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ORIGINAL RESEARCH

THE RELATIONSHIP BETWEEN THE COTININE LEVEL IN URINE AND VITAMIN D IN THE UNIVERSITY STUDENTS

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Abstract
Background: Vitamin D deficiency is today acknowledged as a pandemic. Vitamin D deficiency and insufficiency are associated with many chronic diseases, including common cancers, cardiovascular diseases, metabolic syndrome, and infectious and autoimmune diseases.
Objective: This study aims to investigate the relationship between the cotinine level in urine and Vitamin D.
Methods: This study employed a descriptive and relational screening design. It was conducted with 74 smoking university students between January 2019 and March 2020. Data were collected through socio-demographic form and Fagerstrom test for nicotine dependence. Besides, the participating students' blood and urine samples were taken in a suitable environment.
Results: The average age of the participating students was 21.50±2.09. Of all the students, 71.6% were males, 62.2% were exposed to the sun between 12 p.m. and 2 p.m., and the average number of cigarettes smoked daily was 13.52±8.22. The average Vitamin D level in blood was 32.4±15.3 (ng/mL), and the average cotinine level in urine was 1.60±.32 (ng/L). No statistically significant relationships were found between the Vitamin D level and the cotinine level (p<0.05).
Conclusion: Smoking causes diseases and death in many people, and it is a changeable risk factor. Nursing practices on the struggle against smoking are effective. No significant relationships were found between the Vitamin D level in blood and cotinine level in urine. Similar studies are recommended to be conducted with larger groups and participants from different age groups.

KEYWORDS
vitamin D; cotinine; smoking; risk factors; universities, tobacco

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BACKGROUND

Vitamin D is among the vitamins that dissolve in fat. Since in suitable biological environments, it can be synthesized endogenously, it is a sterol group with hormones and hormone precursors. Vitamin D has the most important effect on calcium, phosphor metabolism, and bone mineralization (Bringhurst et al., 2005; Champe et al., 2007). However, studies conducted recently have shown that Vitamin D deficiency and insufficiency could be related to several chronic diseases such as common cancers, metabolic syndrome, cardiovascular diseases, infectious diseases, and autoimmune diseases (Holick, 2007; Hyppönen et al., 2008).

More than 1 billion people worldwide have Vitamin D deficiency of insufficiency. Today, Vitamin D deficiency is acknowledged as a kind of pandemic (Manavi et al., 2015; Wacker & Holick, 2013). A recent study conducted by Uçar et al. (2012) in Ankara, Turkey, reported a very high prevalence of Vitamin D deficiency (51.8%) and 20.7% Vitamin D insufficiency. Sufficient Vitamin D intake and maintenance of the optimum Vitamin D level in serum are highly important both for the bone, calcium, and phosphor mechanism and for general health and well-being. Vitamin D deficiency and insufficiency is a global health problem and could be a risk for wide-spectrum acute and chronic diseases (Pludowski et al., 2013).

The main source of Vitamin D is the formation of Vitamin D3 (cholecalciferol) in skin photochemically from 7-dehydrocholesterol by the type B ultraviolet (UVB) lights *endogenously.* Vitamin D3 is converted to its inactive products as a result of high exposure to sunlight. Vitamin D in the diet is available in the form of ergocalciferol (Vitamin D2) found in plants and cholecalciferol (Vitamin D3) in animal tissues. Vitamin D is mainly in the fish, egg yolks, and liver (Öngen et al., 2008; Uçar et al., 2012). Vitamin D 25(OH)D levels of below 20 ng/mL show Vitamin D deficiency, between 21 and 29 ng/mL show Vitamin D insufficiency, over 30 ng/mL show sufficient level of Vitamin D (ideal level is between 40 and 60), and over 150 ng/mL show vitamin D intoxication (Holick et al., 2011; Uçar et al., 2012).

The use of the sun as a source of Vitamin D is quite difficult due to factors such as cloudy weather affecting solar radiation, air pollution, ozone density, altitude, time of the day, season, differences in skin color, etc. (Tsiaras & Weinstock, 2010). One of the important causes of air pollution, which makes it challenging to use the sun as the source of Vitamin D, is tobacco use. Tobacco use is one of the biggest public health problems globally (Centers for Disease Control and Prevention, 2019; World Health Organization, 2020). The World Health Organization (2017) indicated the smoking prevalence among individuals aged 15 and over as 27.5%. Smoking is more common among university students than in general society (Hossain et al., 2017; Sezer et al., 2018; Tucktuck et al., 2018; Vatansev et al., 2019). Vatansev et al. (2019) reported the smoking rates as 11.6% among medical faculty students. According to a study, including nursing department students, 34.3% of the students smoked (Çifçi et al., 2018). Every year, more than 8 million people lose their life due to this reason. Of these deaths, approximately 1.2 million are caused by exposure to tobacco. Tobacco use is a significant risk factor for cardiovascular diseases, respiratory system diseases, and several cancer types (Centers for Disease Control and Prevention, 2019; World Health Organization, 2020). Continuing to smoke is assumed to be related to the rewarding effect of nicotine. Tobacco use disorder is a difficult disease to treat due to several biological, psychological, and sociological reasons.

The association of Vitamin D with neuropsychiatric diseases, which has recently been subject to much concern, has made the status of Vitamin D in tobacco addicts a topic of interest. Vitamin D is a steroid hormone whose effects mainly on the endocrine and skeleton system have been investigated in previous studies. Recent literature has shown that Vitamin D is related to several diseases ranging from neuropsychiatric to cancer diseases (Feldman et al., 2014; Spedding, 2014; Yüksel et al., 2014). However, the literature still includes little data on the relationship of tobacco consumption with low Vitamin D levels (Afzal et al., 2013; Shinkov et al., 2015).

The studies that investigated the association of tobacco use disorder with Vitamin D showed that 81.9% of tobacco users in the study conducted by Sengezer et al. (2016) had insufficient Vitamin D levels. Exposure to environmental tobacco smoke was reported to be associated with low Vitamin D levels in blood circulation and sinus tissues (Manavi et al., 2020). Besides, a 28-year cohort study conducted with the participation of 9791 individuals showed that 25(OH)D was not associated with other cancers but with high-risk of tobacco-related cancers, which indicated a different, potential relationship between Vitamin D and tobacco (Afzal et al., 2013).

Cotinine is the predominant metabolite of nicotine used as a biomarker for exposure to tobacco smoke. Cotinine concentrations can be detected in blood, urine, or saliva. The urine cotinine concentration levels are higher than those in blood or saliva. This makes urine a more sensitive matrix to detect low-concentration exposure (Avila-Tang et al., 2013). In light of this information, this study aimed to investigate the relationship between the cotinine level in urine and Vitamin D.

METHODS

Study Design

This study employed a descriptive and relational screening design. It was carried out in a university located in eastern Turkey between January 2019 and March 2020.

Sample

The target population was 109 students who were enrolled in the health-related departments of the university and who met the research criteria. The sample was 74 people calculated according to the "formula used in cases where the size of the universe is known" (Kiliç, 2012; Naing et al., 2006). The study included students who attended the school between the dates mentioned above and volunteered to participate in the study to reach the sample size. The inclusion criteria of the study included individuals who smoked, those who did not use sun cream, exposed to sunlight in hands, face, and arms for at least three times a week and for 15 minutes, not obese or cachectic, not pregnant or breastfeeding, had no chronic diseases, did not use medicine regularly, and those who had no limits for consuming liver, eggs, and butter in their diet.

Measures

The data collection tools included the socio-demographic form and the Fagerstrom test for nicotine dependence. Blood and urine samples of the students who answered the questions in the data collection forms were taken on an empty stomach in the morning.

The socio-demographic form. This form has 12 questions that aim to collect data about tobacco use, exposure to sunlight, and dietary preferences.

The Fagerstrom Test for Nicotine Dependence (FTND). The test was developed by Fagerstrom and Schneider (1989) to identify the level of physical nicotine dependence. The reliability and validity of the test for our country were performed by Uysal et al. (2004); Cronbach's Alpha reliability coefficient was reported to be 0.56. Each item of the scale is scored as "0", "1", "2", and "3". The scores of the scale range between 0 and 10. The scores obtained from the scale increases as the nicotine dependence increases. The total scores obtained from the test indicate nicotine dependence in 5 groups in which 0-2 points indicate very low dependence, 3-4 points indicate low dependence, 5 points indicate medium dependence, 6-7 points indicate high dependence, and 8-10 points indicate very high dependence. The scale's Cronbach's alpha value was found 0.72 in the present study.

Data Collection

After the students were informed about the study through the informed consent form, written approval was obtained from those who volunteered to participate in the study. Blood and urine samples were taken in a suitable environment from the students who filled in the Socio-demographic Form and the Fagerstrom Test for Nicotine Dependence; the samples were taken by the researchers who were specialized in nursing. This procedure took about 25-30 minutes. *Taking Urine Samples and Analysis*: The researcher gave the students capped urine collection containers and asked them to sample urine. The urine samples were taken to screw-capped urine specimen collection, and the sample urines were centrifuged at 5000 rpm for 10 minutes. After the urine samples were centrifuged, supernatants were taken to the Eppendorf tubes. The cotinine analysis in urine was performed on the same day using the Human Elisa Urine Kit. After the procedures were completed in line with the guidelines, the results were printed using the microplate reader for necessary statistical analyses.

Taking the Blood Samples and Analysis: 2 ml full blood samples taken by three researchers who were experts in the field were taken to biochemical gel tubes and centrifuged at 5000 rpm for 20 minutes after they waited in room conditions for 20 minutes. The volunteer serums from the centrifuged blood samples were taken to Eppendorf tubes and numbered. Vitamin D levels in blood were analyzed on the same day using the Human Elisa Serum Kit. After the procedures were completed in line with the guidelines, the results were printed using the microplate reader for necessary statistical analyses.

Data Analysis

Data were analyzed in the SPSS package program using descriptive statistics, Kolmogorov- Smirnov, and Spearman correlation tests. Statistical significance was set at <0.05.

Ethical Considerations

Before the study was conducted, approval was received from the Agrı Ibrahim Cecen University Scientific Research Ethics Committee (Document date and number: 30/01/2018-E.2686), and permission was obtained from the institution where the study was conducted. Written consent was received from the students after they were given information about the purpose of the study.

RESULTS

The average age of the participating students was found at 21.50±2.09. Of all the participants, 71.6% were males, and 62.2% were exposed to the sun between 12 p.m. and 2 p.m. An analysis of the tobacco use of the students showed that the average number of cigarettes smoked daily was 13.52±8.22, the average age for starting smoking regularly was 17.18±2.47 years, the average age of trying smoking for the first time was 14.59±3.72 years, and the average duration of smoking was 4.88±2.82 years (**Table 1**).

Table 1 The Participants' Socio-demographic Characteristics (*N*=74)

		n	**%**
Gender	Female	21	28.4
	Male	53	71.6
What time of day are you exposed to the sun more?	8-12	0	0
	12-14	46	62.2
	14-17	28	37.8
	$\overline{X}$ **±SD**		
Age	21.50±2.09 (min= 19, max= 27)		
How many cigarettes do you smoke per day?	13.52±8.22 (min= 1, max= 40)		
How old were you when you started smoking regularly?	17.18±2.47 (min= 12, max= 26)		
When did you try smoking for the first time?	14.59±3.72 (min= 5, max= 24)		
How long have you been smoking? (years)	4.88±2.82 (min= 0.1, max= 12)		

The blood Vitamin D level of the study participants was 32.4 ± 15.3 ng/mL. The lowest Vitamin D level was 1.3 ng / mL, and the highest level was 67.3 ng/mL. The urine cotinine level of the individuals was 1.60 ± .32 ng/L, with 2.34 ng /L as the highest level and 1.25 ng/L as the lowest level (**Table 2**).

Table 2 Average Vitamin D and Cotinine Levels

	$\overline{X}$ **±SD**	**Min.**	**Max.**
Vitamin D Level (serum) (ng/mL)	32.4±15.3	1.3	67.3
Cotinine Level (urine) (ng/L)	1.60 ± .32	1.25	2.34

When the relationship between the participants' cotinine level in the urine and the vitamin D level in the blood was examined, a negative relationship was found. However, no statistically significant association was detected (*p*>0.05) (**Table 3**).

Table 3 Relationship between the Vitamin D Level in Blood and Cotinine Level in Urine

		Vitamin D Level (serum) (ng/mL)
Cotinine level (urine) (ng/L)	*r*	-.010
	p	.930

DISCUSSION

Cotinine in urine is one of the commonly used and accepted bioindicators used for identifying tobacco use or exposure (Thomas et al., 2020). While the urine cotinine levels of non-smokers are below 100 ng/ml, the threshold between active and passive smokers ranges between 20 and 100 ng/ml (Haufroid & Lison, 1998). According to the SRNT Sub-Committee on Biochemical Verification, the urine cotinine value that can commonly be used for distinguishing between smokers and non-smokers is 50 ng/ml (Benowitz et al., 2002). A study that identified the urine cotinine cut-off point distinguishing between passive or active smokers reported the average concentrations as 2200 ± 800 µg l−1 for active smokers. The cut-off point to distinguish active smokers in terms of minimal and high amounts of exposure to tobacco smoke was reported to be 2100 µg l−1 in the same study (Zielińska-Danch et al., 2007).

This study found the average cotinine concentration level of the participating individuals as 1.60 ± .32 ng/L in their urine samples. The literature indicates that this value is in the same category as non-smokers. In their study conducted with health professionals with an average age of 30.3 ± 6.6, Temel et al. (2009) found the average cotinine level as 949.5 ng/ml among non-smokers. It was reported that nicotine metabolism varied between individuals. It was affected by diet, age, gender, pregnancy, and use of medicine and the polymorphisms in the CYP 2A+ gene (Holloway, 2014).

An individual's Vitamin D status is identified by analyzing the 25(OH)D level in blood. Values of less than ten ng / mL show severe Vitamin D deficiency, less than 20ng/mL show Vitamin D deficiency, and between 20 and 30 ng/mL show Vitamin D insufficiency. If the 25(OH)D level is higher than 30 ng/mL, the Vitamin D level is considered to be sufficient, and if this value is more than 150 ng/mL, it is accepted as vitamin intoxication (Fidan et al., 2014; Holick, 2007; Hossein-Nezhad & Holick, 2013; Wacker & Holick, 2013).

The average Vitamin D level of the participants in this study was found to be 32.4±15.3 (ng/mL). In their retrospective study, Solak et al. (2018) found the average serum 25-hydroxyvitamin D level as 15.2 ± 8.8 ng/ml. Other studies investigating the Vitamin D levels also reported that the 25-hydroxyvitamin D level was lower than 20ng/mL (Katrinaki et al., 2016; Mansoor et al., 2010; Yu et al., 2015). This finding of the study is different from the literature, which might result from the studies conducted in other regions and with different age groups.

This study found no relationships between serum Vitamin D and urine cotinine levels. This finding might result from the fact that the urine cotinine level was in the same category as non-smokers. A study conducted with Korean adults reported that the relationship between smoking and Vitamin D level was significant. The researchers also recommended that the causal relationships between smoking and Vitamin D should be investigated through systematic cohort studies (Lee et al., 2019).

Exposure to cigarette smoke was reported to have negative effects on pregnant women. The study reported no differences between the mothers and babies in terms of the serum Vitamin D levels; however, Vitamin D was lower in the group exposed to cigarette smoke (Banihosseini et al., 2013). Also, cotinine blood serum concentrations are reported to affect factors such as gender, ethnicity, dietary supplement intake, exposure to the sun, and Vitamin D concentrations. Actively smoking black women were reported to have the highest Vitamin D deficiency and insufficiency levels than other women (Manavi et al., 2015).

Public health nurses have roles and responsibilities in diagnosing and eliminating the changeable disease risk factors that may affect the health of individuals, families, and societies to protect, maintain and improve health (Bialous et al., 2017). Cigarette use causes diseases and death in many people and is considered to be a changeable risk factor; randomized controlled studies have revealed that nursing practices on the struggle against smoking are effective (Pardavila-Belio et al., 2015; Rice et al., 2017).

Limitation

The primary limitation of the present study is that the urine cotinine level was at a close level with non-smokers. Also, the young age group and small sample size brought some limitations. Future studies are recommended to be conducted with high cigarette exposure groups, more participants, and individuals from various age groups.

CONCLUSION

Smoking causes diseases and death in many people, and it is a changeable risk factor. Nursing practices on the struggle against smoking are effective. This study found no statistically significant relationships between Vitamin D levels in blood and cotinine levels in urine. Similar studies are recommended to be conducted with larger groups and different age groups.

Declaration of Conflicting Interest
The authors report no actual or potential conflicts of interest.

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Author Contributions
All authors contributed to the study conception and design. ABB conceptualized the study. ABB, NU, and MY collected data. ABB and NU conducted the analyses. ABB, NU, and SKA drafted the original version of the manuscript and provided critical revisions. All authors have read and approved the final manuscript.

Data Availability Statement
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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