Section: Research Paper

IMPACT OF NON-SURGICAL PERIODONTAL THERAPY ON THE LEVELS OF MELATONIN IN THE SALIVA OF SUBJECTS HAVING PERIODONTAL DISEASES

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ABSTRACT

Background: Periodontitis is a chronic condition affecting the supporting tissues of teeth leading to an imbalance of the antioxidant-oxidant system. Melatonin being an immune modulator and antioxidant also controls circadian rhythms and is a hormone.

Aim: The present study aimed to assess the impact of non-surgical periodontal therapy on the levels of melatonin in the saliva of subjects having periodontal diseases

Methods: 90 subjects were divided into 3 groups of equal participants (n=30) having periodontitis (Group I), gingivitis (Group II), and healthy periodontium (Group III). Saliva samples (unstimulated) were collected at baseline and 30 days following scaling and root planing in Group I and II. The data were analyzed statistically using the t-test and significance with $p<0.05$ for results formulation.

Results: The study results showed that the levels of serum melatonin were lower in subjects having chronic periodontitis (Group I) compared to the other two groups. The difference was

statistically significant with $p<0.05$. A marked improvement was seen in the levels of serum melatonin in subjects having gingivitis and chronic periodontitis.

Conclusion: A significant negative correlation is seen between the severity of periodontal disease and the levels of serum melatonin. A significant increase in the levels of serum melatonin is seen after scaling and root planing depicting the protective role of melatonin in periodontal disease making it an effective biomarker in periodontitis.

Keywords: Melatonin, periodontal disease, periodontal therapy, periodontitis, scaling, and root planing.

INTRODUCTION

Periodontitis is a chronic inflammatory condition damaging the supporting tissues of teeth. The main etiologic factor for periodontitis is dental plaque having gram-negative microorganisms where the bacterial products can evoke an exaggerated immune response. The immune response is faced by adaptive and innate immunity resulting in periodontal tissue destruction with the secretion of matrix metalloproteinases, osteoclastogenic products, and inflammatory cytokines.[1] The classic feature of periodontitis is the production of free radicals. An increase in nitrogen and reactive oxygen species leads to oxidative damage in the periodontal tissues and is also linked to an imbalance in the antioxidant/oxidant system.[2]

An indoleamine hormone, melatonin is secreted from the pineal gland governing the biological clock and circadian rhythm of the human body along with functions such as osteopromotion and immunomodulation. Being an antioxidant, melatonin can help in reducing the negative impact of damage from free radicals. In the oral cavity, antioxidant actions depict its involvement in pathogenic periodontal processes.[3] The ability of antioxidants to bind to the metals decreases bacterial development in an in-vitro environment linking it to periodontitis. Levels of melatonin can alter in periodontitis owing to bacterial invasion and oxidative stress. On adequate treatment, periodontal inflammation can decrease causing an imbalance in antioxidant/oxidant status.[4]

One of the best treatment options for periodontitis is scaling and root planing where the biofilm of the bacteria is removed decreasing the burden of the bacteria and the formation of the free radicals. However, previous literature research is done on the association between periodontal disease and serum melatonin. The available literature data is limited on the impact of periodontal disease treatment on the levels of serum melatonin. Melatonin helps treat periodontal disease as it slows the process of alveolar bone loss, increases the immune response, prevents tissue damage, and lowers oxidative stress.[5]

As the evidence is limited concerning the effect of non-surgical treatment of periodontal disease on the levels of serum melatonin. The present study aimed to assess the impact of non-surgical periodontal therapy on the levels of melatonin in the saliva of subjects having periodontal diseases.

MATERIALS AND METHODS

The present clinical study aimed to assess the impact of non-surgical periodontal therapy on the levels of melatonin in the saliva of subjects having periodontal diseases. After explaining the

hazards, benefits, and nature of the study, informed consent was taken from all the subjects in written and verbal format. The study was commenced at the Department of Periodontology of the Institute.

The study included 90 participants recruited from the Outpatient Department of Periodontology. The inclusion criteria for the study were subjects that were systemically healthy, in the age range of 30-60 years, having periodontitis and gingivitis induced from the biofilms, were systemically healthy, and had more than 20 teeth present. The exclusion criteria were subjects on medication affecting salivary flow, having xerostomia, subjects on night shift, lactating females, pregnant females, nicotine users, medications affecting the periodontium, and having systemic diseases affecting the periodontal health.

Included 90 subjects were divided into three groups of 30 participants each where Group I had subjects with periodontitis following the 2017 classification of peri-implant and periodontal diseases, Group II had subjects having gingivitis, and Group III had subjects having healthy periodontium.

After assessing the oral cavity, unstimulated saliva was collected from all the subjects along with the recording of CAL (clinical attachment level), BOP (bleeding on probing), PPD (probing pocket depth), and PI (plaque index).[6] The unstimulated saliva was collected from the subjects of Group I and II at baseline and 30 days after the scaling and root planing. The collected saliva samples were subjected to centrifugation at 3000 rpm for 15 minutes. The supernatant was then collected and autoclaved which was then stored at -20^{o}C till completion of the assessment. For assessing the melatonin levels, ELISA (enzyme-linked immunosorbent assay) was done with a commercial kit.

The data gathered were analyzed statistically using SPSS software version 21.0 and a t-test. The results were expressed in numbers and percentages and mean and standard deviations. The level of significance was taken at $p<0.05$.

RESULTS

The present clinical study aimed to assess the impact of non-surgical periodontal therapy on the levels of melatonin in the saliva of subjects having periodontal diseases. Included 90 subjects were divided into three groups of 30 participants each where Group I had subjects with periodontitis following the 2017 classification of peri-implant and periodontal diseases, Group II had subjects having gingivitis, and Group III had subjects having healthy periodontium. The mean age of the study subjects was 45.7±4.1 years, 42.3±6.64 years, and 38.3±5.12 years respectively for Groups I (periodontitis), II (gingivitis), and group III (healthy periodontium) and the respective age range was 37-51, 31-52, and 30-46 years. There were 8 males and 22 females in Group I, 20 males and 10 females in Group II, and 16 males and 14 females in Group III as described in Table 1.

On intergroup comparison of various periodontal parameters in Groups II and I, in Group I, the plaque index significantly decreased from 2.51±0.27 to 1.04±0.14 from baseline to 30 days and in Group II, from 1.74±0.23 to 0.93±0.18. The difference between the two groups was

statistically significant with p<0.001. Bleeding on probing also decreased significantly from baseline to 30 days in both groups I with periodontitis and II with gingivitis with p<0.001. Probing pocket depth also decreased significantly in Groups I and II with p<0.001. The clinical attachment level in Group I with periodontitis decreased significantly from 5.12±0.79 at baseline to 4.84±0.82 after 30 days of assessment with p=0.007. The salivary melatonin levels in the gingivitis group increased significantly from 18.74±1.05 to 27.54±3.76 with p<0.001. In the periodontitis group, salivary melatonin levels increased significantly from 13.56±2.33 to 29.96±4.44 with p<0.001 as summarized in Table 2.

For plaque index, a significant difference was seen with a higher plaque index in gingivitis compared to the healthy group with p<0.001. At 1 month, a higher plaque index was seen in the healthy group with no treatment (1.03±0.27) compared to treated gingivitis group II (0.93±0.18). However, the difference was statistically non-significant with p=0.55. A significantly higher plaque index was seen in the periodontitis group compared to the gingivitis group with p<0.001. However, at 1 month, a non-significantly higher plaque index was seen in the periodontitis group with p=0.08. At baseline, a significantly higher plaque index was seen in Group I with periodontitis compared to healthy subjects with p<0.001. However, at 1 month, a non-significantly higher plaque index was seen in Group I with periodontitis (p=0.86) (Table 3).

At baseline, significantly lower bleeding on probing (BOP) was seen in the healthy group compared to gingivitis with p<0.001. At 1 month, a non-significant difference in BOP was seen in the gingivitis and healthy group with p=0.08. Significantly higher bleeding on probing was seen in the periodontitis group compared to Group II with gingivitis at baseline with p=0.008. At 1 month, a non-significant difference was seen in BOP for the gingivitis and periodontitis group with p=055. A significant difference was seen in the healthy and periodontitis group for BOP at baseline as well as 1 month with p<0.001 (Table 3).

For probing pocket depth (PPD), at baseline, PPD was significantly higher for the gingivitis group compared to healthy subjects with p<0.001. However, at the 1-month assessment, a non-significant difference was seen in PPD for the healthy and gingivitis group with p=0.08. A significantly higher PPD was seen for the periodontitis group (I) compared to the gingivitis group at baseline and 1 month both with p<0.001. At baseline, a significantly higher PPD was seen in periodontitis group I compared to healthy periodontium group III with p<0.001. However, a non-significant difference was seen in PPD at 1 month for periodontitis and healthy periodontium group with p=0.06 (Table 3).

Concerning serum melatonin, at baseline, serum melatonin was significantly higher in healthy subjects at 31.34±8.71 compared to gingivitis subjects with 18.74±1.05 and p<0.001. However, at 1 month, a non-significant difference in melatonin levels was seen in the healthy and gingivitis group with p=0.07. At baseline, melatonin levels were significantly higher for gingivitis subjects compared to periodontitis subjects with p<0.01. However, at 1 month, a non-significant difference was seen in gingivitis and periodontitis subjects with p=0.22. A significantly higher serum melatonin level was seen in healthy subjects compared to periodontitis subjects with

p<0.001. However, at 1 month, a non-significant difference was seen in healthy and periodontitis subjects with p=0.68 (Table 3).

Characteristics	**Group III (Healthy)**	**Group II (Gingivitis)**	**Group I (Periodontitis)**
Mean age (years)	38.3±5.12	42.3±6.64	45.7±4.1
Age range (years)	30-46	31-52	37-51
Gender			
Males	16	20	8
Females	14	10	22

Table 1: Demographic characteristics of the study subjects

Groups	**Factors**	**Number (n)**	**Assessment time**	**Mean ± S. D**	**p-value**
II	**Plaque Index**	30	Baseline	1.74±0.23	**<0.001**
		30	30 days	0.93±0.18	
	Bleeding on probing	30	Baseline	1.62±0.46	**<0.001**
		30	30 days	0.82±0.35	
	Probing pocket depth	30	Baseline	4.03±0.52	**<0.001**
		30	30 days	2.56±0.37	
	Salivary melatonin	30	Baseline	18.74±1.05	**<0.001**
		30	30 days	27.54±3.76	
I	**Plaque Index**	30	Baseline	2.51±0.27	**<0.001**
		30	30 days	1.04±0.14	
	Bleeding on probing	30	Baseline	2.15±0.56	**<0.001**
		30	30 days	0.94±0.53	
	Probing pocket depth	30	Baseline	6.05±0.84	**<0.001**
		30	30 days	4.56±0.84	
	Clinical attachment level	30	Baseline	5.12±0.79	0.007
		30	30 days	4.84±0.82	
	Salivary melatonin	30	Baseline	13.56±2.33	**<0.001**
		30	30 days	29.96±4.44	

Table 2: Intergroup comparison of various periodontal parameters in Groups II and I

Groups	**Number (n)**	**Factors**	**Assessment time**	**Mean ± S. D**	**p-value**
III	**30**	**Plaque index**	Baseline	1.03±0.27	**<0.001**
II	**30**			1.74±0.23	
III	**30**		1 month	1.03±0.27	0.55

II	**30**			0.93±0.18	
II	**30**		Baseline	1.74±0.23	**<0.001**
I	**30**			2.51±0.27	
II	**30**		1 month	0.93±0.18	0.08
I	**30**			1.03±0.27	
III	**30**		Baseline	1.03±0.27	**<0.001**
I	**30**			2.51±0.27	
III	**30**		1 month	1.03±0.27	0.86
I	**30**			1.04±0.13	
III	**30**	**Bleeding on probing**	Baseline	0.56±0.26	**<0.001**
II	**30**			1.62±0.46	
III	**30**		1 month	0.56±0.26	0.08
II	**30**			0.82±0.35	
II	**30**		Baseline	1.62±0.46	**0.008**
I	**30**			2.15±0.56	
II	**30**		1 month	0.82±0.35	**0.55**
I	**30**			0.94±0.53	
III	**30**		Baseline	0.56±0.26	**<0.001**
I	**30**			2.15±0.56	
III	**30**		1 month	0.56±0.26	**<0.001**
I	**30**			0.94±0.53	
III	**30**	**Probing pocket depth**	Baseline	2.85±0.52	**<0.001**
II	**30**			4.03±0.52	
III	**30**		1 month	2.85±0.52	0.08
II	**30**			2.56±0.37	
II	**30**		Baseline	4.03±0.52	**<0.001**
I	**30**			6.05±0.84	
II	**30**		1 month	2.56±0.37	**<0.001**
I	**30**			4.56±0.84	
III	**30**		Baseline	2.85±0.52	**<0.001**
I	**30**			6.05±0.84	
III	**30**		1 month	2.85±0.52	0.06
I	**30**			4.56±0.84	
III	**30**	**Serum melatonin**	Baseline	31.34±8.71	**<0.001**
II	**30**			18.74±1.05	
III	**30**		1 month	31.34±8.71	0.07
II	**30**			27.54±3.76	
II	**30**		Baseline	18.74±1.05	**<0.001**
I	**30**			13.56±2.33	
II	**30**		1 month	27.54±3.76	0.22
I	**30**			29.96±4.44	
III	**30**		Baseline	31.34±8.71	**<0.001**
I	**30**			13.56±2.33	
III	**30**		1 month	31.34±8.71	0.68

I	**30**			29.96±4.44	

Table 3: Intergroup comparison of various periodontal parameters in Groups I, II, and III

DISCUSSION

The present study assessed 90 subjects and was divided into three groups of 30 participants each where Group I had subjects with periodontitis following the 2017 classification of peri-implant and periodontal diseases, Group II had subjects having gingivitis, and Group III had subjects having healthy periodontium. On intergroup comparison of various periodontal parameters in Groups II and I, in Group I, the plaque index significantly decreased from 2.51±0.27 to 1.04±0.14 from baseline to 30 days and in Group II, from 1.74±0.23 to 0.93±0.18. The difference between the two groups was statistically significant with $p<0.001$. Bleeding on probing also decreased significantly from baseline to 30 days in both groups I with periodontitis and II with gingivitis with $p<0.001$. Probing pocket depth also decreased significantly in Groups I and II with $p<0.001$. The clinical attachment level in Group I with periodontitis decreased significantly from 5.12±0.79 at baseline to 4.84±0.82 after 30 days of assessment with $p=0.007$. The salivary melatonin levels in the gingivitis group increased significantly from 18.74±1.05 to 27.54±3.76 with $p<0.001$. In the periodontitis group, salivary melatonin levels increased significantly from 13.56±2.33 to 29.96±4.44 with $p<0.001$. These results were consistent with the studies of Cugini MA et al[7] in 2000 and Lui J et al[8] in 2011 where similar results were seen for phase I periodontal therapy for gingivitis and periodontitis as in the present study.

On assessing the plaque index, a significant difference was seen with a higher plaque index in gingivitis compared to the healthy group with $p<0.001$. At 1 month, a higher plaque index was seen in the healthy group with no treatment (1.03±0.27) compared to treated gingivitis group II (0.93±0.18). However, the difference was statistically non-significant with $p=0.55$. A significantly higher plaque index was seen in the periodontitis group compared to the gingivitis group with $p<0.001$. However, at 1 month, a non-significantly higher plaque index was seen in the periodontitis group with $p=0.08$. At baseline, a significantly higher plaque index was seen in Group I with periodontitis compared to healthy subjects with $p<0.001$. However, at 1 month, a non-significantly higher plaque index was seen in Group I with periodontitis ($p=0.86$). These findings were in agreement with the findings of Cutando A et al[9] in 2007 and Hagh LG et al[10] in 2013 where similar results were seen for plaque index showing a reduction in plaque index following non-surgical periodontal treatment.

The study results showed that at baseline, a significantly lower bleeding on probing (BOP) was seen in a healthy group compared to gingivitis with $p<0.001$. At 1 month, a non-significant difference in BOP was seen in the gingivitis and healthy group with $p=0.08$. Significantly higher bleeding on probing was seen in the periodontitis group compared to Group II with gingivitis at baseline with $p=0.008$. At 1 month, a non-significant difference was seen in BOP for the gingivitis and periodontitis group with $p=055$. A significant difference was seen in the healthy and periodontitis group for BOP at baseline as well as 1 month with $p<0.001$. These results were in line with the studies of Beikler T et al[11] in 2014 and Srinath R et al[12] in 2010 where authors reported reduced BOP following periodontal treatment as in the present study.

Concerning the probing pocket depth (PPD), at baseline, PPD was significantly higher for the gingivitis group compared to healthy subjects with $p<0.001$. However, at the 1-month assessment, a non-significant difference was seen in PPD for the healthy and gingivitis group with $p=0.08$. A significantly higher PPD was seen for the periodontitis group (I) compared to the gingivitis group at baseline and 1 month both with $p<0.001$. At baseline, a significantly higher PPD was seen in periodontitis group I compared to healthy periodontium group III with $p<0.001$. However, a non-significant difference was seen in PPD at 1 month for periodontitis and healthy periodontium group with $p=0.06$. These results were comparable to the studies of Tekbas OF et al[13] in 2008 and Wei D et al[14] in 2010 where authors suggested reduced PPD following non-surgical periodontal therapy.

On assessing the serum melatonin, at baseline, serum melatonin was significantly higher in healthy subjects at 31.34±8.71 compared to gingivitis subjects with 18.74±1.05 and $p<0.001$. However, at 1 month, a non-significant difference in melatonin levels was seen in the healthy and gingivitis group with $p=0.07$. At baseline, melatonin levels were significantly higher for gingivitis subjects compared to periodontitis subjects with $p<0.01$. However, at 1 month, a non-significant difference was seen in gingivitis and periodontitis subjects with $p=0.22$. A significantly higher serum melatonin level was seen in healthy subjects compared to periodontitis subjects with $p<0.001$. However, at 1 month, a non-significant difference was seen in healthy and periodontitis subjects with $p=0.68$. These findings were in line with the results of Bertl K et al[15] in 2013 and Travis RC et al[16] in 2004 where authors suggested similar alteration in the melatonin levels in periodontal diseases and following treatment as in the present study.

CONCLUSION

Considering its limitations, the present study concludes that a significant negative correlation is seen between the severity of periodontal disease and the levels of serum melatonin. A significant increase in the levels of serum melatonin is seen after scaling and root planing depicting the protective role of melatonin in periodontal disease making it an effective biomarker in periodontitis.

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