International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

The salivary levels of Melatonin and NLRP3 in chronic periodontitis patients with altered quality of sleep – an observational pilot study

*Sowbarnika Harikrishnan [(1)], Harinath Parthasarathy [(2)] *, Deepa Ponnaiyan [(3)], Anupama Tadepalli [(4)]*

Department of Periodontics and Oral Implantology, SRM Dental College, Ramapuram, Chennai-600089, India.

Abstract

Melatonin is an antioxidative and immunomodulatory neuroendocrine hormone. The NLR Family Pyrin Domain Containing-3 (NLRP3) inflammasome of the innate immune system mediates pro-inflammatory responses. Similar to melatonin playing a role in sleep regulation, current research has linked NLRP3 to sleep as well. Both these biomarkers have also shown a strong association to periodontal disease progression as evidenced in literature. The objective of this study is to understand how the salivary levels of melatonin and NLRP3 in chronic periodontitis patients with altered sleep quality presents itself and the need to understand how sleep quality can affect these biomarkers, which indirectly has an association to periodontal disease progression.

Keywords: Melatonin, NLRP3, Periodontitis, Sleep, PSQI

Full length article **Corresponding Author*, e-mail: *drsowbykrishnan@gmail.com*

1. Introduction

Periodontitis is a chronic state of inflammatory condition associated with multiple systemic diseases such as cardiovascular, respiratory, musculoskeletal, neurological and respiratory conditions. Local plaque formation, smoking, diabetes, microbial complexes, immunological reactions could be few of various reasons as to why this disease arises [1]. Sleep is also a key determining factor affecting periodontal disease progression, as it plays a major role in maintaining homeostasis of the body, regulates the immune system and overall proper functioning [2,3].

Melatonin is the prime pineal gland regulated hormone which controls the sleep cycle in the human body. It is controlled by the suprachiasmatic nucleus in the brain and has immense antioxidant properties along with immunomodulatory functions [4]. The average concentration of salivary levels of melatonin is documented to be nearly 50 pg/ml during the night [1]. Inflammasomes play a major role in mediating inflammatory processes during stress or as a pathogenic induced response [5,6]. Studies have shown that bacterial ligands, such as LPS, peptidoglycan, and bacterial RNA, can activate the NLRP3 inflammasome to release IL-1B, leading to alveolar resorption and periodontal tissue destruction [7,8,9]. The PSQI questionnaire was used in this study, this was originally designed by Buysse DJ et al and is meant for the subjective evaluation of sleep disturbances in patients [10]. This study aims to understand how the salivary melatonin and NLRP3 levels appear in chronic periodontitis patients with altered sleep quality and if there seems to be any correlation between the sleep scores and the biomarker levels at a constant state of stress. Understanding this concept might help us treat patients more reliably, keeping in mind their psychosomatic state also.

2. Materials and methods

2.1. Study design and ethical considerations

An observational pilot study was designed to evaluate the salivary levels of Melatonin and NLRP3 in chronic periodontitis patients with altered sleep quality. The study design was approved by the Institutional Review Board, SRM Dental College, Ramapuram, Chennai. Prior informed consent was obtained from the participating sample patients regarding the study. The sample size for this pilot study was set to be an overall 15 patients.

2.2. Study participants

Patients attending the Outpatient Department of Periodontics and Oral Implantology, SRM Dental College, Ramapuram, Chennai were screened for chronic periodontitis and who also had sleep disturbances as evaluated by the PSQI questionnaire.

2.3. Criteria of patient selection

Male or female patients belonging to an age group of 30-64 years with no systemic diseases present, with stage

2 grade A periodontitis (according to the latest AAP 2017 classification) and having a sleep time of <6 hours/day-considered as sleep deprived according to the National Sleep Foundation recommendations (2015) criteria along with the evaluation by the PSQI questionnaire were included in the study. The exclusion criteria involved patients who underwent periodontal therapy or antibiotic treatment within the last 3 months from current screening date, presence of any systemic diseases, history of smoking, history of xerostomia or salivary gland diseases, patients with a history of intake of drugs known to alter melatonin levels, pregnant women, patients with a history of radiotherapy or chemotherapy.

2.4. Subjective evaluation of sleep quality using the Pittsburgh Sleep Quality Index (PSQI) Questionnaire

Sleep quality scores were recorded for the patients using the PSQI questionnaire consisting of 7 individual components totaling to 19 self-rated questions (considered for total scoring) and 5 questions that can be answered by the bed/room partner for better clinical evaluation (not considered for total scoring). This was used as a subjective confirmatory status for altered sleep quality in the recruited cohorts.

2.5. Saliva sampling and processing

The patients were made to sit upright, in a maximum low light environment and saliva collection was done between 8:00am to 12:00 pm. They were asked to quit eating or drinking at least 3 hours prior to procedure, to avoid any interference in results. About 5ml of unstimulated whole saliva was collected in a sterile uricup which was then transferred to a Tarsons conical end centrifuge tube and was centrifuged for 10 minutes at 2700rpm. Following centrifugation, the supernatant was collected and aliquoted into three 0.5ml eppendorf tubes, followed by its preservation at -80℃ until ELISA procedure was performed. The samples were processed and assessed for melatonin and NLRP3 using commercially available ELISA kits (Abbkine Scientific Co. Ltd, Wuhan), at a wavelength of 450Nm. The instructions were followed as per the manufacturer's manual for the respective biomarkers analyzed.

3. Results and Discussions

The demographic data of the study participants have been documented in table 1. The primary parameter values attributed to plaque index, bleeding on probing, gingival index, probing pocket depth, clinical attachment levels for the stage 2 grade A periodontitis cohorts were recorded to give a fair idea about their oral hygiene levels (table 2). The sleep scores were also recorded using the Pittsburgh Sleep Quality Index and the scores averaged between 13-20, all denoting severe sleep quality disturbances. The quality of sleep worsens as the PSQI score increases. Chronic periodontitis in itself is a state of constant inflammation and a disease subjected to increased oxidative damage. The lack of sleep as well has evidences on how it helps majorly in immune regulation and health. The cohort group having chronic periodontitis patients with sleep disturbances is theoretically a state of added immune dysregulation and subjective to oxidative stress, various cytokine activity release etc. Considering the biomarkers in this study, Melatonin and NLRP3, both of them have documented effects on periodontal disease and sleep regulation. On evaluation of the biomarker levels using spearman's correlation coefficient, there was a significantly strong negative correlation between the PSQI sleep scores and melatonin, thus confirming the status of sleep disturbance/deprivation present in the cohort, attributing to the decrease in melatonin levels as sleep score worsens. Similarly, there was a significantly positive correlation between the PSQI sleep scores and the NLRP3 inflammasome levels, denoting an increased state of inflammatory response that can be linked to sleep deprivation. Also, a state of inflammation caused due to periodontal breakdown should also be kept in mind, owing to the multiple allied factors that play a role [11, 12]. Melatonin and NLRP3 were specifically chosen for this study owing to their inter-relationship documented in prior literature and also, both these biomarkers play a role in periodontal inflammation and mediating sleep [13,14]. One of the major reasons as to why there is activation of the NLRP3 inflammasome, is the presence of oxidative stress in the microenvironment [15]. Melatonin inhibits the activation of NLRP3, whose priming stage is controlled by NF-kB [13]. Zheng and colleagues investigated the relationships between melatonin and NLRP3 and discovered that melatonin inhibited NLRP3 activity by inhibiting IL-1 release [16]. These results can be attributed to the current findings in our study, wherein there is a statistically significant negative correlation between NLRP3 and Melatonin levels, thus confirming prior evidences. Hence, with all these primal findings, it is evident that there is an increased presence of inflammatory state that can be seen in chronic periodontitis patients and sleep disturbances add more inflammatory burden to the given situation. Understanding and recognizing the need for this situation is very important since the prognostic factors and treatment plan may alter on individual patient basis, owing to their risk profile.

Table 1: Descriptive statistics for demographic data

Variables	Descriptive data
Age	46.3±9.04
Gender	
(a) Male	9 (60%)
(b) Female	6 (40%)

Table 2: Descriptive statistics for clinical parameters noted and the biomarker levels

	N	Minimum	Maximum	Mean	Std. Deviation	Median	IQR
PLAQUE INDEX	15	1.3	2.6	1.920	.3610	2	0.5
GINGIVAL INDEX	15	.8	1.5	1.140	.2230	1	0.4
BLEEDING ON PROBING	15	1.9	2.3	2.087	.1187	2.1	0.1
PROBING POCKET DEPTH	15	2.7	3.7	3.193	.2463	3.2	0.3
CLINICAL ATTACHMENT LEVELS	15	2.8	3.7	3.207	.2404	3.2	0.3
PSQI SCORE	15	13	20	16.8571	2.53442	18	5
MELATONIN (ng/L)	15	3.22	18.5	7.1658	4.54686	5.20	6.59
NLRP3 (pg/L)	15	45.80	1050.10	486.6501	315.20043	375.10	546

Table 3: Spearman's correlation coefficient

Parameters	Melatonin	NLRP3
PSQI score	rho= -0.740 p=<0.0001**	rho= 0.504 p=0.006*
NLRP3	rho=-0.727 p<0.0001**	rho=1

4. Conclusions

It is of prime importance to treat a patient holistically for achieving long term success of a treatment plan. So far, no proper evidence has been documented owing to the relationship between sleep quality disturbances in periodontitis patients and the magnitude of inflammatory burden correlating with the biomarker levels. The nuance in understanding this determines how well the prognosis of a patient could be estimated. Further studies are required to understand how various treatment regimens alter these inflammatory markers and improve the overall systemic health of a patient.

References:

[1] J.P.Rocca, C.Fornaini, Z.Wang, L.Tan and E.Merigo. (2020). Focal Infection and Periodontitis: A Narrative Report and New Possible Approaches. International Journal of Microbiology, 20(4), 1-9.

[2] F.W.M.G.Muniz, N.M.Pola, C.F.Silva, F.G.Silva and M.Casarin. (2021). Are periodontal diseases associated with sleep duration or sleep quality? A systematic review. Archives of Oral Biology, 129(8), 4-15.

[3] O.M Almughrabi, K.M.Marzouk, R.M.Hasanato and S.SShafik. (2013). Melatonin levels in periodontal health and disease. Journal of Periodontal Research, 48(3), 315–321.

[4] I.M.McIntyre, T.R.Norman, G.D.Burrows and S.M.Armstrong (1987). Melatonin rhythm in human plasma saliva. Journal of Pineal Research, 4(2), 177–183.

[5] J.A.Kummer, R.Broekhuizen, H.Everett, L.Agostini, L.Kuijk, F.Martinon, R.van Bruggen and J.Tschopp (2007). Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society, 55(5), 443–452.

[6] A.Pontillo, M.Girardelli, C.Agostinis, E.Masat, R.Bulla and S.Crovella. (2013). Bacterial LPS differently modulates inflammasome gene expression and IL-1β secretion in trophoblast cells, decidual stromal cells, and decidual endothelial cells. Reproductive Sciences , 20(5), 563–566.

[7] N.S.Ferreira, T.Bruder-Nascimento, C.A.Pereira, C.Z.Zanotto, D.S.Prado, J.F.Silva, D.M.Rassi, M.C.Foss-Freitas, J.C.Alves-Filho, D.Carlos and R.C.ETostes. (2019). NLRP3 Inflammasome and Mineralocorticoid Receptors Are Associated with Vascular Dysfunction in Type 2 Diabetes Mellitus. Cells , 8(12), 6-16.

[8] E.Park, H.S. Na, Y.R. Song, S.Y.Shin, Y.M. Kim and J.Chung. (2014). Activation of NLRP3 and AIM2 inflammasomes by Porphyromonas gingivalis infection. Infection and Immunity, 82(1), 112–123.

[9] D.M. Isaza-Guzmán, M.Hernández-Viana, D.M.Bonilla-León, M.C.Hurtado-Cadavid and S.I.Tobón-Arroyave.(2016). Determination of NLRP3 (rs4612666) and IL-1B (rs1143634) genetic polymorphisms in periodontally diseased and healthy subjects. Archives of Oral Biology, 65: 44–51.

[10] D.J. Buysse, C.F.Reynolds, T.H.Monk, S.R.Berman and D.J.Kupfer. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Research, 28(2), 193–213.

[11] J.R.Christopher, D.Ponnaiyan, H.Parthasarathy and A.Tadepalli. (2021). Association of CST3 Gene with Its Protein: Cystatin C in Health and Severe Periodontal Disease. Genetic Testing and Molecular Biomarkers, 25(6), 405–410.

[12] G.Kumar, D.Ponnaiyan, H.Parthasarathy, A.Tadepalli and S,Veeramani. (2020). Evaluation of Endocan and Tumor Necrosis Factor-α as Inflammatory Biomarkers in Type 2 Diabetes and Periodontal Disease. Genetic Testing and Molecular Biomarkers, 24(7), 431–435.

[13] J.A.García, H.Volt, C.Venegas, C.Doerrier, G.Escames, L.C.López and D.Acuña-Castroviejo. (2015). Disruption of the NF-κB/NLRP3 connection by melatonin requires retinoid-related orphan receptor-α and blocks the septic response in mice. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 29(9), 3863–3875.

[14] C.Smith, K.J.Trageser, H.Wu, F.J.Herman, U.H.Iqbal, M.Sebastian-Valverde, T. Frolinger, E.Zeng and G.M.Pasinetti. (2021). Anxiolytic effects of NLRP3 inflammasome inhibition in a model of chronic sleep deprivation. Translational Psychiatry, 11(1), 52-62.

[15] Z.Cao, Y.Fang, Y.Lu, D.Tan, C.Du, Y.Li, Q.Ma, J.Yu, M.Chen, C.Zhou, L.Pei, L.Zhang, H.Ran, M.He, Z.Yu and Z.Zhou. (2017). Melatonin alleviates cadmium-induced liver injury by inhibiting the TXNIP-NLRP3 inflammasome. Journal of Pineal Research, 62(3), 123-135.

[16] R.Zheng, Y.Ruan, Y.Yan, Z.Lin, N.Xue, Y.Yan, J.Tian, X.Yin, J.Pu and B.Zhang. (2021). Melatonin attenuates neuroinflammation by down-regulating NLRP3 inflammasome via a SIRT1-dependent pathway in MPTP-induced models of Parkinson's disease. Journal of Inflammation Research, 14: 3063–3075.