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# Comparison of melatonin levels in saliva in individuals with healthy periodontium, generalized chronic gingivitis and generalized chronic periodontitis

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***Abstract*---**Introduction: Melatonin, is a hormone secreted by the pineal gland which activates several elements of the immune system that reduce tissue destruction during the inflammatory response, either directly by scavenging free radical, reactive oxygen species and reactive nitrogen species or indirectly by modulating the action of agents such as cytokines and adhesion molecules, which contribute to the advance of cell damage. Aim: The aim of the present study was to compare and evaluate melatonin levels in saliva in individuals with healthy periodontium, generalized chronic gingivitis and generalized

chronic periodontitis. Materials and method: A total of 60 patients were examined and assessed and divided into 3 groups; Group 1: 20 individuals with healthy periodontium, Group 2: 20 individuals with generalised chronic gingivitis and Group 3: 20 individuals with generalised chronic periodontitis. Saliva samples were collected from each sample and melatonin levels were assessed using ELISA, a competitive immunoassay using a capture antibody technique. Results: Melatonin levels in saliva was highest in individuals with healthy periodontium when compared to gingivitis and periodontitis group which was highly significant and inversely proportional correlation was observed between periodontal index and melatonin levels which was statically significant. Conclusion: This study demonstrates the presence of melatonin in gingival tissue. Furthermore, melatonin levels are lowered in gingival tissues of chronic periodontitis patients.

***Keywords*---**melatonin, ELISA, Hormone, gingivitis, periodontitis, healthy periodontium.

## Introduction

Periodontal diseases are multifactorial clinical entities with a diverse aetiology. The most common forms of these diseases are caused by dental plaque, which works in tandem with a slew of local and systemic risk factors such as smoking, diabetes, putative pathogens, and immune deficiencies. [(1)]. Two important types of periodontal disease are gingivitis and periodontitis [(2)]. Gingivitis is an inflammation of the soft tissues surrounding the tooth caused by dental microbial plaque. Gingivitis is influenced by metabolic, genetic, environmental, and other factors. [(3)]. Periodontitis is an inflammatory disease characterised by the destruction of periodontal ligaments, alveolar bone, and soft tissue.[(3,4)] Damage to periodontal tissue is caused by the direct effect of bacterial-derived toxic products and the stimulation of the immune system caused by bacterial infection.[(5)]

Damage to periodontal tissue is caused by the direct effect of bacterial-derived toxic products and the stimulation of the immune system caused by bacterial infection.[(7)] With an increase in free radical production, there is a decrease in the antioxidant defence mechanism. This imbalance in the prooxidant and antioxidant systems may result in additional oxidative damage and destruction of periodontal tissue.[(8)].Furthermore, neutrophil migration to the gingiva in periodontitis causes abnormal ROS production and exacerbates cardiovascular and other diseases by affecting other organs via circulation.[(9)]

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted primarily by the pineal gland, but also by the retina, lens, iris, ciliary body, lacrimal gland, skin, and gut.[(10)] Melatonin, which was discovered in 1917, was not isolated until 1958.[(12)] It is primarily released at night and diffuses passively into saliva via the bloodstream, where it can be reliably measured.[(13)] Despite the presence of melatonin in foods such as fruits, vegetables, and wheat, dietary sources do not significantly contribute to circulating levels of melatonin.[(14)] In adulthood, the

daily variation persists, with peak serum levels occurring between midnight and 2 a.m. and lowest levels occurring between noon and 2 p.m.[11] This prominent night-time peak eventually becomes markedly attenuated with age. [15].Melatonin diffuses passively into saliva through the bloodstream, with salivary melatonin levels accounting for 24–33% of plasma levels.[13]

Melatonin has a wide range of functions, including antioxidant functions and mucosal protection from various irritants. It also protects the mouth and gastrointestinal tract from conditions like stomatitis, esophagitis, gastritis, and peptic ulcers. [16].Melatonin appears to be an important immune system modulator, as it boosts natural and acquired immunity and activates monocytes and neutrophils in vivo.[17] Melatonin is a natural anti-inflammatory.[18] It also promotes bone formation and stimulates type I collagen synthesis.[19,20] The link between periodontal health and melatonin levels is still unknown and unproven.[21,22] The purpose of this study is to compare melatonin levels in saliva in people who have healthy periodontal tissue, generalised chronic gingivitis, and generalised chronic periodontitis.

**Materials and Methods**

A total of 60 subjects with healthy periodontium, generalized chronic gingivitis and generalized chronic periodontitis were selected from the Out Patient Department of Periodontology. **60 Individuals** included in the study was be selected as per the periodontal condition and divided into 3 groups.
GROUP 1: 20 Individuals with generalised healthy periodontium
GROUP 2: 20 Individuals with generalised chronic gingivitis
GROUP 3: 20 Individuals with generalised chronic periodontitis

The selection of subjects was based on the following inclusion and exclusion criteria: Inclusion criteria included systemically healthy individuals with a age group of 18-years and above. Individuals with history of systemic neurological disorder (e.g. epilepsy or shizophrenia), systemic diseases like diabetes mellitus, pregnant females, and under medication that might alter melatonin levels (Diazepam) were excluded from the study.

The research proposal was approved by the Institutional Ethics Committee (IEC). All subjects were explained about the treatment procedure and an informed written consent were obtained from each subject. A detailed casehistory of all 60 individuals were obtained. Also Periodontal Disease Index (SiguarRamdfjord 1959) was recorded by using UNC-15 probe calibrated with millimeter markings.

**Saliva collection and storage** Saliva was collected between 8:00 am to 12:00 pm. The participants were refrained from having meals,drink, chew gum or brush teeth for at least 30 min before sampling. Saliva samples visibly contaminated with blood should be discarded and recollected. For melatonin assessment, saliva samples were collected from subjects at the time of clinical examination. Patients were seated with head slightly down and they were asked not to swallow or move his/her tongue or lips during the collection period. Saliva was allowed to accumulate in patients mouth and he/she was then asked to spit 3ml of accumulated saliva into sterile vessel. Saliva was cleared by centrifugation at

10,000 rpm at room temperature for 15min to remove particulate and bacteria. After centrifugation the clear supernatant was designated as clarified human whole saliva and frozen at 80°c. The stored saliva samples were used for estimation of Melatonin using a Melatonin Enzyme Immunometric Assay Kit.

**Melatonin Measurement**

The melatonin levels in saliva were measured using a competitive immunoassay (Human Melatonin, MT GENLISA ELISA ™,Krishgen, India). Direct Saliva Melatonin ELISA ( (Human Melatonin, MT GENLISA ELISA ™ ,Krishgen, India)is a competitive immunoassay using a capture antibody technique. The polyclonal Kennaway G280 anti-melatonin antibody has been coated onto the micro titer plate, provided in the kit. After the first 16-20 hours over night incubation, melatonin present in the pretreated saliva, controls and calibrators, respectively, compete with biotinylated melatonin during second 3 hours incubation for the binding sites of this highly specific antibody. After washing, the enzyme label, streptavidin conjugated to horseradish peroxidase (HRP) is added, which binds during a third 60 minutes incubation step to the melatonin-biotin-antibody complexes captured on the coated wells. Unbound enzyme label is then removed by a second washing step and TMB substrate (tetramethylbenzidine) is added to the wells. In a fourth 30 minutes incubation step, a chromophore is formed in inverse proportion to the amount of melatonin present in the sample. The colour turns from blue to yellow after the addition of an acidic stop solution and can be measured at 450 nm.

**Statistical analysis**

Data analysis will perform by using SPSS (Statistical Package for social sciences) Version 25:0. Intergroup comparisons for the Periodontal Disease Index (PDI) and the melatonin levels in saliva were done using One Way ANOVA test followed by post hoc Bonferroni test for pair wise comparisons. Pearson's correlation test was used to determine the correlation between PDI and melatonin levels.

**Results**

Melatonin was detected in all samples tested, and the level of melatonin varied among samples and groups. Table 1, shows mean melatonin levels in Healthy Periodontium, Generalized Chronic Gingivitis and Generalized Chronic Periodontitis. Mean melatonin levelsin healthy periodontium group 4.79 ±0.71pg/ml; in generalised chronic gingivitis group was 2.94 ±0.45pg/ml and in generalised chronic periodontitis group was 1.43 ±0.20pg/ml withand 'p value as <0.001 which was statically significant. (Figure 1) Statistically significant difference between the mean melatonin levels in the entire three groups was observed. Highest salivary melatonin levels were seen in healthyperiodontium whereas least melatonin levels were seen in generalised chronic periodontitis subjects. When the salivary melatonin for any two groups was compared, difference was found to be statistically significant. The result of our study showed major difference in healthy periodontium group while minor difference in generalised chronic gingivitis group when compared with generalised chronic periodontitis group.

Table 2, show comparison of Periodontal Disease Index (PDI) scores in Healthy Periodontium, Generalized Chronic Gingivitis and Generalized Chronic Periodontitis. Mean PDI score was of Healthy periodontium group was 0.24; in generalised chronic gingivitis group was 1.34 and in generalised chronic periodontitis group was 3.89 and 'p value as <0.001 which was statically significant. Highest PDI score was seen in generalised chronic periodontitis subjects whereas least PDI score was seen in healthy periodontium subjects. (Figure 2)

PDI score was more in gingivitis subjects as compared to healthy subjects and difference between two groups was significant (p=0.001). PDI score was more in periodontitis subjects as compared to healthy subjects and difference between two groups was significant (p=0.001). PDI score was more in periodontitis subjects as compared to gingivitis subjects and difference between two groups was significant (p=0.001). (Table 2.1)

Table 3 shows correlation between melatonin levels in saliva and Periodontal Disease Index scores using Pearson correlation coefficient (r). When the generalised chronic gingivitis i.e., was considered Pearson correlation coefficient (r) was -0.334 with a 'p' value <0.150. Negative correlation was observed between melatonin levels and Periodontal Disease Index in Generalised Chronic Gingivitis which is statically significant. This suggests that melatonin level in saliva is inversely proportion with Periodontal Disease Index in gingivitis group, which indicates higher the Periodontal Disease Index lower is the level of melatonin in Generalised Chronic Gingivitis group. (Figure 3)

Table 3 shows correlation between melatonin levels in saliva and Periodontal Disease Index scores using Pearson correlation coefficient (r). When the generalised chronic gingivitis i.e., Group III was considered Pearson correlation coefficient (r) was 0.243 with a 'p' value <0.301. This suggests that melatonin level in saliva shows correlation with Periodontal Disease Index in periodontitis group which is statically significant. It indicates that higher the Periodontal Disease Index lower is the level of melatonin in Generalised Chronic Periodontitis group. (Figure 3)

**Discussion**

Gingival bleeding, periodontal pocket formation, and the destruction of connective tissue attachment are all symptoms of periodontal disease. This disease begins with the stimulation of the immune response against bacteria in the dental biofilm. Plaque-induced gingival inflammation is the most common form of periodontal disease in humans, and it can progress to more destructive forms of periodontitis. There is a significant loss of gingival tissue and alveolar bone in the advanced stage of the disease. In the present study saliva was collected to assess melatonin levels and any possible variability of this hormone in periodontal health and disease. Samples were taken during evening (8 to 12 PM) to ensure that the sampling time should not be affected by the diurnal cycle of melatonin, which peaks between midnight and 2 AM.[11]

When compared to generalised chronic gingivitis and periodontitis, higher melatonin levels were associated with healthy periodontium in the current study. The presence of higher levels of melatonin in samples taken from healthy, gingivitis, and periodontitis subjects confirmed this. On the other hand, both chronic gingivitis and chronic periodontitis groups had lower melatonin levels than healthy groups.

Factors that increase the host's susceptibility to periodontal diseases include familial aggregation, single nucleotide polymorphisms, defective neutrophil functions or primed neutrophils, antibodies to bacteria, smoking, stress, and herpesvirus infections.[23] As a result, the aetiology of periodontal diseases is multifactorial. The fact that higher levels of melatonin are found in healthy people suggests that it plays a protective role. Furthermore, the absence of melatonin in periodontitis may highlight the importance of remotely produced molecules in periodontitis pathogenesis.

The generation of free radicals, some of which are derived from oral bacteria and others from inflammation and the induced immune response, is an important aspect of periodontal disease; the activation of pro-inflammatory molecules results in the destruction of periodontal tissues. The tissue oxidative damage in periodontitis is thought to be caused by an increase in both oxygen and nitrogen reactive species [7]. This increase in free radicals is accompanied by a decrease in antioxidant defence; this imbalance may result in significant periodontal tissue deterioration.[24] Melatonin, with its antioxidant and free-radical scavenging properties, plays an important role in the control of this disease. As a result, melatonin levels may be reduced in periodontitis as a result of by-products or mediators that interfere with melatonin levels. In advanced periodontal diseases, a decrease in melatonin levels as an antioxidant may cause an imbalance between the prooxidant and antioxidant systems, leading to further oxidative attack and periodontal tissue deterioration.[24] Melatonin's potential therapeutic effects in periodontitis have been demonstrated in vitro, animal studies, and clinical trials.[25]

There is still no conclusive evidence of a link between periodontal disease and salivary melatonin levels.[21] Cutando et al.[11] discovered an inverse relationship between salivary melatonin and the severity of periodontal disease in humans in a study. As the severity of periodontal disease increases, the salivary melatonin level decreases, indicating that melatonin may act as a bacterial infection protector. Similar findings were reported in another study, in which researchers compared the levels of melatonin in saliva and gingival crevicular fluid in four groups of patients with various degrees of periodontitis, finding that the more severe the periodontitis, the lower the melatonin levels found, with significant differences between the healthy group and the two groups affected by the disease (chronic and aggressive periodontitis). Melatonin levels in saliva and gingival crevicular fluid were found to be similar (with no significant differences) in this study, correlating with previous findings. The authors Gómez-study Moreno's discovered that patients with periodontal disease had significantly lower plasma and salivary levels of melatonin than healthy subjects, while maintaining a similar salivary/plasma melatonin ratio. Only one study, conducted on diabetic people, found that salivary melatonin levels were increased with the worst

periodontal status of all the evaluated studies on melatonin levels in patients with periodontal disease. This was explained by the authors as a result of oral inflammatory mediators. Because of the elevated levels of reactive oxygen species and inflammation found in these patients, the low concentrations of melatonin found in saliva of patients with periodontal disease may be due to its increased use as a free-radical scavenger and anti-inflammatory.

**Conclusion**

From the findings of our study following conclusions could be drawn:

1. Salivary melatonin level decreases as the severity of periodontal disease increases.
2. Salivary melatonin level decreases if tissue destruction and inflammation is more.

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