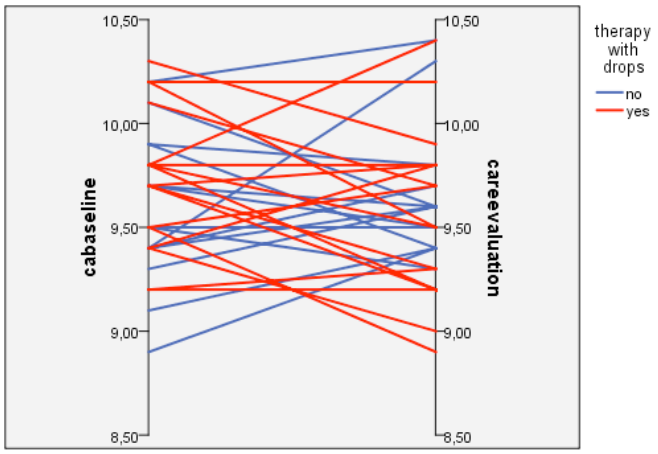

Figure 2: Calcium values at baseline and reevaluation in treated and non-treated children No significant changes were found. No hypercalcemia was detected.

All other parameters did not change during re-evaluation (Table **2**). Figure **3** shows the comparison of

Table 2: Comparison of the Biochemical Analysis of Treated and Non Treated Group

	Baseline		ALL n=36	p	Re-evaluation		P
	Treated n=18	Non-Treated n=18			Treated n=18	Non-treated n=18	
25(OH) Vitamin D	13.42±3.77	16.03±3.07	14.72±3.63	0.03*	23.42±6.65	27.01±7.22	0.13
PTH	30.95±11.38	30.18±15.65	30.6±13.01	0.90	40.05±16.58	35.8±9.7	0.58
Calcium	9.73±0.32	9.65±0.36	9.67±0.36	0.50	9.31±1.08	9.65±0.32	0.27
Phosphorus	4.75±0.43	4.47±0.55	4.68±0.51	0.10	4.8±0.66	4.36±0.44	0.05*
Alkaline phosphatase	236.4±51.9	207±87.1	214.8±74.08	0.07	232±66.5	185±76.98	0.09
Bone alkaline phosphatase	144.9±165.76	83.8±39.9	110.06±114.13	0.16	232±66.5	185±76.98	0.14
Cholesterol	168.5±34.69	148.85±20.82	162.8±31.71	0.15	156.0±23.5	111.3±15.5	0.06
Triglycerides	63.8±18.66	54.23±16.44	60.36±18.10	0.21	53.1±22.07	50.3±1.41	0.70
IGE	207±305	298±695	252±542	0.65	NT	NT	
% increase (Vit D) >40% increase			79.6±64.9 25(69.4)		86.5±75.8 12(66.6)	72.7±50.59 13(72.2)	0.53 1

*p<0.05, NT= non tested

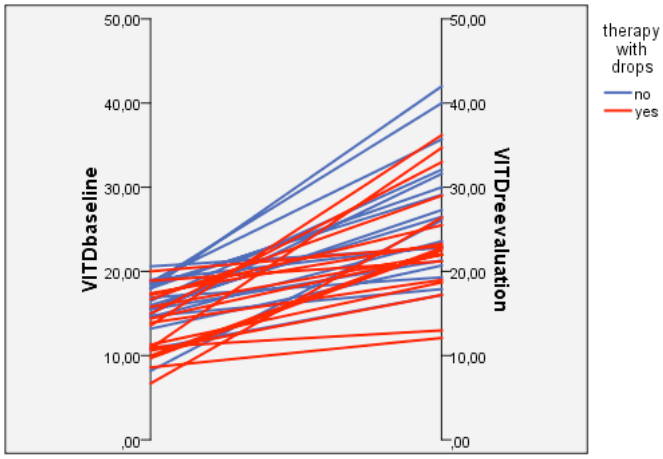

Figure 3: The 25(OH)VitD levels at baseline and re-evaluation by therapy. There was a significant improvement in both groups (p=0.001).

25(OH)VitD levels at baseline and re-evaluation by therapy. No difference was observed between treated children and no treated so the hypothesis of the study could not be supported. Cases who received drops had the same improvement in Vit D levels as the cases that didn't receive drops.

DISCUSSION

The main finding of this study was the significant increase in 25(OH)VitD levels both in cases who receive therapy and those who did not. However, there was no significant difference in the Vit D levels on re-evaluation between the groups. Even though there was a significant difference in baseline measurement, the response rate was equal. This result might have two explanations: either the dose chosen was not the appropriate for healthy children with deficiency or other factors could be therapeutic.

Indeed, the supplementation of Vitamin D in cases with deficiency is still under consideration. Various therapeutic schemes have been suggested by different researchers. The aim of 200,000 IU in a maximum period of three months which can be achieved either by low daily (1000-2000 IU) or higher weakly doses (50,000IU) have revealed equivalent outcomes in young children [8,15]. Even low versus high stoss therapy had similar therapeutic effects [16]. However in recent trials, double or tripled doses have been tested, taking into account the resident area and cultural or religious costume habits which seem to be responsible for an increased prevalence of Vit D deficiency [17-19]. What is more, the form of Vit D administrated also remain a debate even though a study in children showed equal Vit D treatment efficiency either with D2 (Ergocalciferol) or D3 (Cholecalciferol) choice [8]. Having in mind the uncertain information that currently exists regarding the safety or efficacy of vitamin D treatment doses in paediatric population, a low daily dose of D3, Vit D (2000 IU) was used in this study for three months in order to avoid the risk of hypercalcemia as the cases that were included in the study were healthy.

Another factor that could increase Vit D level is ultraviolet radiation. All cases in this study were residents in a sunny city, Athens. The influence of sun in Vit D storage is well documented [20]. Under the influence of UVB of 290–315 nm photons, pro-vitamin D_3, made by keratinocytes, convert to pre-vitamin D_3 which in turn thermally isomerizes to vitamin D3. In addition, most human cells not only have vitamin D receptors but also have the capacity to convert 25-hydroxyvitamin D to the active metabolite 1,25-dihydroxyvitamin D [21,22]. The skin supplies about 90% of vitamin D in humans. This cutaneous synthesis is influenced by the duration of UVB exposure, the amount of skin exposed, the zenith angle of the sun (affected by latitude), the degree of skin melanin pigmentation, the cloud cover and atmospheric pollution. Consequently, Vitamin D production is season depended. A recent Greek study conducted in urban area reported severe deficiency in half of the adolescents which was self treated during summertime [23]. It is worth emphasizing that the recommendations of IOM and the AAP strengthened the need of Vit D supplementation in cases of limited UVB sunlight exposure [1,2]. In this study, outdoor systematic physical activity was reported in one third of the cases during school period (mid September to mid -June) and according to Greek education program, during school hours all children had outdoor leisure time at least 60 minutes daily even in cold days. As reported by the National Meteorological Service during autumn and winter, the mean sunny hours per day in winter in Athens were 3.2 and 1.2 in 2012 and 2013 respectively which were much higher than other parts of Europe [24]. This could be an explanation for the valuable influence of sun on Vit D levels in this city.

Skin type was found to be related to skin Vit D synthesis. Lighter skin color evolved to facilitate Vit D production under conditions of low UVB radiation [25]. Children in this study were mainly Type II in both groups and no different was detected in the response rate between the groups. In addition, the use of sun protection raised controversies. Adult studies reported that creams with SPF>15 prevented the valuable

action of sun lights and reduced levels of Vit D contrary to low SPF use which did not increase parathyroid function or cause osteoporosis [26-28]. In three large studies, Caucasian children with skin type II who had adequate outdoor UVB exposure during spring and summer had adequate vitamin D_3 levels all year, if they did not diligently wear sunscreen with SPF ≥ 15 except during beach vacations [29-31]. Skin protection in this study was reported to be applied mainly during summer vacation. There was a moderate difference in the total days of sunscreens application during last year between the groups. The treated group used protection significantly more and with prolonged use than the non-treated group. This difference might explain the significant lower baseline level of Vit D in this group as a negative correlation of the amount of sun protection use and Vit D levels was detected. Even though this could not be proved from this study and other reviews reported opposite results supporting that sun avoidance practices with broad-spectrum coverage do not reduce vitamin D synthesis [32,33], the results should be further evaluated in a larger group of patients.

It is important to emphasize that sun protection was recommended to reduce the increasing incidence of skin cancer. Public health organizations warn people, especially children, to stay out of the sunlight whenever possible or limit outdoors activity and to wear protective clothing, sunglasses and sunscreens with high protection factor [33,34]. However, there are important references showing that regular moderate continual sun exposure can reduce the incidence of fatal melanoma or increased survival in melanoma patients [33-39]. Therefore, it has been suggested that promoting protection from all midday UV exposures may paradoxically also be promoting the incidence of melanoma. The mechanism that seems to explain these confusing result is the fact that the formed 1,25-dihydroxyvitamin D can inhibit cellular proliferation, induce cellular maturation, inhibit angiogenesis and ultimately cause apoptosis to prevent malignancy such as melanoma, colon, breast, pancreatic and ovarian cancer [40].

Limitation of the Study

Our study included children from the general population living in the northwest suburbian area so the prevalence of Vit D deficiency did not reflect the prevalence of the whole population of this city. The number of the cases re-evaluated was low, thus restricting the power of the study. The significant lower mean level of 25(OH)VIT D at baseline in supplemented group might hide a therapeutic benefit of supplementation therapy even though no different response rate was detected. Solar UV exposure was not calculated and some presumed risk factors as sun exposure, use of sunscreens and diet were only indirectly evaluated by questionnaire administration, so recall bias may have partially influenced our results.

CONCLUSION

The main finding of this study was the significant increase in 25(OH)VitD levels both in cases who receive therapy and those who did not. The supplementation of drops could not be suggested in healthy children. Other factors, i.e. UV radiation of the skin in cases with limited sunscreens, might influence Vit D levels. Albeit Vit D deficiency seems to be a multifactorial consequence, the authors wondered about the amount of sunscreen and SPF advised by the World Health Organization which might have abolished vitamin D production. However, additional research is needed to confirm our estimates and to improve our understanding on the benefits and risks of sun exposure to children's health and to define the cases that actually need supplementation therapy for Vit D deficiency.

ABBREVIATIONS

AAP = American Academic of Pediatrics

CV = Coefficients of Variation

EAR = Estimated Average Requirement

IOM = Institute of Medicine

PTH = Parathormone

RDA = Recommended Dietary Allowance

Vit D = Vitamin D

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