MEBSOLT TETRAD CLUSTER CLASSIFICATION IN MEDICAL STUDENTS AND EFFECT OF FLAXSEEDS ON TOTAL SLEEP TIME

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ABSTRACT

Purpose: The aim of this research was to study if clustering of sleep characteristics of medical students at King George Medical University, Lucknow has any role in the effect of flaxseed on Total Sleep Time (TST).
Methods: Polysomnographic and DLMO data of Ninety-six medical students who were randomly selected have been used in the study. Cluster analysis is a powerful multivariate tool for detecting structures in datasets. In the absence of known classification variable or the kind of relationship between observations, the method of cluster analysis, an unsupervised method is popularly used for knowing subgroups in data. Using agglomerative hierarchical clustering, we grouped data with complete linkage aggregation method on mutual similarity. The goal was to divide the students into groups with relatively homogeneous anthropometric and objective sleep features and study the role of consuming flaxseed for one month on students' sleep time. As an intervention the students were given flaxseeds to eat daily continuously for 30 days. After this treatment, they were again assessed on their sleep characteristics.
Results: Using complete linkage methodology on the pre-treatment sleep characteristics of 96 students, we obtained four clusters dominated by objective sleep characteristics whose centroids were significantly different. TST in clusters is bifurcated at 5 hrs sleep. Influence of flaxseeds on the total sleep time was obtained in first three clusters. Post treatment centroid of third cluster with about 5.5 hours TST was significantly higher than first two clusters.
Conclusion: Mebsolt tetrad variables BMI, TST, sleep latency and DLMO might help to form subgroups of students to study the effect of flaxseed on TST.
Key words: Body mass index (BMI), Cluster analysis, Polysomnography (PSG), Total sleep time (TST), Sleep latency (SL), Dim light melatonin onset (DLMO),

INTRODUCTION

Good of sleep is a necessity for optimal physiological functioning (1). Sleep deprivation can lead to several short term and long-term adverse health effects. Medical Students are more prone to extend their bed time and have a voluntarily short sleep time due to studies and peer pressure in a competitive environment. Researchers have reported ill-health effects on medical students because of compulsive continuous long duty hours, including night duty etc. (2) and thus getting insufficient sleep.

Cluster analysis has been used as a powerful tool in medical science for discovering patterns in a way such that it is homogeneous within subgroups and heterogeneous between cluster subgroups. High degree of heterogeneity is inadequate to capture and classify sleep disorder has been reported by (3). His cluster analysis identified six clusters of obstructive sleep apnoea. Miller etal. (2016) obtained two insomnia clusters depending upon sleep onset and heart rate variability (4) Metabolic disorder, cardiovascular disease are reported to substantially contribute to the prognosis of sleepiness symptoms (Kendzerska etal. 2014) (5). Walters etal. (2021) (6) found two clusters of periodic limb movements in sleep of restless legs syndrome patients with psychiatric conditions. Azad (2015) expressed the need for research to improve general sleep education, identify associated risks and targeted programs to improve sleep for medical students (7). Cluster analysis was previously used for studying health related lifestyle in university students (8). In the present study we tried to explore the clusters on medical student's sleep characteristics which has not been studied earlier. Also, our interest was to see the effect of flaxseed on TST post clustering. Flaxseed has been the focus of growing interest for the nutritionists and medical researchers due to its potential Health benefits (9). It has been shown beneficial in the management of hypertension, hyperlipidaemia, increased blood glucose, and other disorders of metabolism. Its effect on sleep has not been investigated. Homogeneous categories as far as possible with

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variables including age, BMI, length of time of stay of student in KGMU, subjective and objective sleep parameters and the circadian parameter in the form of Dim light melatonin onset (DLMO) in young medical students surely will help in targeted treatment.

METHODOLOGY:
The data was obtained from a larger study involving changes in sleep study and circadian parameters of medical students. The data was obtained from the sleep laboratory, department of physiology King Georges Medical University (KGMU). Proper ethical was obtained from the institutional ethical committee. Polysomnographic and DLMO data of Ninety-six MBBS and BDS students has been used for the study. Ten variables were used to for this purpose. They were Age, gender, time spend in institute from joining, BMI, two predesigned pre-tested structured Questionnaire, Pittsburgh sleep quality index (PSQI) (10) , Epworth sleepiness scale (ESS) (11) for assessing sleep quality and day time sleepiness respectively were used PSG parameters used were Sleep latency, Total sleep time (TST) , sleep efficiency and REM Latency.

Sleep study method
Polysomnography (12) has been used as an effective tool to study the sleep architecture and comprehensively analyse different physiological parameters and objective sleep parameters. The PSG was conducted using SOMNO screen plus EEG32 video equipped polysomnography with a resolution of 16-bit, sampling rate up to 512 Hz, and band pass filter of 0.1 to 128 Hz. Standard electrode placement for EEG (F4-M1, C4-M1, O2-M1 along with alternate electrodes at F1M2, C1M2, O1M2, chin EMG and other channels according to the recommendations of the AASM were used. A Polysomnographic study was conducted for 7 to 8 hrs in the night. A technician attended the polysomnography for the entire night. The report was interpreted by a sleep specialist next day morning, for female subjects the PSG was conducted in an offline mode in their hostel room itself, as an unattended study. Their report was similarly interpreted in the morning.

Dim light melatonin onset
The Dim light melatonin onset (DLMO) is the time at which the salivary melatonin reached 4pg/ml; it is considered a useful parameter for estimation of circadian rhythm (13). An earlier time of DLMO means an advanced Circadian rhythm and vice versa. DLMO was obtained from salivary analysis using Human melatonin Elisa kit from Bio assay technology laboratory. Circadian rhythm was marked by their salivary melatonin level by collecting salivary samples every 30 min beginning at 9:00 PM till the subject goes to sleep, both sleep study and salivary collection were conducted in the sleep laboratory on the same night. Subjects were expected to collect saliva in labelled Eppendorf tubes, collection was conducted in the sitting position in dim light of less than 30 lux (14), the subjects were refrained to eat in the laboratory, about 4 to 5 ml of saliva was collected and promptly refrigerated. Early in the morning salivary samples were centrifuged at 2000 RPM and the supernatant was stored at minus 80 degree and examined when all samples were collected. Elisa test was conducted according to the user instruction, a standard curve plotting the optical density of each sample and was correlated with salivary melatonin concentration. The baseline time is 9:00 PM was considered as 00 min, this was the time from which the half hourly salivary collection window for melatonin estimation began. Time of onset of DLMO is in minutes past after 9:00 PM or the baseline time.

Statistical analysis
Data has been summarised as Mean ± SD. Pearson's correlation coefficient was calculated. The significance was marked for correlation higher than zero at 0.05 level of significance. An unsupervised multivariate statistical technique, hierarchichal Cluster analysis uses the process of partitioning a set of data points according to some measure of similarity (e.g., Euclidean distance). In the cluster analysis, our focus was on the observations in the data set. Using agglomerative hierarchical clustering we group data together with complete linkage aggregation method on mutual similarity. The goal was to divide the students into groups with relatively homogeneous demographic and PSG features.

RESULTS
The summary of N=96 cases studied can be seen in Table 1. More than 20% coefficient of variation was observed for Time spent in KGMU, sleep latency and DLMO. More than 10 percent standard deviation was registered for total sleep time, REM latency and DLMO. So the situation is complex as the variables are varying drastically. The correlation matrix in Table 2 gives mixed pattern of positive, and negative correlations. High similarity correlation was recorded between PSQI and ESS (r=0.5). Looking at the correlation matrix it is difficult to distribute students in subgroups.

After the treatment of flaxseeds for one month to students, the F-ratio showed that the ESS ($p<0.05$) and sleep latency ($p<0.01$) were significantly different. Agglomerative clustering does not tell clearly about the number of

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clusters a data set can have. Dendogram (figure 1) is a graphical representation of a hierarchy of nested solutions. A vertical line on the dendogram reveals the cluster solution at that level. The vertical line cuts dendogram at four places, giving an idea of four clusters in the data set. The outcome using K-means cluster analysis methodology has been shown in Table 3.

The cluster profiles (Table 3), which were the mean characteristics within each cluster for all variables helped us to understand the difference between clusters. In the first cluster, which constitutes 30.2% of the sample, the leading characteristics were BMI and Sleep Latency. Students in this cluster have highest BMI (23.03±3.08 kg/M^2) and sleep latency (16.55±4.58). These students generally have least TST about 4.6 hours per night. These students have second highest DLMO onset which is generally more than two hours (131.4±18.66 minutes).

In contrast, the students of second cluster were the ones who have lowest BMI (21.07±1.91 kg/M^2) and lowest DLMO onset about 1.3 hours. These students' characteristic was such that they have second highest total sleep time (290 minutes) and sleep latency (13.76±5.67). Generally, their total sleep time was a little less than 5 hours. This cluster constitutes 34.4% students of the sample.

Main characteristic of the students of third cluster was that their high total sleep time. In general, the TST was highest about 5.5 hours among the four clusters. The sleep latency was 15.33±5.67, marginally lower than the cluster 1 students. The 21.9% students' sample in this cluster has DLMO 1.6 hours (98.6±16.82 minutes). Students of this cluster generally have BMI about 21.95±2.01 kg/M^2.

Generally speaking, the main characteristic of the fourth cluster is DLMO of 2.5 hours. This is the highest DLMO among four clusters. Also, these students of this cluster have lowest sleep latency, 11.23±3.54. The BMI of these cluster students in general was about 22.5±3.32 kg/M^2. The 13.5% students of the study sample in this cluster have total sleep time about 5.1 hours (309.46 minutes).

Further, the post treatment TST data was analyzed by three-way ANOVA with treatment schedule, clusters and treatment its three factors. The highest mean of TST post treatment was among students of third cluster (353.6 m). The lowest TST was for the students of first cluster (312.0 m). On comparing the post treatment TST, the mean of third cluster was significantly different from first cluster ($p<0.01$). The mean TST of third cluster was significantly higher than the second cluster (327.2, $p<0.05$) and fourth cluster (329.7, $p<0.05$) respectively.

The least change in TST was noticed in third cluster in both placebo (5.6% and flaxseed (7.0%). The greatest change in placebo was in first cluster (11.1%), while in the treated group it was in second cluster (14.1%). In flaxseed treated group, the TST of third cluster was 13.3% higher than the lowest TST of first cluster. However, the TST of third cluster was significantly greater from its pretreatment level in first cluster ($p<0.01$), second cluster ($p<0.05$) and third cluster ($p<0.05$) respectively after flaxseed treatment.

Table 1: General summary of all study variables.

Input Variables	**Means**	**SD**	CV (%)
Age Years	20.2	1.8	8.91
Time Spent in KGMU	6.57	3.17	48.25
BMI	22.05	2.63	11.93
PSQI	11.44	1.86	16.26
ESS	8.67	1.79	20.65
TST (min)	297.53	24.34	8.18
Sleep Latency (min)	14.6	5.13	35.14
%Sleep efficiency	89.72	3.25	3.62
REM Latency (min)	122.85	13.41	10.92
DLMO (min)	108.75	32.35	29.75

Table 2: Correlation matrix (N=96) of variables studied.

	Age Years	**Time Spent in KGMU**	**BMI**	**PSQI**	**ESS**	**TST**	**Sleep Latency**	**%Sleep efficiency**	**REM Latency**	**DLMO-Minutes**
Age Years	1.00	-0.05	0.15	-0.07	0.10	0.10	-0.09	-0.02	0.06	0.13
Time Spent in KGMU	-0.05	1.00	-0.07	-0.17	-0.14	0.06	-0.19	0.14	0.12	-0.18

BMI	0.15	-0.07	1.00	0.24	0.16	0.01	-0.14	0.11	-0.10	0.18
PSQI	-0.07	-0.17	0.24*	1.00	**0.50**	-0.10	0.22	-0.01	-0.12	-0.01
ESS	0.10	-0.14	0.16	**0.50****	1.00	-0.09	0.31	-0.22	-0.13	0.19
TST	0.10	0.06	0.01	-0.10	-0.09	1.00	-0.13	0.12	-0.01	-0.13
Sleep Latency	-0.09	-0.19	-0.14	0.22*	0.31**	-0.13	1.00	0.02	0.15	-0.02
%Sleep efficiency	-0.02	0.14	0.11	-0.01	-0.22*	0.12	0.02	1.00	0.09	-0.06
REM Latency	0.06	0.12	-0.10	-0.12	-0.13	-0.01	0.15	0.09	1.00	-0.13
DLMO	0.13	-0.18	0.18	-0.01	0.19	-0.13	-0.02	-0.06	-0.13	1.00

BMI=basal metabolic rate, PSQI=pittsburg sleep quality index, ESS=Epworth sleepiness scale,TST=Total sleep time, DLMO = Dim light melatonin onset

p-value: * Significant; ** Highly significant

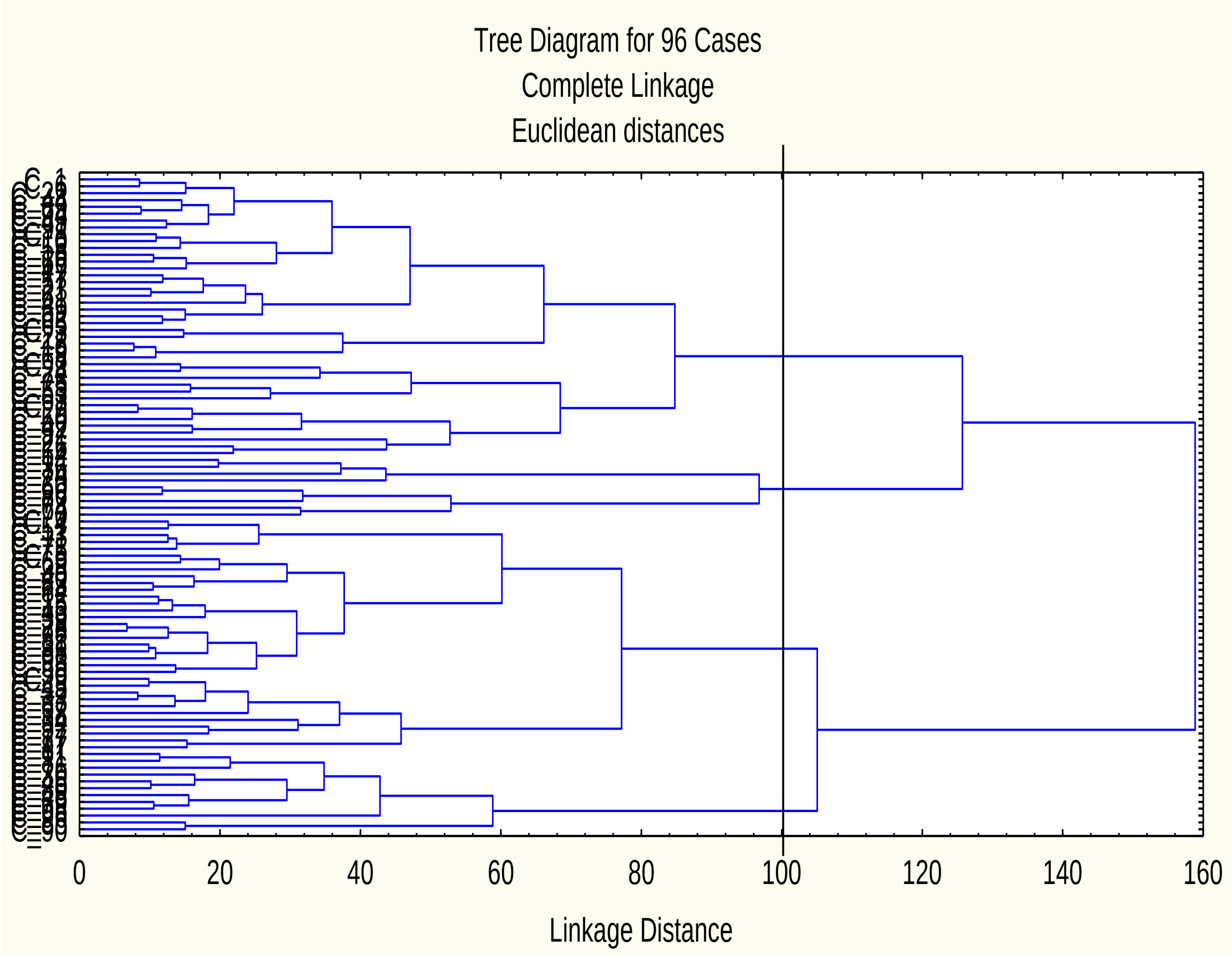

Figure 1 : Dendogram of medical students indicating four clusters in study sample.

Table 3 : Centroids of the four clusters before treatment

Variables	Cluster 1, n=29		Cluster 2, n=33		Cluster 3, n=21		Cluster 4, n=13	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age Years	20.24	1.50	20.03	2.02	20.00	2.00	20.85	1.52
Time Spent in KGMU - months	6.03	2.87	6.97	3.26	7.19	3.54	5.77	2.92
BMI	23.03	3.08	21.07	1.91	21.95	2.01	22.52	3.32
PSQI	11.76	2.20	11.42	1.77	11.14	1.74	11.23	1.54
ESS	9.21	1.97	8.09	1.86	8.62	1.40	9.00	1.41
TST	279.24	19.76	290.06	13.43	327.14	15.14	309.46	16.37
Sleep Latency	16.55	4.58	13.76	5.09	15.33	5.67	11.23	3.54
%Sleep efficiency	89.69	2.94	89.36	2.90	91.05	3.88	88.54	3.31
REM Latency	121.21	15.64	123.94	10.78	123.62	14.18	122.54	14.02
DLMO- Minutes	131.38	18.66	78.18	18.28	98.57	16.82	152.31	8.32

Table 4: Correlations of cases in four clusters with BMI <25 kg/M^2 .

Variables		Cluster 1	Cluster 2	Cluster 3	Cluster 4
	n	21	32	18	10
Age vs Time spent in KGMU - months		0.47*	-0.22	-0.29	-0.46
PSQI vs ESS		0.66*	0.53*	0.18	0.38
PSQI vs Sleep Latency		0.11	0.42*	0.14	0.34
ESS vs %sleep efficiency		-0.27	-0.58*	-0.02	-0.24
BMI vs DLMO		-0.44*	-0.51*	0.04	-0.30
TST vs DLMO		-0.47*	-0.16	-0.08	0.19

When cluster samples were classified on BMI <25 kg/M^2, it was found that the majority ones had BMI <25 kg/M^2 in every cluster. However, the second cluster was most homogeneous with 97% samples had BMI lower than 25 kg/M^2. The correlations on selected variables have been shown in Table 4. It is evident that the linear relationship of sleep variables got enhanced on sub grouping of students with BMI and particularly among students of cluster 1 and cluster 2, however, none of the variables were correlated in Cluster 3 and 4 respectively.

DISCUSSION:

A good quality sleep is important for mental and physical health of students. Research on sleep pattern in undergraduate medical students is of interest to know their academic performance and stress. Sleep deprivation and disturbance of the circadian rhythm can seriously impair daytime functioning. In order to prevent disturbance of circadian rhythm to ensure better sleep, student's bed and wake-up time should be same as far as possible. Insufficient sleep leads to reduction in T-cells compromising the immune system (15). This is probably the first report to cluster out medical students on subjective and objective sleep characteristics. The interest was to know, if there was an effect of flaxseed treatment on TST, if yes, then on which sub-group of students.

This study aims to categorize cluster variables to improve sleep quality and health in medical students. The clustering was to reveal subgroups from heterogeneous data such that each individual cluster has greater homogeneity than the whole. Some students have less sleep than and some have more. It means instead of uniform; appropriate medical attention could be advised to student depending upon their TST.

Interestingly, prior to intervention, PSQI and percent sleep latency were identical in cluster 1 and cluster 3. In general, the variation of variables in clusters was smaller than what we see in Table 1. Interestingly it came to almost half for DLMO in all four clusters. DLMO was significant different between clusters in our study. Brown etal (2021) (14) classified DLMO of humans using neural network and found decreasing mean error in DLMO. The variables whose standard deviation (SD) was high in the sample before clustering were decreased for TST and DLMO.

In this study Melatonin, BMI, sleep onset latent time and total sleep time (Mebsolt) four sleep variables were significant in getting four clusters so we named it the phonemenon of Mebsolt tetrad to characterise sleep patterns of medical students. BMI was significantly different between clusters. The revised guidelines by Raatikainen etal. 2013 categorize overweight as a BMI of 23.0 – 24. However, BMI <18.5 kg/m2 were categorized as underweight and 18.5-22.9 normal or lean. BMI a significant variable in our study could be a feature related to sleep quality. This is analogous to a study by Giri etal (18) which rreported a positive correlation between sleep disturbances and body mass index. This sleep loss in medical students could be a reason for poor academic performance (19). Another study by Basu etal (20) highlighted the relationship of poor-quality sleep-in medical students to several factors including lower age group, hostel residence and BMI.

Generally speaking, medical students have sleep less than the required hours. A delayed bed time as seen in these students often results insufficient sleep along with an irregular sleep wake pattern this delayed circadian rhythm is associated with poor academic performance. Six hours sleep can be considered a satisfactory sleep for students. In the present study, the average sleeping duration of students was around 5 hours. It was distributed less than and more than 5 hours in first two clusters and last two clusters respectively. About 65 percent students have sleep less than 5 hours in our study. In his Figure 1 Rathod etal. (21) showed that the daily hours of sleep is independent of BMI<25 kg/M^2. While, authors found that TST of students BMI<25 kg/M^2 have linear relationship with DLMO (r = -0.44, p<0.05).

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Two clusters have higher sleep latency but they are distinct with highest and lowest TST. The DLMO is common with the other two clusters. The lowest DLMO might be associated to lowest BMI. While the highest DLMO is featured with high TST but lowest sleep latency.

Conclusion

Authors concluded that Mebsolt tetrad features may help in subgrouping medical students on sleep. Also, one should preferably look for clustering for deeper insight into the significant effect of flaxseed treatment among students subgroups.

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