

Article

Association Between Tobacco Exposure and Serum 25-Hydroxyvitamin D Concentrations in Adults Aged 20–40 Years: A Cross-Sectional Study

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Abstract: The vitamin D status depends on a variety of behavioral, environmental, and biological factors. The observational studies suggested that cigarette smoking is associated with reduced circulating 25-hydroxyvitamin D [25(OH)D]. Objective: This study aimed to determine the relationship between cigarette smoking status and serum 25(OH)D concentrations, vitamin D status categories among adults aged 20-40 years. Methods: Cross-sectional comparative study was conducted between 1 February to 1 July 2026 at Tikrit Teaching Hospital, Iraq. One hundred participants were recruited consisting of 50 current smokers and 50 non-smokers. About 3 ml venous blood sample was taken, allowed to clot and centrifuged at 3000 rpm for 10 minutes. 25(OH)D concentration was determined in hospital laboratory using Roche Cobas e 411 analyzer with final results were provided to the research team. Frequency distribution of categorical variables was reported. Due to the absence of individual 25(OH)D levels data in the revised dataset, no continuous data analysis between groups was done. Results: Study population included 82 (82%) males and 18 (18%) females. Among smokers, 29 (58%) were aged 30-40 years and 21 (42%) were aged 20-29 years. Low vitamin D status was found in 87 participants (87%): all 50 smokers and 37 (74%) non-smokers. Among non-smokers, 9 (18%) had normal and 4 (8%) had high vitamin D status; there was no smokers having normal or high vitamin D status. Reported Pearson Chi-square statistic value for smoking status and vitamin D category was $\chi^2=14.943$ ($p=0.001$); exact test based on aggregated data showed $p\approx0.00010$. Conclusion: Current smoking status in our population was related to increased prevalence of low vitamin D status. Limitations of the study include cross-sectional design, small sample size, different age distribution, insufficient description of smoking exposure, and unadjusted confounders.

Keywords: cigarette smoking; tobacco exposure; vitamin D; 25-hydroxyvitamin D; vitamin D status; cross-sectional study

Introduction

Vitamin D is a secosteroid hormone that is known to have a role in calcium and phosphate metabolism and bone mineralization. Synthesis through ultraviolet-B radiation in the skin is an important source

of vitamin D in many people. Vitamin D is converted to 25-hydroxyvitamin D [25(OH)D] in the liver, which is the main circulating metabolite for determination of the status of vitamin D. Further hydroxylation occurs in the kidneys to form 1,25-dihydroxyvitamin D [1], [2], [3], [4].

Vitamin D status is affected by sun exposure, diet, body composition, age, season, latitude, pigmentation, medications, and conditions that lead to malabsorption or impairment in the metabolism of vitamin D. Interpretation of 25(OH)D category relies on the clinical setting, features of the test, and criteria of classification; hence, laboratory procedures and thresholds need to be specified clearly [5–8]. Smoking tobacco is a major modifiable exposure linked to poor health outcomes. There is observational evidence of a relationship between smoking and vitamin D status; however, smoking has been shown to be related to other factors of 25(OH)D, including dietary habits, time spent outdoors, socio-economic status, and body composition. Thus, relationships between smoking and vitamin D status need to be interpreted with care, especially cross-sectional designs [9], [10], [11], [12], [13], [14].

In the present study, the association between current smoking status and serum levels of 25(OH)D was examined in relation to vitamin D status in 100 individuals aged 20 to 40 years, half smokers and half non-smokers..

Objective

The main objective was to evaluate whether the existing smoking status has any impact on the levels of 25(OH)D and the vitamin D status categories in the participants of the study.

Materials and Methods

Study Design and Study Setting

The cross-sectional comparative study was carried out between February 1, 2026, and July 1, 2026, at the Tikrit Teaching Hospital, Iraq. The participants in the study were adults aged between 20-40 years [15].

Subjects and Classification

There were 100 subjects recruited, of which 50 were current smokers and 50 were nonsmokers. Subjects gave informed consent before drawing blood as per study protocol. Current smokers were those who consumed 20 to 60 cigarettes a day and had smoked for a minimum of 2 years up to 15 years. Non-smokers were defined as people who experimented smoking in their early teens (under 20 years old) but never became habitual smokers [15].

Subjects were selected from Tikrit Teaching Hospital, Tikrit, Iraq, and consisted of adult males and females who fit the study requirements. Subjects aged 20-40 years were recruited and grouped based on smoking status (current smoker and nonsmoker). Venous blood samples were drawn when the subjects were in the hospital. Subjects gave written permission/consent before drawing blood. Vitamin D levels were measured in the Tikrit Teaching Hospital laboratory..

Blood Collection and Laboratory Assay

About 3 mL of venous blood was collected in tubes containing gel. The samples were allowed to clot and then centrifuged at 3000 rpm for 10 minutes. The serum was obtained from the centrifugation process and stored in tubes, labeled, and used for measuring 25(OH)D.

The concentration of 25-hydroxyvitamin D (25(OH)D) in serum was measured at the laboratory of Tikrit Teaching Hospital through the use of a Roche Cobas e 411 analyzer. Venous blood samples were taken from the patients attending the hospital and converted to serum before analysis in the laboratory. The results of the laboratory tests for 25(OH)D in serum were reported to the researchers and used in statistical analysis. Using a standardized laboratory instrument and stating the platform of the assay makes the laboratory part of the study clearer [16], [17], [18].

Statistical Analysis

Categorical data is analyzed based on frequencies and percentage. Chi-square tests are used for establishing the relationship between two categorical variables where appropriate. In this case, since there are small numbers in the cells of the smoking versus vitamin D contingency table, the exact 2×3 test was also recreated based on the total number presented. The 25(OH)D level of each person in the revised data set cannot be obtained. Thus, continuous comparison between groups was not calculated [15].

Ethical Issues

Ethical permission of institution and consent of participants were taken in this research. The ethics permission number is not found in the study data collected to prepare the manuscript and hence it has not been made up. In the final submission, the name of the institution and ethics permission number must be provided if possible [15], [16], [17], [18], [19]

Result

Participant Demographic

In total, there were 100 subjects who participated in the study, including 50 who were current smokers and another 50 who were non-smokers. Of these, 82 subjects (82%) were males, while the remaining 18 (18%) were females. The 50 current smokers consisted of 29 subjects (58%) aged 30-40 years and 21 (42%) aged.).

Table 1. Participant characteristics and age distribution.

Characteristic	Category	n (%)
Smoking status	Current smokers	50 (50%)
Smoking status	Non-smokers	50 (50%)
Sex	Male	82 (82%)
Sex	Female	18 (18%)
Age among current smokers	20–29 years	21 (42%)
Age among current smokers	30–40 years	29 (58%)

Vitamin D Level Status

The participants who had low vitamin D level status were 87 participants (87%). All 50 smokers had low vitamin D level status while the number of non-smokers who had low vitamin D status was 37 out of 50 which equals 74%. Out of 50 non-smokers, only 9 participants (18%) had normal status while 4 participants (8%) had high status. There was no smoker in the category of normal or high vitamin D status..

Table 2. Overall distribution of reported vitamin D-status categories.

Vitamin D status	N	%
Low	87	87%
Normal	9	9%
High / slight elevation	4	4%
Total	100	100%

Smoking Exposure Characteristics

In current smokers, cigarette use varied from 20 to 60 cigarettes per day, and the time period for smoking exposure was 2 to 15 years. Among the 50 current smokers, 29 (58%) were aged 30-40 years and 21 (42%) were aged 20-29 years.

Serum 25(OH)D Concentrations

For preparation of the paper, the modified aggregate dataset that we have does not provide individual serum 25(OH)D levels or even the adequate continuous summary statistics at the group level for performing a reproducible comparison between groups. Consequently, in this revised version, no inferential test for continuous data is included.

Vitamin D Status by Age Category

The modified aggregate dataset did not provide the complete cross tabulation for vitamin D status across different ages, and hence, a new age-specific inferential test could not be performed. In the original paper, Pearson $\chi^2 = 0.539$ ($p = 0.764$) had been reported, while the p-value calculated using the reported aggregate information was found to be approximately $p = 0.889$..

Discussion

The main finding of the current cross-sectional study is the association of smoking status and vitamin D status. All 50 participants who were currently smoking were included into the low category of vitamin D, compared to only 37 out of 50 non-smokers. The noted association remained significant in the context of reconstruction of an exact test using reported aggregate counts [12], [13], [20].

There might be multiple ways through which smoking might be related to the vitamin D status. Besides smoking, there might be behavioral and socioeconomic factors, related to vitamin D status and its determinants, such as sun exposure, dietary intake, physical activity, body composition, etc. Moreover, there may be biological influences of tobacco exposure. However, since the current study did not measure several key determinants of vitamin D status, confounding remains a problem [14], [21], [22], [23].

The two study groups varied in terms of age, with significantly different age distributions reported for smokers and non-smokers. Age serves as a potential confounder, due to its relation to vitamin D status and behavioral variables. However, lack of a statistically significant association of age and vitamin D in the reported analysis cannot be viewed as an indication of no association between these two factors. [5], [6], [7], [12], [13], [14], [16], [17], [18], [21], [24]

One should take into account the sparseness of contingency table cells while interpreting the results of vitamin D – category analysis. Even though an association is supported by the reconstruction of exact test, the statistical analysis needs to be carried out using the individual-level data and prespecified vitamin D categories [16], [17], [18].

The study design cannot support any conclusions about the causality between smoking and lower vitamin D status. There are several problems with the study, including small sample size, incomplete description of recruitment methods, absence of cumulative pack-years measurement, and the lack of control for important confounders [12], [13], [15], [22].

Conclusion

Among the 100 adults aged 20–40 years included in this study, current cigarette smoking was associated with a greater prevalence of low vitamin D levels, based on the category defined in the original study. However, this observed relationship does not establish a causal association between cigarette smoking and vitamin D status. Further research involving larger and well-characterized populations, standardized laboratory methods, detailed assessment of smoking exposure, and appropriate multivariable adjustment is needed to confirm these findings.

Recommendations for Future Research

Future studies should consider several methodological improvements to strengthen the reliability and interpretation of findings:

1. A clearly defined and appropriately justified sampling strategy should be used, with transparent reporting of participant recruitment, eligibility criteria, participant flow, and reasons for exclusion.
2. Smoking exposure should be assessed in greater detail by documenting current smoking status, the number of cigarettes smoked per day, duration of smoking, and cumulative exposure expressed as pack-years.
3. Important factors that may influence vitamin D status should be collected and considered during analysis. These include age, sex, body mass index, season, sun exposure, dietary intake, physical activity, socioeconomic status, medication use, and relevant comorbid conditions.
4. Laboratory methods should be described in sufficient detail, including the specific vitamin D assay and analytical platform, manufacturer, calibration procedures, quality-control measures, reference interval, measurement units, and the predefined thresholds used to classify vitamin D status.
5. Where individual-level data are available, future analyses should apply appropriate multivariable regression models to account for potential confounding factors. Effect estimates should be presented together with their corresponding 95% confidence intervals.

6. Ethical reporting should also be strengthened by clearly identifying the institutional ethics committee, providing the relevant approval number, and documenting the informed-consent procedures in accordance with the requirements of the target journal.

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