EXAMINATION OF CORRELATIONS BETWEEN VITAMIN D AND MELATONIN LEVELS WITH SLEEP AMONG WOMEN AGED 18–49 YEARS

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SUMMARY

Objectives: Although clinical research is still going on to determine any relationship between vitamin D and sleep regulation, only few studies have identified the role of vitamin D metabolism in sleep disorders. The current study aims to examine the incidence of vitamin D deficiency/insufficiency in the sample group and its effects on sleep quality and melatonin level.

Methods: A cross-sectional study was designed. A total of 79 women aged 18–49 years who applied to the research and training hospital between 1 October and 30 November 2021 participated in the study. Data were collected using a socio-demographic questionnaire prepared by the authors and the Pittsburgh Sleep Quality Index (PSQI). Blood samples were taken from the participants, also, 25-OH-vitamin D3 and melatonin levels in serum samples were measured by ELISA.

Results: The participants ($n = 79$) were aged 29.61 ± 11.14 years. The mean total PSQI scores of the participants were calculated as 5.77 ± 2.70. We determined that 64.6% of the participants had vitamin D deficiency, 21.5% had vitamin D insufficiency, and 13.9% of the participants were vitamin D sufficient. The mean melatonin level was found to be 24.77 ± 27.77 ng/L. We determined that an increase in the melatonin levels decreases the risk of vitamin D deficiency. Besides, our findings showed a good positive correlation between serum melatonin and 25 OH vitamin D3 levels ($r = 0.544$, $p < 0.001$).

Conclusion: Our results indicate that the correction of vitamin D insufficiency can positively affect melatonin levels, therefore, it may positively contribute to the treatment of sleep disorders related to melatonin deficiency.

Key words: vitamin D deficiency, melatonin, sleep quality, woman

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INTRODUCTION

It is known that vitamin D has an important role in the endocrine system, calcium homeostasis, and bone metabolism. However, evidence over the last twenty years has indicated that vitamin D is also effective on very divergent biological functions such as induction of cell differentiation, inhibition and immunomodulation of cell growth, and control of other hormonal systems (1). Groups at particular risk are pregnant women, non-white people (black people, Hispanics, or individuals with increased skin melanin pigmentation), obese children and adults, and children and adults who are not directly exposed to the sun. About 20–100% of elderly men and women in the USA, Canada and Europe suffer from vitamin D deficiency (2). Vitamin D is associated with many biological functions and systems in the body including calcium homeostasis, prevention of falls and fractures, hypertension, diabetes, metabolic syndrome, cancer, and autoimmune and infectious diseases.

It should be noted that vitamin D deficiency is a global public health problem. Approximately 1 billion people worldwide have vitamin D deficiency, 50% of the population have vitamin D insufficiency. The prevalence of vitamin D deficiency (<20 ng/dl) was found to be 80% in shift workers, 78% in indoor workers, whereas it was 48% in outdoor workers. Therefore, shift workers and indoor workers were identified as high-risk groups for vitamin D deficiency (3). Previous reports indicate that vitamin D levels of workers in most occupational groups are much lower than the levels considered necessary for a healthy body (4). While 47% of African American infants and 56% of Caucasian infants in the US were vitamin D deficient, more than 90% of infants in Iran, Turkey, and India suffer from vitamin D deficiency. Considering vitamin D deficiency rates in the adult population, 35% of adults in the US suffer from vitamin D deficiency, the prevalence was more than 80% among the adult population in Pakistan, India, and Bangladesh. The results of another study showed that 61% of the elderly population in the US, 90% in Turkey, 96% in India, 72% in Pakistan, and 67% of the elderly population in Iran suffer from vitamin D deficiency (5).

Although clinical research is still going on to determine the relationship between vitamin D and sleep regulation, only few studies have identified the role of vitamin D metabolism in sleep disorders. However, vitamin D metabolism is thought to play an important role in sleep disorders and its severity (6).

Low levels of vitamin D have also been found to affect hypothalamus-controlled sleep/wake cycles and are associated with shorter sleep durations. It is known that less sleep time is the underlying reason for sleep disorders including obstructive sleep apnoea, restless legs syndrome, or narcolepsy in adults and paediatric groups (7).

Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone mainly synthesized and secreted by the pineal gland. Extra-pineal melatonin sources are bone marrow, skin, platelets, lymphocytes, retina, and gastrointestinal tract gland. Melatonin was first isolated from bovine pineal gland by Aaron Lerner in 1958 and has been extensively studied by researchers since then (8). The effect of melatonin is not limited to regulating circadian and seasonal rhythms, it modulates the immune system and it also has anti-inflammatory properties (9). Low melatonin concentration was measured in a variety of pathophysiological conditions such as neurodegenerative diseases including Alzheimer's disease, headache, obesity, hypertension, and diabetes (10). Similarly, low melatonin levels have been found to be effective in seasonal depression and sleep disorders (11).

Although both vitamin D and melatonin are essential for cellular physiology, their rhythms are completely opposite. While vitamin D is synthesized in the skin with the help of the sun's ultraviolet rays, melatonin is secreted by the pineal gland and its level increases at night (12).

Although vitamin D deficiency is associated with sleep disorders, there is a lack of solid evidence on the role of vitamin D supplementation in the prevention or treatment of sleep disorders. Therefore, the current study aims to examine the incidence of vitamin D deficiency/insufficiency, which is very common among women of childbearing age, in the sample group and its effects on sleep quality and melatonin level. Our findings revealed that the correction of vitamin D insufficiency can positively affect melatonin levels and therefore can positively contribute to the treatment of sleep disorders related to melatonin deficiency.

MATERIALS AND METHODS

Research Design

A cross-sectional study was designed.

Sample and Population

The population of the study consists of women aged 18–49 years who applied to the internal medicine clinic of the research and training hospital. A sample selection procedure was not performed. The study was carried out with 79 women who applied to the clinic between 1 October and 30 November 2021, who met the criteria and gave consent to participate in the study. Those who used vitamin D supplements in the last 3 months, individuals with chronic liver and kidney disease, any skin disease, type 1 diabetes, endocrine and metabolic diseases, alcoholics and pregnant women, as well as night shift workers were not included in the study.

Data Collection Methods

Data were collected face-to-face using a socio-demographic questionnaire prepared by the authors and the Pittsburgh Sleep Quality Index (PSQI). Also, blood samples were taken to measure the vitamin D and melatonin levels of the participants after 8–10 hours of fasting. Since a dynamic change was observed in plasma melatonin concentration during the day, blood samples were taken between 8.30 a.m. and 11 a.m. The samples were collected within 4 and a half hours at the latest after sunrise according to local conditions.

The socio-demographic questionnaire consists of questions about the participants' age, height, weight, smoking and alcohol use, daily sunlight exposure duration, and questions about nutrition (number of meals per day, variety of food consumed, etc.) status.

Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) was developed by Buysse et al. to identify people with sleep disorders and to determine sleep problems. The validity and reliability study for the Turkish version of the 24-item scale was carried out by Ağargün (13). The total score that can be obtained from the scale varies between 0–21 and a score above 5 indicates poor sleep quality. The overall Cronbach's alpha coefficient of the scale was calculated as 0.804.

Measurement of Serum 25-OH-Vitamin D3 and Melatonin Levels

Blood samples were collected in a serum clot activator tube, centrifuged at 3,000 rpm (10 minutes at room temperature) and serum samples were stored at −80 °C for the ELISA assays. The levels of 25-OH-vitamin D3 (Sun Red, Cat. No. 201-12-5419) and melatonin (Sun Red, Cat. No. 201-12-1014) in the serum samples were analysed using an ELISA kit according to the manufacturer's instructions. Briefly, standards and serum samples were added to a well plate. After that, biotin labelled primer antibody and Streptavidin-HRP were added to the well plates and incubated at 37 °C temperature for 1 h. Then the upper liquid was discarded from the plate and the well plates were washed 6 times using wash buffers. Chromogen solutions were added to the wells and were incubated at 37 °C temperature for 10 minutes in a rotating shaker (dark). Stop solution was added to each well. The absorbance of each microwell was measured at 450 nm using a spectrophotometer (Thermo Multiskan Go). Afterwards, the amounts of 25-OH-vitamin D3 and melatonin were calculated according to the standard curve. Although there is no consensus on the optimal level of 25-OH-vitamin D, a majority of the guidelines accept a vitamin D level above 20 ng/ml (50 nmol/L) as sufficient, between 10 and 20 ng/ml (25–50 nmol/L) as insufficient, and above 10 ng/ml (25 nmol/L) as deficiency (14). Furthermore, melatonin concentration shows a diurnal dynamic variation. Especially, whereas during the day its concentration is maintained at 5 pg/mL levels and this molecule up to the 50–100 pg/ml levels at night. Clinically, the normal reference values of the melatonin molecule in both women and men are accepted as 0–30 pg/mL during the day and 30–150 pg/mL at night (15).

Data Analysis

R software ver. 2.15.3 (R Core Team, 2013) was used to analyse the data. Findings were reported as the minimum, maximum, mean, standard deviation, first quartile, third quartile, frequencies, and percentages. The Shapiro-Wilk test was applied to determine whether the data is normally distributed. The Mann-Whitney U test was used for comparing the variables between two non-normally distributed groups and the Kruskal-Wallis test was conducted for analysing more than two groups. The correlation between 25-OH-vitamin D3 and melatonin was determined by the Spearman's correlation coefficient. The Pearson's correlation coefficient analysis was performed to measure the strength of the inter-variable relationships. A p-value less than 0.05 was accepted as statistically significant.

Ethical Permissions

Prior to the research, ethical permission was obtained from the Non-Interventional Clinical Research Ethics Committee of Amasya University (dated 9 September 2020, No. 19096) and research permission (dated 18 August 2020, No. 68724985-044) was obtained from the Provincial Directorate of Health to carry out the study in the hospital. Also, informed consent forms were obtained from the study participants.

RESULTS

The participants were aged 18–49 years with a mean age of 29.61 ± 11.14 years. Participants' average daily duration of sun exposure was 2.22 ± 1.37 hours. We found that 38.0% of the participants' sleep quality was good, whereas 62.0% had poor sleep quality. Furthermore, it was determined that 64.6% of the participants had vitamin D deficiency, 21.5% were vitamin D insufficient, and finally, 13.9% of the participants had vitamin D sufficiency. The mean total PSQI scores of the participants were calculated as 5.77 ± 2.70. The socio-demographic characteristics of the participants are given in Table 1.

No statistically significant differences were determined in the PSQI total scores of women regarding their age, BMI, cigarette smoking and alcohol use status ($p > 0.05$). The comparison of the vitamin D and melatonin levels with PSQI results is given in Table 2.

***Table 1.** Socio-demographic characteristics of participants (N = 79)*

Characteristics	n	%
BMI		
Underweight	8	10.1
Normal	39	49.4
Overweight	19	24.1
Obese	13	16.5
Use of hijab or head-cover		
Using	44	55.7
Not using	35	44.3
Cigarette smoking		
Never	65	82.3
Still using	9	11.4
Quitted	5	6.3
Alcohol use		
Never	75	94.9
Still using	3	3.8
Quitted	1	1.3
Omega-3 supplement use		
Yes	11	13.9
No	68	86.1
Pittsburgh Sleep Quality Index		
Good sleep quality	30	38.0
Poor sleep quality	49	62.0
Vitamin D level		
Deficient	51	64.6
Insufficient	17	21.5
Sufficient	11	13.9
Total	79	100.0

***Table 2.** Comparison of vitamin D and melatonin levels with PSQI sleep status (N = 79)*

Characteristics	PSQI		p-value
	Good sleep quality Median (Q1, Q3)	Poor sleep quality Median (Q1, Q3)	
Vitamin D conc. (nmol/L)	16.86 (12.72, 36.70)	14.93 (13.13, 31.54)	0.801[a]
Melatonin level (ng/L)	16.88 (8.90, 34.66)	15.04 (9.85, 26.51)	0.852[a]
Vitamin D level – n (%)			
Deficient	18 (35.3)	33 (64.7)	0.683[b]
Insufficient	8 (47.1)	9 (52.9)	
Sufficient	4 (36.4)	7 (63.6)	

PSQI – Pittsburgh Sleep Quality Index
[a]Mann-Whitney U test
[b]Pearson's chi-square test

Table 3. Comparison of vitamin D and melatonin levels

Characteristics	Melatonin Median (Q1, Q3)	p-value
Vitamin D		
Deficient	11.48 (8.46, 17.37)	<0.001* c
Insufficient	28.81 (16.21, 47.17)	
Sufficient	34.85 (21.97, 76.61)	

cKruskal-Wallis test
*p<0.05

As shown in Table 3, a statistically significant difference was determined between vitamin D and melatonin levels ($p<0.001$). Accordingly, it was determined that the melatonin concentration of the participants with vitamin D deficiency was lower than those with insufficient and sufficient vitamin D levels ($p=0.001$ and $p<0.001$, respectively).

Multinomial logistic regression analysis was performed to examine the combined effect of the melatonin levels and PSQI scores on the vitamin D levels. As shown in Table 4, higher melatonin concentrations, a lower risk of vitamin D deficiency and OR value above 1 indicates that the likelihood of the relevant category increases as the value increases. The probability of adequate vitamin D increases by 1.081 times per 1 unit increase in melatonin level.

Furthermore, our results indicate a moderate positive correlation between melatonin and 25 OH vitamin D3 levels ($r=0.544$, $p<0.001$). The correlation between vitamin D and melatonin levels is shown in Figure 1.

DISCUSSION

The effects of vitamin D and melatonin hormone levels on sleep patterns are one of the most frequently examined topics in recent years. Our findings showed that 62% of women had poor sleep quality. Consistent with our results, Şahin et al. found that 63.6% of adults had poor sleep quality (16). Similarly, Landry et al. showed that 64% of adults over 55 years suffer from poor sleep quality (17). The fact that the majority of the population suffers from poor sleep quality suggests that many different variables affect sleep. Studies examining different variables affecting sleep may play an important role in improving sleep quality. Within this context, the current study aims to examine the incidence of vitamin D deficiency/insufficiency, which is very common among women of childbearing age, in the sample group and its effects on sleep quality and melatonin level.

Vitamin D insufficiency is a common problem in almost all age groups in many countries. Our findings showed that 64.6% of the women had vitamin D deficiency and 21.5% had vitamin D insufficiency (Table 1). The average annual population prevalence in the US, Canada, and Europe is 24.0%, 36.8%, and 40.4%, respectively (18, 19). Moreover, Ciğerli et al. found that 66% of 2,488 patients in a university hospital had vitamin D deficiency and 24% had vitamin D insufficiency (20). Based on these reports, it can be argued that vitamin D deficiency/insufficiency is a common global problem in Turkey and around the world as well. The findings of our study indicate that there is a vitamin D deficiency problem in this region.

No significant differences were found in the PSQI scores for age, BMI values, cigarette smoking, and alcohol use status. Some

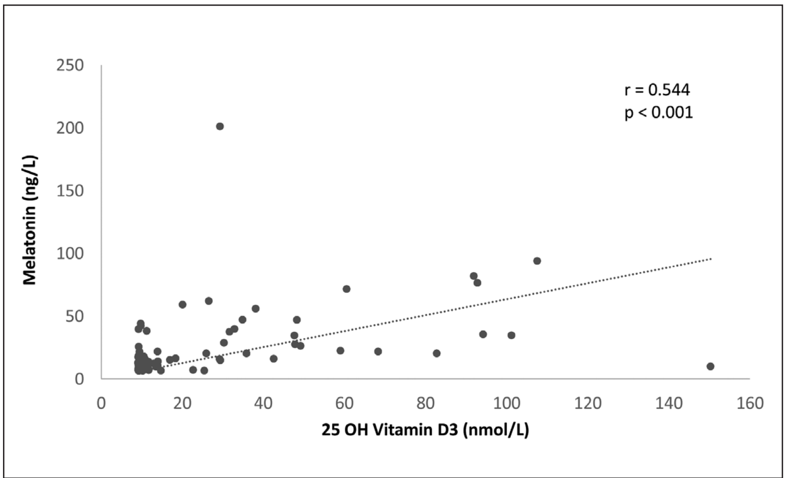

Fig. 1. Correlation between vitamin D and melatonin levels.

Table 4. Relation of vitamin D level to melatonin level and PSQI scores

Characteristics		B	OR (95% CI)	Wald	p-value
Vitamin D (insufficient)	Melatonin	0.075	1.078 (1.035–1.123)	12.869	<0.001*
	PSQI (good)	0.536	1.710 (0.482–6.060)	0.691	0.406
Vitamin D (sufficient)	Melatonin	0.078	1.081 (1.036–1.127)	13.017	<0.001*
	PSQI (poor)	0.123	1.131 (0.252–5.070)	0.026	0.873

PSQI – Pittsburgh Sleep Quality Index; OR – odds ratio; CI – confidence interval
*p<0.05; multinomial logistic regression analysis, vitamin D deficiency reference category.

previous research also indicated that there was no link between obesity and sleep quality. In a study involving 927 women aged 16–40 years, in another study carried out by Hulth et al. with nurses working on rotating shifts, and in other study with 2,446 adults, no significant correlation was found between individuals with BMI > 30 and above and their PSQI scores (21, 22). On the other hand, many studies found that obesity negatively affects sleep quality (23, 24). The different results obtained in our study were thought to be caused by parameters other than BMI that affect sleep quality. We believe that objective measurement of BMI values and sleep quality index, as well as keeping parameters such as stress and vitamin D constant might affect the results.

We found that vitamin D levels did not have a significant effect on PSQI scores. Vitamin D has both direct and indirect effects on the regulation of sleep (Table 2). The increasing evidence suggests that there is a relationship between vitamin D deficiency/insufficiency and sleep disorders; and vitamin D is thought to have a role in the regulatory mechanisms of sleep-wake rhythms (25). Some previous research supports our findings. A previous study found that 43.5% of 92 pregnant women had poor sleep quality and there was no significant relationship between vitamin D levels and PSQI scores (26). This result, i.e., no difference between the groups, was probably caused by the insufficient sample size. Some studies examining the relationship between vitamin D and sleep quality have obtained different results; these studies indicate that vitamin D has both a direct and an indirect role in sleep regulation. Furthermore, vitamin D may also indirectly affect sleep quality through non-specific pain syndromes such as restless legs syndrome and obstructive sleep apnoea syndrome (25). There is increasing evidence that vitamin D may play a role in the regulation of sleep. According to the National Health and Nutrition Examination Survey (NHANES), lower vitamin D levels are associated with shorter sleep durations (27). Moreover, it was found that sufficient vitamin D levels are required for maintaining sleep and help reduce night wake-ups (28). On the other hand, some studies reported that vitamin D is effective not only on sleep duration but also on sleep quality. Jung et al. carried out a cross-sectional study with indoor workers in the manufacturing industry between January–December 2015 and found a significant association between serum vitamin D deficiency and poor sleep quality (29). In a cohort study conducted with women in the first trimester of pregnancy in Singapore, vitamin D deficiency was found to be associated with poor sleep quality (30).

Our results show that vitamin D levels were positively correlated with melatonin levels (Table 4). In the studies, sleep quality in women was examined through PSQI scores, blood samples were also taken to measure melatonin levels. A decrease in the melatonin levels causes poor sleep quality (31). Furthermore, it is known that vitamin D plays a role in the production of the hormone melatonin which affects circadian rhythms and sleep regulation (25). The fact that there are no studies that have examined the relationship between vitamin D and melatonin levels is the novelty of the current study. Our findings indicated that an increase in the melatonin levels reduces vitamin D insufficiency. Moreover, our findings revealed a good positive correlation between melatonin and 25 OH vitamin D3 levels in all samples ($r = 0.544$, $p < 0.001$) (Fig. 1). It has been determined that melatonin deficiency, which is one of the factors affecting sleep quality and thought to cause sleep disorders, is related to vitamin D. Therefore, elimination of vitamin D deficiency/insufficiency, which is a common problem among the public, with exposure to sunlight, food and vitamin D supplements, and elimination of melatonin deficiency with the help of supplements may help to overcome sleep quality problems and sleep-related disorders.

CONCLUSION

The effects of vitamin D and melatonin levels on sleep patterns is one of the most frequently examined topics recently. Previous experimental research revealed that vitamin D is effective in many body systems. Besides, vitamin D is important in sleep duration and quality. The relation of vitamin D to melatonin is also critical for normal development. In the present study, a significant relationship was found between vitamin D and melatonin levels. The obtained important results might be a reference for further studies. Accordingly, the following recommendations were made based on our findings:

- The relationship between vitamin D and melatonin should be examined with larger sample sizes.
- We thought that particular factors such as BMI, nutritional status, and daily sun exposure duration affect vitamin D and melatonin levels, however, we could not obtain a significant correlation. In this regard, these variables should be examined in different groups.
- Screening applications can be carried out to ensure all populations have adequate levels of vitamin D, which is found to be effective on the sleep hormone.

Authors' Contributions

ZİS and BY designed the study, analysed the data and wrote the manuscript; AK and ADD collected blood samples; BY performed the ELISA experiments; ZİS and SİI performed the socio-demographic questionnaire and Pittsburgh Sleep Quality Index assessments. All authors critically revised the manuscript and approved the final version of the submitted manuscript.

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Conflict of Interests

None declared

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