

# Sleep quality and its association with psychological distress and sleep hygiene: a cross-sectional study among pre-clinical medical students

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## ABSTRACT

**Objective:** This study aimed to investigate subjective sleep quality and its association with demographics, psychological health, and sleep hygiene related behaviors in pre-clinical medical students. **Methods:** In this cross-sectional study, a self-administered questionnaire consisting of demographics, sleep hygiene behaviors, Pittsburgh Sleep Quality Index (PSQI), and Depression, Anxiety and Stress Scale-21 (DASS21) was handed out to all medical students of Tehran University of Medical Sciences at pre-clinical stage. **Results:** The questionnaire was filled out by 553 (89.7%) of 616 students approached. About 60% of our sample had a global PSQI score of more than 5 (cut off of poor sleep quality) with mean global PSQI score of 6.32 (SD=2.72). The prevalence of moderate to extremely severe depression, anxiety and stress scores were 26.1%, 29.61%, and 14.5% respectively. Poor sleep quality was associated with later year in the school, psychological distress and several lifestyle behaviors. Constructing a multivariate logistic model, depression, anxiety and some sleep hygiene behaviors were significantly associated with higher PSQI score. **Discussion:** Our findings suggest that poor sleep quality is a common problem among pre-clinical medical students and is associated with some psychological symptoms and sleep hygiene behaviors. This issue demonstrates necessity of interventions to improve the sleep quality in this population group.

**Keywords:** sleep; Students; Medical; behavior.

## INTRODUCTION

Pre-clinical medical students are in a critical period of acquiring the necessary knowledge and experience that would be useful for the rest of their education and career. Therefore, this period necessitates complete physical and mental health. In addition, high level of academic burden on students, exposes them to an increased risk of different sleep problems and its related consequences. In one study prevalence of daytime sleepiness is estimated to be as high as about 50% amongst students, whilst it is only 36% amongst the general population<sup>1</sup>. In another study, it was shown that 40.6% of medical students suffer from poor sleep quality<sup>2</sup>.

It can be hypothesized that several lifestyle behaviors, environmental and psychological factors were associated with poor sleep quality and quantity. Sleep hygiene is a set of behaviors that is believed to have a positive effect on both sleep quality and quantity. Alcohol use, caffeine and energy drinks, stimulants and frequent use of technology such as phone or computers before sleep are among common behaviors that are incompatible with sleep hygiene<sup>3-9</sup>. Environmental conditions like living in dormitories have been associated with poor sleep quality in previous studies<sup>10,11</sup>. Psychological problems such as depression, anxiety, and stress have been associated with sleep disorders such as insomnia<sup>12</sup>.

Clinical stage of medical training starts at the fourth year in the usual curriculum of the Iranian medical schools and it's associated with the night calls and shifts. Whilst public student communities and some special populations have been studied<sup>3,13</sup>, to the best of our knowledge, no extensive study on the sleep condition and its associated factors among medical students at pre-clinical stages has been carried out. In this study, we aimed to focus on this distinct subgroup of students and we aimed to (1) assess sleep quality amongst different subgroups of medical students of Tehran University of Medical Sciences at the pre-clinical stage, and then to (2) identify specific lifestyle behaviors, environmental and psychological factors associated with poor sleep quality amongst them.

## MATERIAL AND METHODS

### Participants and procedures

This is a cross-sectional questionnaire-based study conducted from December 2013 to January 2014 and all medical students of Tehran University of Medical Sciences at first, second, and third years (616 students) were invited to participate. Medical students at this level of education are at pre-clinical stage and do not have night calls or shifts. The questionnaires were administered on paper. Time for the distribution of the self-administrated questionnaires was chosen carefully paying attention to the exams schedule of the students such that they had no exam over a period of 2 weeks before and after filling out the questionnaires. The study protocol was approved by institutional review board and ethic committee of Tehran University of Medical Sciences.

## MEASURES

### Demographics and sleep hygiene

The survey included demographic questions assessing age, gender, and year at medical school, marital and residential status and the number of their roommates. The latter includes the number of people who shared the same living environment in the dormitory (e.g, a suite or a room). We also assessed students' sleep hygiene and lifestyle behaviors by asking them 10 questions with responses including 'never', 'sometimes', 'often' and 'almost always' in regard to taking afternoon naps lasting at least one hour, going to bed at different times from day to day, getting out of bed at different times from day to day, using bed for activities other than sleeping, consumption of caffeinated drinks such as coffee, teas and energy drinks before bedtime, using electronic devices before bedtime, going to bed late in order to study for exams, difficulty sleeping because of different sleep schedules of roommate(s), having an uncomfortable room and sleeping on an uncomfortable bed. We customized questions based on previous similar studies<sup>4,14</sup>. Each question was analyzed separately with no global score.

### Sleep quality

Pittsburgh Sleep Quality Index (PSQI) was used to assess students' subjective sleep quality. This is an internationally recognized tool for the evaluation of sleep quality during a preceding month and consists of 19 items which are combined to form seven subscales: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. Each item was scored on a 3 point scale (ranging from 0 for non-difficulty to 3 for severe difficulty) and the combination of seven subscales yields the global PSQI score which ranges from 0 to 21. Scores greater than 5 have been shown to be indicative of poor sleep quality.

The PSQI has a high degree of internal consistency with an overall reliability coefficient (Cronbach's  $\alpha$ ) of 0.83 in the original article. The global cutoff score of 5 for distinguishing poor from good sleep quality has a sensitivity of 86.9% and a specificity of 86.5%<sup>15</sup>. Although these figures were calculated based on a study with different sample properties (52 controls, 34 poor sleepers with major depression, 62 physician-referred poor sleepers; with mean ages of 59.9, 50.9 and 44 respectively), the PSQI has been previously used in several studies to assess medical students' sleep quality<sup>3,16</sup>. Reliability and validity of the Persian version of PSQI (PSQI-P) were verified in a study conducted by Farrahi Moghaddam et al.<sup>17</sup> The overall reliability coefficient of the questionnaire was 0.73 in this study.

### Depression, anxiety, and stress

Depression, Anxiety, and Stress Scale-21(DASS-21) was used to assess students' mental health. Recommended cutoff points for each subscale are as follows: Depression [normal (0-9), mild (10-13), moderate (14-20), severe (21-

27), and extremely severe (28+)], Anxiety [normal (0-7), mild (8-9), moderate (10-14), severe (15-19), and extremely severe (20+)], Stress [normal (0-14), mild (15-18), moderate (19-25), severe (26-33), and extremely severe (34+)]<sup>18</sup>. Reliability and validity of the Persian translation of DASS-21 were assessed and verified in a study conducted by Asghari et al.<sup>19</sup>. It had an acceptable internal consistency with an overall reliability coefficient (Cronbach's  $\alpha$ ) of 0.94 for total score and 0.85, 0.85, and 0.87 for depression, anxiety, and stress subscales respectively. The overall reliability coefficient of the questionnaire was 0.90 in this study.

### Statistics

The student's independent t-test or analysis of variance test was applied to assess the differences in global PSQI between two or more than two independent groups respectively. Chi-square test was used to compare categorical variables in good *vs.* poor sleepers. Pearson's correlation coefficient was applied to assess association between the number of roommates and PSQI score. Data was tested for normality before applying parametric analysis. All variables with a *p*-value <0.1 in univariate analysis plus gender were entered into a multiple logistic regression model.

As global PSQI score cannot be calculated if any data were missing, questionnaires with missing PSQI values were eliminated from the data analysis. Other missing data were of small amount and missing pattern were similar between independent groups, so after checking for random distribution, a pairwise deletion method was used. All analyzes were done using the general statistical package Stata SE 12.0 (StataCorp LP, College Station Texas) and *p*-values less than 0.05 were considered significant.

## RESULTS

### Demographics

The questionnaire was filled out by 553 (89.7%) of the 616 students approached (63 questionnaire were not returned or eliminated due to missing data in PSQI), including 268 (48.5%) males and 281 (50.8%) females. Of these, 177 (32%) were first-year, 84 (15.2%) second-year, and 292 (52.8%) third-year medical students (our data is compatible with the total number of students at each year) with a mean age of 21.69 (SD=1.11) ranging from 19 to 27 years.

The majority of students declared that they live with their families or in university dormitories (262 [47.4%] or 230 [41.6%] respectively), and the remaining (58 [10.5%]) either live alone, or with a friend or other forms. From those who live in dormitories, the number of roommates ranges from 1 to 9, with the mean of 4.15 (SD=1.47) roommates. Only 4 (0.7%) students in this survey were married, all others were single.

### PSQI

The mean component scores and mean global scores are shown in Table 1. Among all students, the mean global PSQI score was 6.32 (SD=2.72). There were 332 (60.0%) participants

**Table 1.** PSQI global and subscale means.

Sleep quality	Mean	SD
Global PSQI	6.32	2.72
Subscales		
Subjective sleep quality	1.08	0.71
Sleep latency	0.97	0.86
Sleep duration	1.19	0.84
Habitual sleep efficiency	0.22	0.19
Sleep disturbances	0.89	0.47
Use of sleep medications	0.12	0.14
Daytime dysfunction	1.84	0.90

PSQI=Pittsburgh Sleep Quality Index.

with a global PSQI score >5 which are classified as "poor sleepers", and 221 (40%) with a score of  $\leq 5$  which are classified as "good sleepers". Subjective sleep quality, sleep duration and daytime dysfunction had means above 1, identifying these components as the main contributors to the global PSQI score<sup>3,20</sup>.

Analysis of sleep timing shows that 129 (23.32%) students go to bed before midnight and 424 (76.68%) after midnight. Mean sleep duration was 6.32 (SD=1.43), and only 184 (33.3%) reported sleeping 7 hours or more as the "minimum" required sleep time in this generation according to National Sleep Foundation's recommendation<sup>21</sup>, considering that "optimal" sleep duration may be even longer<sup>22</sup>. The mean global score of the different demographic subgroups is shown in Table 2. According to the data in this table, earlier class years reported better sleep quality compared with the later class years (*p*-value=0.026).

A post-hoc Tukey test showed that global PSQI score of first year and second year students differs significantly (*p*-value=0.02), while other pairs doesn't have any significant difference. There was no significant difference in sleep quality based on gender and residency status. There was also no significant association between number of roommates and global PSQI score ( $r=-0.02$ , *p*-value=0.85). Association between sleep quality and marital status couldn't be investigated because of the small frequency of married students.

### DASS-21

Mean DASS-21 score for depression, anxiety, and stress were 9.70 (SD=6.99), 6.93 (SD=5.96), 6.01 (SD=3.42) respectively. Frequency and percentage of students with psychological distress are shown in Table 3. An overall prevalence of moderate to very severe depression, anxiety and stress was founded to be 26.1%, 29.61% and 14.5%, respectively. The students with better psychological status had significantly lower global PSQI scores (*p*-value<0.0001).

A post hoc Tukey test showed that all pairs of psychological levels have significant difference in global PSQI score (*p*-value<0.05) except mild *vs.* moderate and moderate *vs.* severe levels in depression, anxiety and stress category; in addition to severe *vs.* extremely severe levels in depression and anxiety category; and normal *vs.* mild levels in anxiety category.

**Table 2.** Association between demographics and sleep quality (mean PSQI score).

Characteristics	Number (%)	Mean (SD) PSQI Score	p-value
Year in the curriculum			
1		5.97 (2.47)	
2	84 (15.2%)	6.93 (3.35)	0.026a
3	292 (52.8%)	6.36 (2.63)	
Missing data	0 (0%)		
Gender			
Male	268 (48.5%)	6.21 (2.63)	
Female	281 (50.8%)	6.44 (2.80)	0.311
Missing data	4 (0.7%)		
Residency			
With family	262 (47.4%)	6.18 (2.76)	
Dormitory	230 (41.6%)	6.58 (3.07)	0.496
Other <sup>b</sup>	58 (10.5%)	7.17 (2.63)	
Missing data	2 (0.5%)		
Marital status <sup>c</sup>			
Single	548 (99.1%)	6.30 (2.70)	
Married	4 (0.7%)	8.50 (4.66)	
Missing data	1 (0.2%)		
Number of roommates			
1-3	67 (29.1%)	6.31 (2.45)	
4-6	141 (61.3%)	6.54 (2.62)	0.913
7-9	22 (9.6%)	6.32 (2.83)	
Missing data	0 (0%)		

PSQI=Pittsburgh Sleep Quality Index  
SD=Standard Deviation

<sup>a</sup> Post- hoc test showed that the difference is between the first year group and the second year group ( $p$ -value=0.02)

<sup>b</sup> Including alone, with friend or other undetermined forms.

<sup>c</sup> Because of low number of married students,  $p$ -value was not calculated.

## Sleep hygiene

Frequency and percentage of responses “never or sometimes” and “often or almost always” to each question are as shown in Table 4. We grouped the responses to summarize the data, however statistical analysis without grouping the responses showed similar results. According to these results, poor sleepers tend to ‘take daytime naps lasting one hour or more’, ‘go to and out of bed at different times from day to day’, ‘drink caffeine-containing drinks within 4 hours before bedtime’, ‘use electronic devices before bedtime’, ‘go to bed late because they have to study’, ‘have a disturbed sleep due to the different sleep-wake schedules of their roommates’, and/or ‘sleep in an uncomfortable room’.

### Associated factor with poor sleep quality

To evaluate factors associated with poor sleep quality, we constructed a multivariate logistic model from all variables with a  $p$ -value < 0.1 in univariate analysis plus gender. Global PSQI score > 5 was considered as the dependent variable in the logistic regression model. As presented in Table 5, depression, anxiety and some sleep hygiene behaviors were found to be significantly associated with poor sleep quality.

**Table 3.** Association between psychological disorders and sleep quality (mean global PSQI score).

Psychological disorder	Number (%)	Mean Global PSQI score (SD)	p-value
Depression <sup>a</sup>			
Normal	295 (53.3%)	5.52 (2.30)	
Mild	98 (17.7%)	6.53 (2.21)	<0.0001
Moderate	101 (18.3%)	7.40 (2.62)	
Severe to extremely se-vere	38 (6.9%)	9.32 (3.87)	
Missing data	21 (3.8%)		
Anxiety <sup>b</sup>			
Normal	319 (57.7%)	5.67 (2.37)	
Mild	62 (11.2%)	6.39 (2.39)	<0.0001
Moderate	101 (18.3%)	6.98 (2.70)	
Severe to extremely se-vere	59 (10.7%)	8.61 (3.18)	
Missing data	12 (2.2%)		
Stress <sup>c</sup>			
Normal	381 (68.9%)	5.22 (2.84)	
Mild	72 (13%)	5.82 (2.35)	<0.0001
Moderate	53 (9.6%)	7.10 (2.48)	
Severe to extremely se-vere	24 (4.3%)	7.85 (2.47)	
Missing data	23 (4.2%)		

PSQI=Pittsburgh Sleep Quality Index

SD=Standard Deviation

<sup>a</sup> Post- hoc test showed that the difference is significant in all levels ( $p$ -value<0.05) except mild vs. moderate, moderate vs. severe and severe vs. extremely severe levels.

<sup>b</sup> Post- hoc test showed that the difference is significant in all levels ( $p$ -value<0.05) except normal vs. mild, mild vs. moderate, moderate vs. severe and severe vs. extremely severe levels.

<sup>c</sup> Post- hoc test showed that the difference is significant in all levels ( $p$ -value<0.05) except mild vs. moderate and moderate vs. severe levels.

## DISCUSSION

Pre-clinical students go through an intensive course before entry into the clinical wards and contact with patients. It is thus useful to monitor the well-being of these students before they proceed to the next stage. There are limited researches investigating sleep habits of this subgroup which can have both health and academic implications. Our study aimed to evaluate sleep quality and its related factors amongst medical students at the pre-clinical stage.

Our results showed that over half of participant in our study revealed poor sleep quality and about 60% of our sample had a global PSQI score of more than 5 (cutoff point for poor sleep quality) with mean global PSQI score of 6.32 (SD=2.72)<sup>3,16,20</sup>. This can be explained by several factors that were partially investigated in this study. We observed no significant difference in sleep quality among male and female students, which is consistent with the findings of another study conducted by Cates et al.<sup>20</sup>. However, some studies have reported that female medical students have a poorer sleep quality compared with male medical students<sup>16,23</sup>. It seems likely that cultural differences between countries may play a role in the differences between these results.

The differences in sleep quality between students of different year groups observed in this study is in contrast with the

**Table 4.** Association between sleep hygiene and sleep quality.

Question	Never & Sometimes (%)	Often & Almost Always (%)	Missing data	p-value <sup>a</sup>
I take daytime naps lasting one hour or more	334	217	2	
Poor sleepers <sup>b</sup>	179 (53.9%)	151 (45.5%)	2 (0.6%)	<0.0001
Good sleepers <sup>c</sup>	155 (70.1%)	66 (29.9%)	0	
I go to bed at different times from day to day	428	120	5	
Poor sleepers	237 (71.4%)	91 (27.7%)	4 (1.2%)	<0.0001
Good sleepers	191 (86.4%)	29 (13.2%)	1 (0.5%)	
I go out of bed at different times from day to day	447	79	27	
Poor sleepers	248 (74.7%)	63 (19%)	21 (6.3%)	<0.0001
Good sleepers	199 (90%)	16 (7.2%)	6 (2.7%)	
I use bed for activities other than sleeping	335	216	2	
Poor sleepers	193 (58.1%)	138 (41.6%)	1 (0.3%)	0.084
Good sleepers	142 (64.3%)	78 (35.3%)	1 (0.5%)	
I drink caffeine containing drinks such as tea, coffee or energy drinks within 4 hours before bedtime	401	151	1	
Poor sleepers	221 (66.6%)	110 (33.1%)	1 (0.3%)	<0.0001
Good sleepers	180 (81.4%)	41 (18.6%)	0	
I use electronic devices such as TV, computer or cell phone before bedtime	105	446	2	
Poor sleepers	47 (14.2%)	283 (85.2%)	2 (0.6%)	<0.0001
Good sleepers	58 (26.2%)	163 (73.8%)	0	
I go to bed late because I have to study my lessons	375	171	7	
Poor sleepers	216 (65.1%)	112 (33.7%)	4 (1.2%)	0.049
Good sleepers	159 (71.9%)	59 (26.7%)	3 (1.4%)	
My roommates prevented me from sleeping because of their different sleep-wake schedule	433	53	67	
Poor sleepers	248 (74.7%)	43 (43%)	41 (12.3%)	0.001
Good sleepers	185 (83.7%)	10 (4.5%)	26 (11.8%)	
I sleep on an uncomfortable bed	512	35	6	
Poor sleepers	303 (91.3%)	26 (7.8%)	3 (0.9%)	0.071
Good sleepers	209 (94.6%)	9 (4.1%)	3 (1.4%)	
I sleep in an uncomfortable room	517	33	3	
Poor sleepers	302 (91%)	28 (8.4%)	2 (0.6%)	0.003
Good sleepers	215 (97.7%)	5 (2.3%)	1 (0.5%)	

<sup>a</sup> p-value is referred to categories of subjects (poor sleepers vs. good sleepers)

<sup>b</sup> defined as PSQI global score > 5a defined as PSQI global score >5

<sup>c</sup> defined as PSQI global score ≤ 5

results of previous studies<sup>3</sup>. Later years reported worse sleep quality compared with the former class years. One explanation for this phenomenon can be that later year students face a relatively heavier educational burden and this issue maybe affects the psychological distress among them. Another hypothesis is that as students get older, they take more time with their friends instead of their families in the university and dormitories, so this fact can adversely affect sleep hygiene.

High prevalence of psychological distress and its strong association with poor sleep quality amongst medical students were also observed in the present study. Similar results have been reported by previous studies using DASS-21. A depression prevalence of 37.2%, an anxiety prevalence of 63%, and a stress prevalence of 23.7% among Malaysian medical students have been reported<sup>24</sup>. According to a study in Saudi Arabia, the

prevalence of moderate to extremely severe level of depression, anxiety, and stress among Saudi Arabian medical students were 52.4%, 46%, and 56% respectively<sup>25</sup>.

It seems that high academic burden on medical students plays a major role in the prevalence of psychological distress. Although sleep disturbance can be either a cause or a symptom of psychological distress or simply be a comorbidity, there is some evidence that shows psychological distress can be caused by insomnia<sup>26</sup>. In view of these, prompt attempt should be made to better understand the nature of relation between sleep status and psychological distress. Studies are needed to find out the most important causes and consequences of psychological distress.

Sleep hygiene has a considerable role in the quality of sleep among medical students. Poor sleep hygiene have been

**Table 5.** Multiple regression model of factors associated with poor sleep quality.

Characteristics	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value(For Adjusted OR)
Year in the curriculum			
1	1		
2	0.68 (0.54-1.48)	0.91 (0.52-1.61)	0.76
3	1.13 (0.77-1.65)	0.98 (0.64-1.51)	0.94
Gender			
Female <sup>a</sup>	0.78 (0.55-1.10)	1.34 (0.93-1.97)	0.12
Psychological disorder			
Depression <sup>b</sup>	2.22 (1.28-3.24)	1.86 (1.17-2.82)	0.007
Anxiety	2.63 (1.75-3.82)	1.93 (1.15-2.69)	0.009
Stress	1.20 (0.69-2.11)	1.48 (0.90-2.44)	0.12
Sleep hygiene behaviors			
I go to bed at different times from day to day <sup>c</sup>	2.10 (1.60-2.77)	1.87 (1.07-3.26)	0.03
I go out of bed at different times from day to day	2.03 (1.53-2.71)	1.27 (0.77-2.08)	0.35
I drink caffeine containing drinks such as tea, coffee or energy drinks within 4 hours before bedtime	1.76 (1.43-2.18)	2.20 (1.46-3.32)	<0.0001
I use electronic devices such as TV, computer or cell phone before bedtime	1.64 (1.33-2.01)	1.87 (0.74-4.69)	0.18
I go to bed late because I have to study my lessons	1.27 (1.02-1.58)	1.11 (0.63-1.95)	0.73
My roommates prevented me from sleeping because of their different sleep-wake schedule	1.55 (1.20-2.02)	1.25 (0.84-1.88)	0.27
I sleep on an uncomfortable bed	1.44 (1.06-1.94)	1.07 (0.62-1.85)	0.80
I sleep on an uncomfortable room	1.79 (1.32-2.46)	1.14 (0.69-1.88)	0.61

PSQI=Pittsburgh Sleep Quality Index

CI=Confidence Interval

<sup>a</sup> Male is considered as reference<sup>b</sup> Normal is considered as reference<sup>c</sup> Response never is considered as reference

Global PSQI score &gt; 5 is considered as the dependent variable in the logistic regression model.

previously reported to associate poor sleep quality by several studies<sup>3,27</sup>. However, after controlling for confounders, besides depression and anxiety, only irregularity of the time to go to bed, and caffeine use were the significant explanatory variables. In other words, our results show that some of the sleep hygiene behaviors are not so important when the psychological symptoms, irregularity sleep schedule, and caffeine intake were controlled and there is confounding between sleep hygiene and psychological symptoms.

To the best of our knowledge, there is no extensive study investigating association between psychological symptoms and sleep hygiene behaviors, and their relationship with sleep quality. As next steps, studies with longitudinal designs (that allow for cross-lagged analysis) are proposed to better understand the most important and effective components of sleep hygiene behaviors and their potential confounders.

This is a cross sectional study with a high response rate of 89.7%, and it's the first study that focuses on pre-clinical students' sleep quality in an Iranian population. The use of a standardized measure for sleep quality and psychological distress is another advantage of the present study. To the best of our knowledge, no previous study has examined the relationship between psychological distress and sleep quality using both DASS-21 and PSQI standard measures. However, there are several limitations in our study. First of all, the cross-sectional

methodology limits our ability to measure sleep quality changes as the students go through the different levels of the preclinical stage, and also prevents us from finding causality relationship between the different factors.

It is still unclear that factors associated with poor sleep quality are a cause or consequence or simply a comorbidity, so determining the most appropriate intervention needs further investigations. The use of objective measure of sleep pattern such as polysomnography could also lead to additional data, however, it seems that there is a strong relation between subjective and objective measurements<sup>28</sup>. Further longitudinal and interventional studies are needed to overcome these limitations.

## CONCLUSION

To summarize, this study investigated the sleep quality and its related factors amongst pre-clinical medical students of Tehran University of Medical Sciences. We showed that poor sleep quality is a common problem in the population of students, and it's associated with later year in the school, psychological distress and poor sleep hygiene. This study provides a better vision of factors associated with poor sleep quality and points to the need for further evaluation of sleep problems.

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**Conflict of interest:** The authors declare that they have no conflict of interest.

**Compliance with ethical standards:** All procedures performed in studies involving human

Participants were in accordance with the ethical standards of the institutional the study was approved by the institutional ethnic committee (IR.TUMS.REC.1394.967).

**Informed consent:** Informed consent was obtained from all individual participants included in the study verbally.

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