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Research article

Vitamin D Status in an Italian Pediatric Cohort: Is There a Role for Tobacco Smoking Exposure?

Clemente et al. Vitamin D and Tobacco Smoking Exposure

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What is already known on this topic?
Inadequate vitamin D status has been previously reported in children from Italy's northern and southern regions, suggesting that it is relatively independent of the latitude.

What this study adds?
Smoke exposure was found to be a significant risk factor for hypovitaminosis D. This finding highlights the importance of ensuring healthy and smoke-free environments for children.

Abstract
Introduction: Vitamin D deficiency is a common public health issue worldwide. The purpose of this study was to investigate the vitamin D status and its potential determinants in children residing in Sardinia (40°N), Italy.
Methods: A total of 182 children (males: 51.7%; median age: 9 years) were enrolled over a 12-month period. Serum 25(OH)D was measured by an immune-chemiluminescence assay. A questionnaire was used to gather information on other variables, including passive smoke exposure.
Results: Mean (SD) serum 25(OH)D was 25.2 (8.3) ng/mL for the whole group. The majority (n=123, 67.6%) of children had vitamin D sufficient values >20 ng/mL, while about 1/3 had vitamin D insufficient/deficient values (≤20 ng/mL (n=59, 32.4%). Among the variables investigated, passive smoke exposure was significantly associated with insufficient 25(OH)D levels ($p<0.0001$).
Conclusion: Our results further prove that hypovitaminosis D is common in the Italian children and documented that passive smoke exposure is a significant risk factor for hypovitaminosis D.
Keywords: Vitamin D deficiency, hypovitaminosis D, passive smoke exposure, lifestyle habits

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Introduction

Vitamin D deficiency is a common public health issue worldwide, affecting people of any age. Its etiology results from the variable and complex interactions among environmental, genetic, and epigenetic factors (1). Although the exact cut-off level for defining childhood hypovitaminosis D is still debated, vitamin D insufficiency is defined as serum 25(OH)D levels between 12-20 ng/mL and deficiency as <12 ng/mL; both are associated with increased risk for rickets (1, 2). Hypovitaminosis D affects a majority of children in both northern and southern regions of Italy (44°- 40°N), suggesting that vitamin D status is relatively independent of region latitude (3, 4).
Recent studies have shown a significant association between tobacco smoke exposure and vitamin D levels in children (5-8). The purpose of this study was to investigate the vitamin D status and its potential determinants in children residing in Sardinia, Italy.

Methods

Experimental subjects
A retrospective observational study was conducted among children aged 1-16 years who lived in Northern Sardinia (40°N), Italy, enrolled from June 2018 to May 2019. This study's data was obtained during a clinic visit at the University Hospital of Sassari. The intake of vitamin D supplements after the first year of life was the study's exclusion criterion. Our study was conducted in accordance with the ethical standards of the regional committee on human experimentation. Informed consent was obtained for each participant.
Collected data included blood levels of vitamin D, demographic data, body height and weight, and body mass index (BMI) z-score. Each participant's parents filled out a specifically designed questionnaire that investigated family-related factors, residence (rural/urban), sunlight exposure, regular use of total protection sunscreens, Fitzpatrick skin types, fortified milk intake, and passive smoke exposure. The questionnaire also inquired about the presence of chronic diseases and prolonged pharmacological treatments.
Vitamin D assay
The serum 25(OH)D levels were measured using the immune-chemiluminescence Liaison® 25 OH vitamin D Total Assay (CLIA, DiaSorin Spa, Saluggia (VC), Italy) following the manufacturer's instructions. Vitamin D status was classified as sufficiency (serum 25(OH)D >20 ng/mL), insufficiency (serum 25(OH)D 12–20 ng/mL), and deficiency (serum 25(OH)D <12 ng/mL), according to the "Global Consensus Recommendations on Prevention and Management of Nutritional Rickets" (2).
Statistical analysis
Qualitative data were summarized as absolute and relative (percentage) frequencies. Means and standard deviation (SD) or medians and interquartile ranges (IQR) were used for quantitative variables. Comparison of quantitative variables among different levels of serum vitamin D (3 groups) were performed using one-way ANOVA or its non-parametric version (Kruskall Wallis test). Post-hoc analysis was performed using Dunn's test and Bonferroni correction. Differences in qualitative variables were assessed using Fisher's exact test. Spearman's correlation coefficients were calculated to explore the

relationship between serum vitamin D levels and siblings. Moreover, univariate and multivariate logistic regression analyses were performed to assess the relationship between serum vitamin D levels (cut-off <0.20) and sample characteristics. Stata 15 statistical software (StataCorp LLC, Texas, USA) was used for every statistical computation. P-values of less than 0.05 were considered statistically significant.

Results

A total of 182 children were enrolled in the study period, median (IQR) age was 9 (6-12; range 1-16) years; 51.7% were male. Sixty-nine of the participants were siblings. Demographic and clinical characteristics of study population stratified by vitamin D status are shown in Table 1.

Mean (SD) serum 25(OH)D value was 25.2 (8.3) ng/mL for the whole group. The majority (n=123, 67.6%) of children had vitamin D sufficient values >20 ng/mL; 56 (30.8%) had values ≥ 30 ng/mL, and 67 (36.8%) had values in the 21 to 29 ng/mL range.

Among the children with serum vitamin D values ≤20 ng/mL (n=59, 32.4%), 55 had insufficiency (25(OH)D, 12-20 ng/mL), and only 4 (2.2%) had deficiency (<12 ng/mL). The latter underwent further laboratory investigations to rule out active rickets.

A history of daily tobacco smoke exposure was found in 27 (14.8%) children, of whom 16 (59.2%) had vitamin D ≤20 ng/mL. Only 43 (27.7%) of the 155 children not exposed to tobacco smoke had vitamin D ≤20 ng/mL (p=0.001). Among the lifestyle factors investigated through the questionnaire, only smoke exposure showed a significant association with vitamin D status. Multivariate logistic regression analysis confirmed a significantly increased risk (OR, 6.0; 95% CI, 2.1-17.6; p=0.001) of hypovitaminosis D (serum 25(OH)D levels ≤20 ng/mL) in children exposed to passive smoke (Table 2, Figure 1).

Discussion

Consistent with the results of our preliminary report of a smaller cohort of children (3), we found about one third of the healthy children living in Northern Sardinia had hypovitaminosis D. In our study population, the median global serum 25(OH)D value was 25.2 ng/mL, substantially similar to the value of 28.2 ng/mL reported in a recent Italian cross-sectional study by Galeazzi et al. (4). Consistent with Galeazzi et al. (4), we observed 25(OH)D levels were highest in summer and lowest during winter and spring, reflecting seasonal variations in sun exposure. However, unlike other studies, we documented mean values above the threshold of 20 ng/mL in winter.

The results of this questionnaire-based study showed that tobacco smoke exposure is a significant risk factor for hypovitaminosis D. To our knowledge, this is the first time such an association has been demonstrated in Italian children.

Previous studies have identified active smoking as a risk factor for vitamin D insufficiency in adolescents (9), but only a few studies have reported the effects of passive tobacco smoke exposure on vitamin D status in otherwise healthy children (6-8). In a US study of 2,263 subjects aged 3-17 years, vitamin D deficiency was observed in 15.1% of children not exposed to tobacco smoke, 20.9% of children exposed to secondhand smoke, and 18.0% of adolescent smokers (7). A Danish study investigated environmental, dietary, and genetic determinants of serum 25(OH)D levels during pregnancy and early childhood. In 298 children aged 4 years, the following determinants were identified: lower maternal age at birth, higher pre-pregnancy BMI, lower genetic vitamin D score, older siblings, tobacco smoke exposure, and female sex (5). More recently, a Japanese questionnaire-based study evaluated the association between smoke exposure and vitamin D deficiency in a large cohort of young children, showing that the two factors were significantly associated with each other (OR 1.35; 95% CI, 1.14–1.59) (6).

The mechanisms by which tobacco smoke exposure might affect vitamin D status are likely complex and not fully understood. It has been hypothesized that tobacco smoke might interfere with vitamin D metabolism in multiple ways, including skin and renal activation of vitamin D and dysfunction in the parathyroid hormone (PTH)-vitamin D axis (10). Smoke exposure has been reported to be associated with altered dietary intake of vitamin D and calcium, through malabsorption but also by modifying taste (10). Moreover, through a yet unknown mechanism, smoke exposure alters normal PTH response to low vitamin D levels, resulting in simultaneous decreases in vitamin D, calcium and PTH (10). Whether this is secondary to PTH impaired secretion or to its faster degradation, or both, it is not known, but the consequence is certainly hypocalcemia and low bone mineral density. In this regard, a study cohort of 1,422 individuals followed for 28 years (age 3 to 18 years) observed that exposure to passive smoking in childhood, determined by parental smoking and serum cotinine (metabolite of nicotine) concentrations, was an important determinant of impaired bone health with reduced bone mass, density, and strength indices measured later in adulthood (11). In addition, the toxicity of high cadmium and lead contents in cigarette smoke determines low levels of vitamin D by impairing both its intake and its activation, as it causes renal glomerular and tubular dysfunction. Vitamin D activation decreases also by skin aging and by dysregulation of the Cytochrome P450s' genes related to its metabolism, due to smoke exposure (10).

Some in vitro studies have provided further insight into the mechanism by which tobacco smoke may affect vitamin D status. A Korean study demonstrated that cigarette smoke extracts can inhibit the vitamin D-induced translocation of Vitamin D Receptor (VDR) in human alveolar basal epithelial cells. The subsequent treatment of 1,25-(OH)2-D3 induced translocation of VDR from nucleus to microsomes in a dose-dependent manner (12). More recently, Mathyssen et al (13) found that cigarette smoking reduces the production of the active form of vitamin D in lung epithelial cells and also alters the normal expression of the VDR.

Study Limitations

The findings of this study should be interpreted in light of some limitations and biases mainly due to its nature, i.e., retrospective observational design, where information collected through questionnaire was self-reported. Another limitation is the lack of a follow-up evaluation, which is necessary to describe and investigate the medium-term implications for vitamin D deficiency. Other limitations are related to the small sample size non-representative of the overall Italian pediatric population.

Conclusion

Our results provide further evidence that hypovitaminosis D is common in the Italian pediatric population, and suggest that smoke exposure is a significant risk factor for hypovitaminosis D. Given that vitamin D plays a crucial role in various body's physiological processes including the development and maintenance of a healthy skeleton, mineral homeostasis, and immune system regulation, our findings are relevant to both clinical practice and public health, even more so considering that smoke exposure and other unhealthy lifestyle habits are preventable environmental factors.

Taken together, the available data suggest the complexity of the factors influencing serum vitamin D, whose levels in children result from a variable combination of environmental factors, family lifestyle habits, epigenetic and genetic determinants. Experimental and observational prospective studies are needed to further evaluate causal relationship between smoke exposure and vitamin D status in children.

Compliance with ethical standards

Conflicts of Interest: All authors declared no conflict of interests related to this paper.

Funding information: The authors declare that they have no financial relationships relevant to this article to disclose.

Ethical Standards: This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the regional committee on human experimentation (Comitato Etico ATS Sardegna, 29 May 2018, Protocol number: PG/2018/68).

Informed consent: Parents gave informed consent for each child participating in the study.

Author contributions

MGC and DA equally contributed to the study. RA, MGC, LA and DA had primary responsibility for the design and execution of the study, data collection, preliminary data analysis and writing the manuscript. AB, LA, CL, SB, MA, LS, MVP and GS participated in data collection, data analysis and the writing of the manuscript. RA, MGC, DA, SB, MEB, and CL supervised the design and execution of the study, performed the final data analyses and contributed to the writing of the manuscript. All authors of this manuscript have read and approved the final version submitted.

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Table 1. Demographic and clinical characteristics of study population (n=182) stratified by vitamin D status (sufficiency, serum 25(OH)D >20 ng/mL; insufficiency, serum 25(OH)D of 12-20 ng/mL; deficiency, serum 25(OH)D <12 ng/mL).

Variables		<12 ng/mL (n= 4)	12-20 ng/mL (n= 55)	>20 ng/mL (n= 123)	p-value
Males, n (%)		3 (75.0)	27 (50.0)	64 (51.6)	0.68
Median (IQR) age, years		10.0 (4.5-13.5)	10 (7-12)	8 (5-11)	0.07
Median (IQR) weight, kg		29 (16.3-42.0)	36 (18.8-42.5)	24 (17.2-36.0)	0.02[1]
Median (IQR) height, m		1.37 (1.02-1.47)	1.40 (1.16-1.52)	1.25 (1.10-1.41)	0.03[2]
Median (IQR) BMI, kg/m^2		17.0 (15.2-19.9)	16.4 (15.0-19.3)	15.8 (14.6-18.1)	0.25
Median (IQR) BMI z-score		-0.47 (-1.67;0.60)	-0.52 (-1.09;0.25)	-0.38 (-1.37;0.47)	0.03
Residence, n (%)	Rural	0 (0.0)	9 (16.4)	9 (7.6)	0.17
	Urban	4 (100.0)	46 (83.6)	110 (92.4)	
Sun exposure, n (%)	<15 days	1 (25.0)	2 (3.7)	7 (5.7)	0.36
	15-30 days	1 (25.0)	9 (16.7)	23 (18.9)	
	>30 days	2 (50.0)	43 (79.6)	92 (75.4)	
	Not exposed	1 (25.0)	6 (11.1)	11 (8.9)	
Use of sunscreens, n (%)	Non-regular	1 (25.0)	19 (35.2)	40 (32.5)	0.73
	Regular	2 (50.0)	28 (53.7)	72 (58.5)	
Formula milk (between 1-3 years), n (%)	No	3 (75.0)	36 (66.7)	66 (54.1)	0.22
	Yes	0 (0.0)	16 (29.6)	54 (44.3)	0.06
Fitzpatrick class, n (%)	Maternal	1 (25.0)	2 (3.7)	2 (1.6)	0.02[3]
	II	0 (0.0)	6 (11.1)	3 (2.4)	
	III	0 (0.0)	10 (18.5)	26 (21.1)	
	IV	2 (50.0)	23 (44.6)	56 (45.5)	0.43
	V	2 (50.0)	14 (25.9)	35 (28.5)	
	VI	0 (0.0)	1 (1.9)	3 (2.4)	
Nephrotic syndrome, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Kidney failure, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Liver failure, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Liver disease, n (%)		0 (0.0)	0 (0.0)	1 (0.8)	1.00
Antiepileptic drugs, n (%)		0 (0.0)	3 (5.6)	0 (0.0)	0.03[4]
Systemic corticosteroids, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Rifampicin, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Highly active antiretroviral therapy, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Celiac disease, n (%)		0 (0.0)	2 (3.6)	1 (0.8)	0.28
Inflammatory bowel disease, n (%)		0 (0.0)	0 (0.0)	0 (0.0)	-
Asthma, n (%)		0 (0.0)	3 (5.5)	7 (5.7)	1.00
Diabetes mellitus type 1, n (%)		0 (0.0)	2 (3.6)	1 (0.8)	0.28
Passive smoke exposure, n (%)		1 (50.0)	15 (53.6)	11 (17.7)	0.001[5]
Mean (SD) serum 25-OH-D level, ng/mL		8.8 (0.5)	16.7 (2.6)	29.5 (6.3)	<0.0001

1. <10 ng/mL versus > 20 ng/mL, p-value= 0.02
2. <10 ng/mL versus > 20 ng/mL, p-value= 0.01
3. <10 ng/mL versus > 20 ng/mL, p-value= 0.002
4. <10 ng/mL versus > 20 ng/mL, p-value= 0.008
5. <10 ng/mL versus > 20 ng/mL, p-value= 0.002

Table 2. Relationship between hypovitaminosis D (serum 25(OH)D levels ≤ 20 ng/mL) and variables analyzed.

Variables		Univariate analysis		Multivariate analysis	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Male		1.0 (0.5-1.8)	0.88	0.9 (0.3-2.3)	0.83
Age, years		1.1 (1.0-1.2)	0.02	1.04 (0.89-1.21)	0.64
Weight, kg		1.0 (1.0-1.0)	0.05	-	
Height, m		6.7 (1.5-29.6)	0.01	-	
BMI, kg/m^2		1.1 (1.0-1.1)	0.30	-	
BMI z-score		1.06 (0.87-1.30)	0.56	-	
Urban residence		0.5 (0.2-1.2)	0.12	-	
Sun exposure		1.1 (0.6-1.9)	0.76	-	
Regular use of sunscreens		0.8 (0.4-1.5)	0.52	-	
Formula milk		0.5 (0.2-0.9)	0.03	1.0 (0.4-2.9)	0.96
Fitzpatrick class	II	4.6 (1.1-19.1)	0.04	9.6 (0.9-105.3)	0.06
	III	0.9 (0.7-1.2)	0.54	-	
	IV	1.0 (0.5-1.8)	0.87	-	
	V	0.9 (0.5-1.7)	0.76	-	
	VI	0.7 (0.1-6.9)	0.76	-	
Passive smoke exposure		5.3 (2.0-14.0)	**0.001**	6.0 (2.1-17.6)	0.001

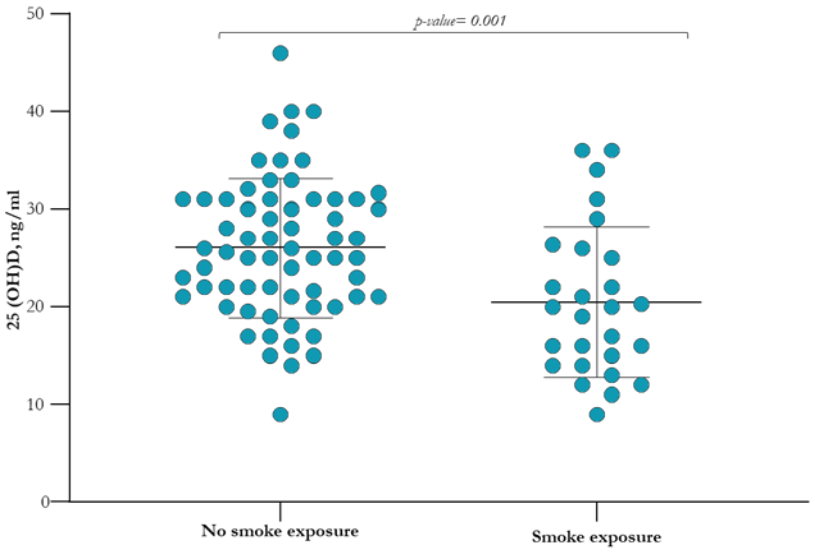

Figure 1. Serum 25(OH)D values in children with and without tobacco smoke exposure.