ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
June2025 • Vol. 38

Impact of sleep and chronic stress on periodontal health of health care workers: A case-control study

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Aim: To evaluate the impact of altered sleep routine and stress on periodontal health in night shift working healthcare workers and to also assess and compare the melatonin ,cortisol and malonodialdehyde levels in night shift working healthcare workers .
Materials and methods: 44 nurses and paramedic staffs working in night shift pattern and in day regular time pattern were recruited for the study . Periodontal parameters were assessed and unstimulated saliva sample was obtained from all the patients. Athen's insomnia scale (AIS) and Pittsburgh Sleep Quality Index was recorded to assess sleep quality and quantity .Salivary melatonin levels was assessed using commercially available Human melatonin assay kit . Biochemical analysis was done to evaluate the cortisol levels of the participants and TBARS assay was used to analyse the malonodialdehyde levels.
Results: Salivary melatonin levels were higher in day shift workers while salivary malondialdehyde levels were higher in night shift workers and the results were statistically significant with p<o.o1. Salivary cortisol and malondialdehyde levels were elevated thereby correlating oxidative stress with periodontal parameters .
Conclusion: The night shift healthcare workers had higher cortisol and decreased melatonin levels which in turn impacts their systemic and periodontal health.

Keywords: sleep quality, chronic stress, melatonin, cortisol, malanodialdehyde

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Introduction

Periodontitis is a multifactorial chronic inflammatory disease caused by dysbiotic plaque biofilm and is more prevalent (50%) among the adult Indian population.[1] In a susceptible host, recurrent bacterial insult disrupts the inflammatory pathways resulting in persistent inflammation and tissue destruction of the attachment apparatus.[2] Despite the primary cause being the interplay between host immune response and periodontopathic bacteria, other environmental factors also increase the susceptibility to disease progression.[3,4] Evidence suggests that among those environmental factors are sleep- quality, pattern, and duration which play a prudent role in the progression of periodontal disease.[5,6]

Sleep and circadian rhythms are crucial for immune system regulation thereby increasing the disease susceptibility. Shift work, working at night, mutation in circadian genes, mental depression, poor diet habits, and certain metabolic diseases are the common causes of circadian rhythm disruption. Sleep disruption may increase the acute phase response, which ultimately leads to the release of multiple pro-inflammatory cytokines resulting in periodontal tissue destruction.[7]

Melatonin, a sleep hormone produced by the pineal gland, coordinates physiological processes such as the sleep-wake cycle, the intake of food and water, hormone secretion, and metabolism. It has a protective effect against highly reactive free-radical scavengers, protecting cells from inflammatory processes, and oxidative damage. Melatonin secretion can be suppressed by circadian misalignment and exposure to bright light at night .[8]

Both melatonin and cortisol are closely related to the circadian cycle and when disrupted result in immune dysfunction.[9] When melatonin level dips, more corticotropic releasing hormone(CRH) is released, culminating in increased cortisol secretion. One of the most common causes of circadian rhythm disruption is night shift work. Night-shift healthcare workers, especially nurses, perceive increased stress levels due to the job's demanding nature and altered sleep patterns. Thus, salivary cortisol level assessment can be chosen as a biomarker of psychosocial stress reflecting the activity of the Hypothalamus Pituitary axis(HPA). Given that periodontal disease has been linked to shift work within the concept of low-grade chronic inflammation, it is proven that psychological stress is the known risk determinant for periodontitis.

Sleep promotes anti-oxidative processes and deprivation which leads to oxidative stress burden. Lipids and Poly Unsaturated Fatty Acids (PUFAs) are common targets of oxidative stress and undergo lipid peroxidation and oxidation. This damage can be measured by assessing the production of malondialdehyde (MDA) levels. Salivary MDA levels are linked to an increase in reactive oxygen species (ROS) activity in periodontal disease and other oral disorders.[10]

Only a few recent studies have investigated the association between altered sleep and periodontitis with conflicting results between altered sleep pattern and periodontitis.[11,12] It is quite surprising that there is no research on the relationship between altered sleep patterns and periodontal health among healthcare workers. Hence, we hypothesized that altered sleep patterns weaken the immune system, reduce antioxidant production, and aggravate periodontal inflammation. To test this hypothesis, salivary melatonin, cortisol, and malondialdehyde levels have been assessed to associate them with

periodontal inflammatory status. In light of this background, this study was designed to evaluate the association between altered sleep patterns and periodontitis in night-shift working healthcare workers.

Materials and methods

This case-control study was approved by the institutional review board and ethical clearance was obtained from the Ethics Committee of Sri Ramachandra Institute of Higher Education and Research, Chennai. Nurses and paramedic staff working night and day shifts at Sri Ramachandra Medical College and Hospital, Chennai, were included in this study. A sample size of 44 subjects comprising 22 participants in each group was estimated based on the true probability of exposure for rejection of the null hypothesis with a type 1 error of 5 % and a power of 90 %. Only participants who work for a minimum of 8 hours, with no other systemic disease, not under any medications, and non–smokers have been included in this study. 22-day and 22-night shift nurses were recruited after obtaining written informed consent following the Declaration of Helsinki.

Clinical parameters assessment

Periodontal parameters including bleeding on probing (BOP), Probing Depth (PD), Clinical Attachment Loss (CAL), oral hygiene index simplified OHI(S), and Periodontal inflammatory surface area (PISA) were assessed by a single trained examiner using UNC 15 probe. Sleep quality was assessed using Pittsburg's sleep quality scale and Athen's insomnia scale.[13,14] Stress was assessed using Holmes-Rahe's Life Stress Inventory Rating Scale.[15] It enables measurement of the stress level of participants.

Biochemical parameters assessment

Unstimulated whole saliva samples were collected from the volunteers. Subjects were advised to abstain from food intake for 8 to 12 hours and oral hygiene measures for 2 hours before sample collection. The samples were collected before 7 a.m. in a fasting state. During saliva sampling, the participants were seated upright in a chair in a room with low-intensity lighting, since light can alter melatonin secretion. 1.5-2 ml of pooled saliva was collected by passive drooling method and transferred into 2 ml sterile micro-centrifuge tubes. The collected samples were then processed by centrifugation and stored at -40 centigrade until further analysis.

Salivary melatonin estimation

A commercially available Human melatonin assay kit (Elab Biosciences, USA Human MT ELISA kit- Lot no. JYIJNMD3RM) was used for salivary melatonin assessment. The manufacturer's protocol was followed. The optical density was measured with a spectrophotometer at 450 nm immediately after pipetting the stop solution.

Salivary malondialdehyde estimation

Briefly, Lipid peroxidation in C2C12 myotubes was measured using a Thiobarbituric Acid Reactive Substances (TBARS) assay as previously described (Keles et al. 2001).[16] Calculating TBARS concentration was based on the molar extinction coefficient of malondialdehyde.

Salivary cortisol assessment

Salivary cortisol level was measured by Electro chemiluminescence (ECL) method Roche cobas 8000 as previously described by Pires et al .[17] All the detection experiments were conducted in the dark as it is a light-sensitive procedure.

Statistical analysis

All Statistical analysis was done by IBM SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

Mean and SD were used to summarize the data of salivary, serum biomarkers, and periodontal parameters and significance was assessed by Chi-Square test and Kruskal Wallis test.

Correlation between biomarkers and periodontal parameters was done with Spearman's correlation test. The correlation coefficient of value 1 is considered an association, with>1 as a positive association and <1 as a negative association. A 'P' value of <0.05 was considered a statistically significant difference. A 'P' value of <0.01 was considered a highly statistically significant difference.

Results

1. Demographic variables

A total of 44 samples were included in the study (n=22). The mean age of the day shift group was 30.27 ± 7.304 years, and that of the night shift group was 29.82 ± 4.876 years, with a P value of 0.72 which was not statistically significant. All of the sampled population were female participants.

2. Assessment of sleep quality and quantity

Sleep quality and quantity were assessed using Pittsburg's Sleep Quality Index (PSQI) and Athen's Insomnia Scale respectively. Athen's insomnia score for the day shift group was lower than night shift group with a P value of <0.01, which was statistically significant as shown in Figure 1 a. PSQI score was higher in the day shift compared to the night shift group with a P value of <0.01, which was statistically significant as shown in figure 1b.

3. Assessment of stress level

Stress level was assessed using Home's and Rahe's stress score. The stress score for the day shift group was 94.32 ± 54.89, and for the night shift group was 110.59 ± 46.33 with a P value of 0.352, which was not statistically significant as shown in Figure 1 c.

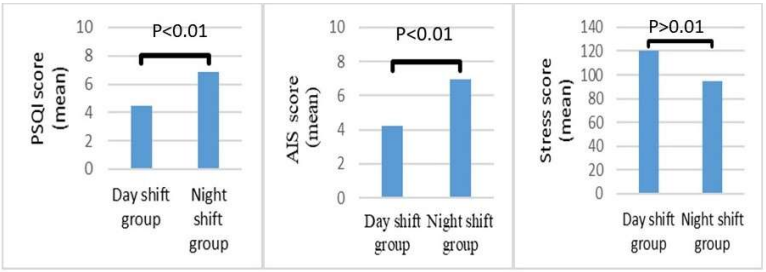

Figure 1: Stress and sleep scores for day and night shift groups AIS- Athen's Insomnia Scale, PSQI- Pittsburg's Sleep Quality Index

4. Assessment of clinical parameters

Periodontal parameters such as probing depth, clinical attachment loss, bleeding on probing(BOP), and PISA score were assessed between the night and day shift health care workers. BOP and PISA scores were significantly higher on the night shift when compared to day shift workers with p<0.01 as shown in Figure 2b,d. Similarly, clinical attachment loss was higher in the night shift group than the day shift group as shown in Figure 2c.

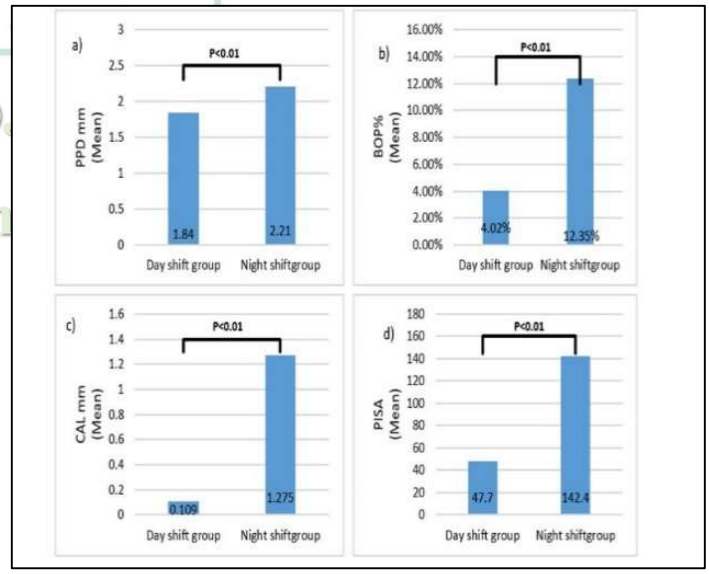

Figure 2: Mean values of periodontal parameters between two groups- a) PPD, b) BOP,c) CAL and d) PISA

5. Biochemical assessment

Salivary melatonin levels were higher in day shift workers (2.36 ± 2.128 pg/ml) compared to night shift workers (1.23 ± 2.329) with p-value <0.01, which was statistically significant as shown in Figure 3a. Salivary malondialdehyde levels were higher in night shift workers (4.16 ± 1.833) than day shift group (3.78 ±1.24) with the P value of <0.01, which was statistically significant as shown in Figure 3b. Salivary cortisol levels were higher in night shift workers (1.19± 0.56) than day shift group (0.51 ± 0.43) with a P value of <0.01, which was statistically significant as shown in Figure 3c.

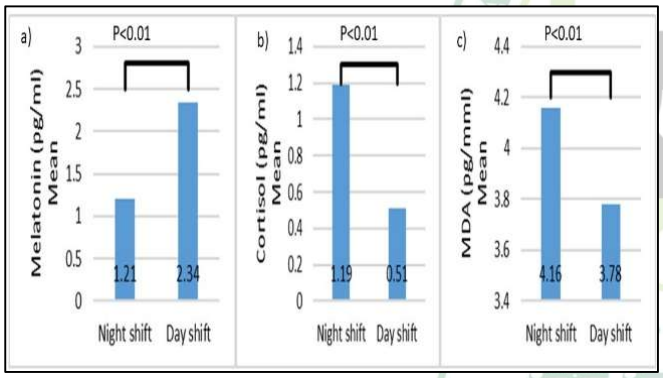

Figure 3: Comparison graph of salivary melatonin (a), cortisol (b), and malondialdehyde (MDA) (c) levels among day and night shift groups.

6. Correlation of salivary biomarkers with periodontal parameters

Salivary melatonin levels were negatively correlated with periodontal parameters (PPD, CAL, BOP %, and PISA) with a P value of <0.01 which was statistically significant as shown in Table 1. Salivary cortisol levels showed a positive correlation with PPD, CAL, BOP %, and PISA with a P value of < 0.05, which was statistically significant as shown in Table 1. Salivary malondialdehyde levels were moderately associated with PPD, CAL, BOP %, and PISA which showed a significant correlation between oxidative stress with periodontal parameters as shown in Table 1.

Table 1: Spearman's correlation analysis of salivary melatonin, cortisol, and MDA levels with stress score, sleep scores, and periodontal parameters.

	Melatonin		Cortisol		MDA	
	r value	P value	r value	P value	r value	P value
Stress score	-.232**	0.01	0.904*	0.088	0.446	0.047
Athen's score	-.704**	0.00	0.524	0.071	0.772	0.0264
PSQI score	-0.672**	0.00	0.556*	0.09	0.59	0.04
PPD	-0.430**	0.04	0.650*	0.06	0.39	0.203
CAL	-0.480**	0.04	0.623*	0.02	0.44	0.063
BOP%	-0.465**	0.04	0.361*	0.12	0.45	0.16
PISA	-0.574**	0.00	0.716*	0.037	0.214	0.164
Melatonin	1.00	-	-0.704**	0.00	-0.518*	0.05
Cortisol	-0.704**	0.00	1.00	-	0.202	0.188
MDA	-0.118	0.44	0.202	0.188	1.00	-

Discussion

The primary objective of the study was to evaluate associations between altered sleep patterns and psychological stress with periodontal status among night-shift healthcare workers. Sleep deprivation has been associated with alterations of innate and adaptive immune parameters, leading to a chronic inflammatory state.[18]

In this study, both sleep quantity and quality were assessed using Athen's Insomnia Scale (AIS) and Pittsburgh Sleep Quality Index (PSQI) score respectively. AIS evaluates nighttime sleep disturbance and daytime dysfunctions that occur at least thrice a week. AIS consists of eight items: the first five relate to sleep induction, awakenings at night, total sleep duration, and sleep quality; while the last three refer to sleepiness and functioning capacity during the day. The total score is calculated (range: 0–28), with higher scores denoting poor quality and quantity of sleep. In this study, Athen's insomnia scale (AIS) scores were significantly higher in night-shift healthcare workers than day day-shift healthcare workers (P <0.01). It was in accordance with the previous study by Tsuchiya et al., 2015 reported that lower sleep quantity assessed

by AIS was associated with periodontitis in the Japanese population.[19]

The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire comprising 7 components that assess the sleep quality and disturbances over a 1-month time interval. The total score of these seven components yields one global score.10 In this study, PSQI scores were significantly higher in night shift healthcare workers than day shift healthcare workers (P <0.01), indicating that the night shift group had irregular sleep patterns and poor quality of sleep compared to day shift working persons. These are in accordance with previous studies that showed night shift workers with overall poor quality and quantity of sleep were associated with increased severity of periodontitis.[20,21] Islam et al, and Karaasalan et al., reported that sleep quality assessed using PSQI was poorer in night shift workers.[12.22] A previous study by Singh et al. found that poor sleep quality assessed using PSQI was significantly associated with poor periodontal status in the Indian population.[11]

The role of stress on periodontal health has been widely studied previously using different stress analysis scales.[23] Owing to the working environment, night-shift healthcare workers are more prone to chronic stress. Psychosocial stressors may have an impact on the Corticotrophin Releasing Hormone (CRH)/HPA axis, which physiologically lowers immunity, and increases the likelihood of infection and inflammation.[24] In our study, stress was assessed using Holmes-Rahe's Life Stress inventory rating scale. It comprised 43 stressful life events that could predispose to illness and a cumulative score of '<150' indicates a slight risk of stress-related health breakdown, '150-299' indicates a moderate risk of illness (50% of chance in stress-related health breakdown in next 2 years), a score of '>300' indicates person is at high risk of illness.[25] Our results showed that the stress score was higher in the night shift group than day shift group. This finding is in accordance with the previous studies that reported a higher stress score in night shift workers.[26,27] While day shift healthcare workers are typically working in routine situations, night shift healthcare workers are typically working in crucial emergencies. This eventually increases the likelihood that they will become stressed and have elevated cortisol levels, which in turn affect their immune response. As a result, night shift healthcare professionals are more prone to immune system impairment.

The study results showed that periodontal parameters such as PPD, CAL, BOP%, and PISA score were significantly higher in night-shift healthcare workers than in day shift group (P <0.01). This could be attributed to the fact that poor sleep quality and quantity could predispose the night shift group to melatonin levels. Melatonin plays an important role in immune regulatory function thereby leading to increased periodontal inflammation in the night shift group. These findings were from previous study by Han et al., where they found that night shift workers had 27% higher odds of developing periodontitis than daytime workers.[28] Further these findings were strengthened by previous studies by Thomas et al., and Karaasalan et al., which have reported a similar increase in the incidence of periodontitis in persons working night shifts or with altered sleep pattern.[29,30]

To the best of our knowledge, this is the first study correlating salivary melatonin, cortisol, and Malondialdehyde levels with periodontal status in night-shift healthcare workers. In order to understand

the possible biological plausibility between sleep, stress, and periodontal health, we chose to analyze salivary biomarkers such as melatonin, cortisol, and malondialdehyde levels in night and day shift healthcare workers.

Nocturnal exposure to light while working causes a disturbance in the circadian rhythms. Among the various circadian rhythm disturbances associated with the endocrine system, changes in hormonal secretions such as melatonin, serotonin, prolactin, glucocorticoids, and adrenocorticotropic hormone have been observed.[31] Changes in melatonin synthesis and secretion are known to affect the function of the immune system and can predispose to disease susceptibility.[32] Studies have reported that Melatonin can inhibit LPS-stimulated proinflammatory cytokines production through a mechanism involving the attenuation of NF-κB activation and also suppress LPS-induced cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS).[33] Our findings showed that melatonin levels are lower in night-shift healthcare workers when compared to the day-shift group. Also in this study, melatonin levels were inversely correlated with periodontal inflammation with P value <0.05. The higher the periodontal inflammatory burden in night shift healthcare workers, the lower the melatonin level due to awakening at night shift. Since melatonin production always peaks at night, disruption of melatonin secretion due to nocturnal awakening and light exposure could potentially predispose periodontal inflammation through immune dysfunction. Our finding is in association with the study by Almughrabi et al., where they found that salivary and GCF melatonin levels were lower in chronic and aggressive periodontitis.[34] Our study results are in accordance with a recent systematic review and meta-analysis by Thodur Madapusi Balaji et al., which concluded that salivary melatonin levels could reflect the periodontal status of a patient and thereby serve as a potential biomarker.[35]

Stress and sleep have a bidirectional relationship, with cascading effects that will disrupt normal physiological functions.[36] Our findings showed that the salivary cortisol level was higher in night shift healthcare workers than day shift healthcare workers and also it was significantly correlated with the severity of periodontal inflammation with a p-value of <0.05. Our findings are in accordance with the previous studies that also showed an increase in salivary cortisol levels in chronic periodontitis patients.[37,38]. In our study, salivary cortisol level was inversely correlated with salivary melatonin level. Mesic et al stated that Sleep disruption was found to be associated with an upregulation of inflammatory cytokines, reactive oxygen species, and a decrease in the production of melatonin.[39]

One of the proposed functions of sleep is promoting anti-oxidative mechanisms and deprivation of which leads to oxidative stress-related cellular damage and chronic inflammatory changes in the body. Previous studies indicate that the MDA level in bodily fluids may be a reliable indicator of the extent of oxidative damage to cells in the body. In this study, salivary MDA level was significantly correlated with salivary melatonin level which showed that altered sleep patterns increase oxidative stress. Its level was also positively correlated with all the periodontal parameters indicating that oxidative stress caused by altered sleep patterns during shift work was associated with a higher degree of periodontal inflammation. This is in accordance with

available evidence showing that MDA levels are increased due to periodontal disease as a result of oxidative stress.[40]

The findings of the present study suggest that the severity of periodontal inflammation (indicated by PPD, CAL, BOP%, and PISA), sleep quality, quantity, and work stress were highly linked to salivary melatonin, cortisol, and MDA levels.

conclusion

This study revealed that healthcare workers function under enormous stress and sleep deprivation. Higher stress levels in the healthcare environment not only negatively impacts overall well-being and systemic health but also oral health. Longer working hours in the night or frequent night shifts reduce the opportunity for sleep and shorten recovery time in nurses, thus endangering their overall general and oral health. Bearing in mind the significance of these problems, it is necessary to explain the importance of sleep, stress management, and good oral hygiene practices to healthcare workers.Preventive changes in job conditions and lifestyle are necessary to improve the overall quality of life in healthcare workers with altered sleep patterns.

Funding Information: No funding was obtained for this study.

Data Availability: Data that support the current study are available from the corresponding author upon reasonable request.

Declarations: We declare no conflict of interest.

Ethical Approval: This case-control study was approved by the institutional review board and ethical clearance was obtained from the Ethics Committee of Sri Ramachandra Institute of Higher Education and Research, Chennai. REF: CSP/21/SEP/99/494.

Consent to participate: Written informed consent was obtained from all the study's participants before being enrolled in the study.

Competing interest: The authors declare that they have no competing interests

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