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Gingival, Plasma and Salivary Levels of Melatonin in Periodontally Healthy Individuals and Chronic Periodontitis Patients: A Pilot Study

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ABSTRACT

Introduction: Periodontal disease is an inflammatory condition affecting tooth supporting structures in which dysregulated immune response and oxidative stress mediate tissue destruction. Melatonin, the pineal gland hormone is a regulator of circadian rhythm, an antioxidant and an immunomodulator. Previous studies have shown lowered melatonin levels in saliva, plasma and gingival crevicular fluid (GCF) of patients with periodontal disease. Till date no study has assessed the melatonin levels in gingival tissues.

Materials and Methods: Five healthy individuals and 15 chronic periodontitis patients were recruited for this pilot study. 5ml of whole saliva, 2 ml peripheral blood and gingival tissue samples were obtained from each individual at 8.00 am in fasting state. Melatonin assay was performed with a commercially available ELISA kit. Statistical analysis was done to assess the difference in mean melatonin levels among the groups.

Results: No statistically significant difference was found in mean melatonin levels between healthy individuals and chronic periodontitis patients in saliva (p=.266) and plasma (p=.933) samples, whereas in gingival tissue samples (p=.015), the melatonin levels were significantly lowered in chronic periodontitis patients compared to healthy individuals.

Conclusion: This study demonstrates the presence of melatonin in gingival tissue. Furthermore, melatonin levels are lowered in gingival tissues of chronic periodontitis patients.

Keywords: Antioxidant, Chronic periodontitis, Gingiva, Melatonin, Plasma, Saliva

INTRODUCTION

Melatonin (N-Acetyl-5-hydroxytryptamine) is an indole amine hormone synthesized predominantly by the pineal gland [1]. Extrapineal synthesis and release of melatonin can occur from organs like ovaries [2], retina [3], gastrointestinal tract [4] and also from cells such as human lymphocytes [5]. Melatonin regulates circadian rhythm and the biological clock in the human body [6]. In addition, it performs numerous other functions like immunomodulation [7] and osteopromotion [8]. Melatonin has potent antioxidant action against reactive oxygen species [9] and can mitigate deleterious effects of free radical damage in the human body.

It is well known that chronic periodontitis is an inflammatory condition of the tooth supporting structures with a microbial aetiology, in which dysregulated immune response and a jeopardized oxidant - anti oxidant balance play a major role in causing tissue destruction and tooth loss. Previous studies have shown increased oxidative stress markers [10,11] and lowered antioxidant status [12] in periodontal disease compared to health. In this context, the presence of melatonin as an antioxidant in saliva and plasma and its association with the pathogenesis of chronic periodontitis has been researched [13]. It has been hypothesized that salivary melatonin can act as a potent local antioxidant boosting the defence mechanism against periodontal pathology [13]. In a recent study melatonin has been estimated from GCF [14]. Till date no study has investigated, melatonin levels in the gingival tissue. This pilot study was designed to measure and compare the levels of melatonin in plasma, saliva and gingival tissue samples obtained from healthy individuals and chronic periodontitis patients.

MATERIALS AND METHODS

This pilot study with a case control design was performed in the Department of Periodontology, Faculty of Dental Sciences of Sri Ramachandra University (Chennai, Tamil Nadu, India) from January 2014 to April 2014. The study was approved by the Institutional Ethics Committee. The Helsinki guidelines of ethical research were followed in our study. Sample collection was done in the Outpatient Department of Periodontology after obtaining written informed consent from the individuals. Melatonin assay was done in the Department of Biochemistry, Sri Ramachandra University (Chennai, Tamil Nadu, India).

The study consisted of a total of 20 participants. They were divided into two groups, healthy individuals (n=5) and chronic periodontitis patients (n=15). The inclusion criteria for healthy individuals were: the presence of clinically healthy gingiva with no evidence of bleeding on probing and local factors (dental plaque and calculus) and absence of probing pocket depth >3 mm. The inclusion criteria for chronic periodontitis patients were the presence of abundant local factors (dental plaque and calculus), generalised bleeding on probing and attachment loss in more than 30% of intraoral periodontal sites [15] with radiographic evidence of bone loss as confirmed by examining an orthopantamogram. The exclusion criteria for both the groups were the presence of systemic disease and habits such as smoking, alcoholism and pan chewing. Individuals who had consumed antibiotics, anti-inflammatory agents, vitamins, antioxidants and melatonin supplements in the last six months were excluded from the study. Pregnant and lactating women were also excluded from the study. All study participants underwent basic haematological investigations prior to sample collection to minimize surgical risks. 5 ml of unstimulated whole saliva, 2 ml of peripheral blood, and gingival tissue samples were collected from all the study subjects at 8.00 am in the morning in the fasting state. The individuals were asked to refrain from eating solid food or drinking fluids from 8 pm the previous night. Saliva was collected without stimulation by aspiration from the vestibule and floor of the mouth using a sterile 5ml syringe and transferred to sterile eppendorf tubes for storage. A 2 ml blood

sample was collected from the antecubetal vein in vacutainers, centrifuged at 3,000 g at 4⁰C for three minutes and the plasma was separated for storage. Gingival tissue samples were collected by excision using a surgical Bard Parker blade from healthy individuals undergoing surgical crown lengthening procedure and from chronic periodontitis patients prior to non-surgical therapy. The excised gingival tissues were wrapped in sterile aluminium foil and transferred to sterile Eppendorf tubes for storage. All samples were stored at – 20°C until processing. A commercially available ELISA kit (IBL International, Hamburg, Germany) was used to quantify melatonin levels in the samples. All samples were processed according to the manufacturer's instructions prior to the ELISA procedure. After thawing; the plasma samples and saliva samples were centrifuged at 3,500 g at 4⁰C for three minutes and the supernatant obtained was used for the study. The gingival tissue samples were thawed and homogenized using phosphate buffered saline and the supernatant was collected. The plasma, saliva and gingival homogenate supernatant samples were purified by graded passage through plastic purification columns provided along with the ELISA kit. After sample purification ELISA procedure was performed according to the instructions provided in the kit and the results were finally obtained by subjecting the 96 well microtitre plate to the ELISA reader at 405 nm. The results were compared with standards and blank wells provided in the kit. The collected data was analysed with statistical software SPSS16.0 version. The Non Parametric Mann Whitney U test was used to compare the melatonin levels in plasma, saliva and gingival tissue homogenates between healthy individuals and chronic periodontitis patients. In this statistical tool, probability value ($p< .05$) was considered statistically significant.

RESULTS

The subjects were categorized into two groups, Group 1: Healthy individuals (n=5) and Group 2: chronic periodontitis patients (n=15). The demographic details of the study population are depicted in [Table/Fig-1]. The mean melatonin levels in plasma, saliva and gingival tissue homogenates in picograms/ml of the study groups is depicted in [Table/Fig-2]. Based on the analysis we found that there was no significant difference in plasma ($p=.933$) and salivary ($p=.266$) levels of melatonin in the two groups. However, there was a statistically significant lowering of melatonin levels in the gingival tissue homogenates of chronic periodontitis patients compared to healthy individuals ($p=.015$).

Variables	Healthy Individuals (n=5)	Chronic Periodontitis (n=15)
Gender (M/F)	3/2	8/7
Mean age (y)	29.6±5.814	41.18±7.064
Plaque index (%)	15±12	90±41
Clinical attachment loss (mm)	0±0	5.2±0.3
Bleeding on Probing (%)	5±3	80±35

[Table/Fig-1]: Demographic data of the study participants

Sample	Groups	Melatonin Levels in Picogram /ml	p-value
Saliva	Healthy individuals	3.95±3.28	0.266(NS)
	Chronic Periodontitis patients	2.8±2.39	
Plasma	Healthy individuals	4.4±2.35	0.933(NS)
	Chronic Periodontitis patients	4.6±3.16	
Gingival tissue homogenates	Healthy individuals	1.58±.0.84	0.015(S)
	Chronic Periodontitis patients	0.54±0.80	

[Table/Fig-2]: Melatonin levels in saliva, plasma and gingival tissue samples of the study groups
Note: NS-denotes not statistically significant and S- denotes statistically significant

DISCUSSION

Tissue destruction in periodontal disease occurs as a consequence of microbial infection, a dysregulated immune response and overzealous generation of free radicals and reactive oxygen species. Numerous studies have shown a jeopardized antioxidant status in plasma, saliva and GCF of chronic periodontitis patients [12]. In this connection, melatonin has been investigated for its antioxidant role in preventing periodontal disease progression. A previous study has shown lowered levels of melatonin in plasma and saliva of chronic periodontitis patients [13]. In a recent study [14], melatonin has been detected and found to be lowered in GCF and saliva samples of patients with chronic periodontitis. With this background information, we set out to measure the levels of melatonin in plasma, saliva and gingival tissue samples of healthy individuals (n=5) and chronic periodontitis patients (n=15).

The results of our study demonstrated detectable levels of melatonin in all the samples (plasma, saliva and gingival tissue) assayed. Our results showed variation in melatonin levels in saliva and plasma samples in the two groups (healthy individuals and chronic periodontitis patients) [Table/Fig-2]. Intergroup comparison revealed a slight elevation in the levels of plasma melatonin in patients with chronic periodontitis as compared to healthy individuals; however the difference was not statistically significant ($p=0.933$). This variation can be explained because plasma melatonin is derived from the pineal gland and many other organs and is controlled by several confounding factors such as circadian rhythm, systemic antioxidant status and the sleep wake cycle. The salivary melatonin levels were slightly lower in the chronic periodontitis patients as compared the healthy individuals. However, the difference was not statistically significant ($p=0.266$). In this regard, our results contradict a previous study that has shown statistically significant lowering of melatonin levels in saliva and plasma of chronic periodontitis patients compared to healthy individuals [13].

The presence of melatonin in gingival tissues has not been investigated previously and to the best of our knowledge the present study is the first to demonstrate detectable levels of melatonin in gingival tissue samples. We are justified in measuring gingival melatonin levels as gingiva is the first tissue to face microbial challenge in periodontal disease pathogenesis. This tissue is consequently infiltrated by numerous immune cells that produce inflammatory mediators and reactive oxygen species. Hence, the gingiva can be considered as the direct site where periodontal destruction occurs, and gingival tissue samples could be regarded ideal for detection of an antioxidant such as melatonin.

We found that the levels of melatonin were lowered in the gingival tissue samples of chronic periodontitis patients compared to healthy individuals. This difference was statistically significant ($p=.015$) Melatonin is a potent antioxidant [9]. It exerts antioxidant effects by rapid diffusion into the mitochondria of cells where it stabilises the enzymes of the electron transport chain [16]. The effects described above could operate in the gingiva in the pathogenesis of chronic periodontitis. Melatonin that is locally present in the gingiva could possibly be destroyed in the process of periodontal destruction due to the oxidant stress and could thereby be lowered in chronic periodontitis.

One cannot rule out the possibility of gingiva as a source of melatonin considering the fact that human gingiva is a target tissue for many hormones. Metabolism of steroidal hormones such as cortisol, estrogen and testosterone [17-19] is known to occur in the gingiva. It is worthwhile to note that glucocorticoid receptors have been demonstrated in the gingiva with high affinity for binding with radio labelled cortisol [20]. Our study has shown the presence of melatonin, a pineal gland hormone in the gingiva. It has been documented that melatonin is predominantly synthesised by the pinealocytes of the pineal gland during the dark phase of the day.

Extra pineal melatonin production has also been documented in organs such as the ovaries [2], lymphocytes [5], and gastrointestinal tract [4]. The role of melatonin synthesised outside the pineal gland is predominantly for antioxidant action [9] , immunomodulatory role [7], and osteopromotive effects [8]. In the gingiva too melatonin could perform the same role. The findings of our study have raised another important query as to how melatonin is synthesised in the gingiva. The biochemical pathway of synthesis requires the amino acid tyrosine and a group of enzymes. A recent study has demonstrated the presence of melatonin synthesising enzymes in the rat salivary gland tissue [21]. Whether these enzymes are present in the gingiva is yet to be investigated. Another possible mechanism to explain the presence of melatonin in gingival tissues could be its synthesis and release from mast cells. Mast cells are important resident leukocytes in the gingiva [22]. A study has shown synthesis and release of melatonin from human mast cells [23]. This phenomenon could operate in the gingiva also. Yet another source for melatonin presence in the gingiva could be its diffusion from systemic circulation to the gingiva. Since gingiva is a highly vascular tissue, there exists a possibility for this mechanism. Another interesting fact that should be analysed is how melatonin levels are maintained and regulated in the gingiva. It is possible that melatonin could be synthesised and released in the gingiva for its local antioxidant role and from the gingiva it could reach the GCF by vascular diffusion.

The findings of our study highlight the role of melatonin as an antioxidant in periodontal homeostasis. The limitations of our study are its low sample size but could be justified since it is only a pilot study. Moreover, we have not identified the enzymes required for melatonin synthesis in the gingiva. Future studies are required to analyse these facts.

CONCLUSION

Our study shows detectable amounts of melatonin in human gingiva which is decreased in chronic periodontitis thereby compromising the antioxidant capacity of the gingival tissues. It may be interesting to see if melatonin can be used as a local drug delivery to reduce oxidative stress and tissue damage in periodontal disease.

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