

## Original Article

# Effect of Sudarshan Kriya Pranayama on periodontal status and human salivary beta-defensin-2: An interventional study

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## ABSTRACT

**Background:** Yogic stretching (asana) has been proven to have an effect on salivary human beta-defensin-2 (HBD-2) concentration, which is an antimicrobial peptide and is an inflammatory marker in periodontal disease. Sudarshan Kriya Pranayama (SKP) is a part of yoga which involves rhythmic breathing. Hence, we aim to evaluate the periodontal parameters and to estimate the salivary HBD-2 level before and after SKP program in periodontitis individuals.

**Materials and Methods:** An interventional study was designed and individuals were divided into three groups: Group I – healthy periodontium, Group II – chronic gingivitis, and Group III – chronic periodontitis. SKP was the interventional tool. The clinical parameters such as plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL), and salivary HBD-2 level were analyzed at baseline and 90 days after the SKP practice. Enzyme-linked immunosorbent assay (ELISA) was used to evaluate salivary HBD-2. McNemar's Chi-square, Paired samples *t*-test, and one-way ANOVA were used to analyze the results.

*P* < 0.05 was considered statistically significant.

**Results:** Following the SKP intervention, the clinical parameters such as PI and GI improved significantly in all the groups (*P* < 0.001); however, Group III showed a significant reduction as compared to the other groups. The mean baseline salivary HBD-2 levels of Group I, Group II, and Group III were 91.78 ng/μl, 110.22 ng/μl, and 157.63 ng/μl which was further decreased to 95.22 ng/μl, 98.22 ng/μl, and 132.88 ng/μl, respectively, following SKP intervention (*P* < 0.001). However, Group III had a higher HBD-2 level at 90<sup>th</sup> day as compared to other groups.

**Conclusion:** There was an improvement in PI and GI with a decrease in salivary HBD-2 in chronic periodontitis patients following SKP. Hence, SKP can be considered as an adjunct to treatment modality in patients with periodontal disease.

**Key Words:** beta-defensins, Human, periodontal disease, yoga

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## INTRODUCTION

Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth.<sup>[1]</sup> The etiology behind this

pathology is multifaceted. Although bacteria play a key role in initiating the disease, the host immune response

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and environment factors are critical for disease progression. Furthermore, the current research discloses that periodontitis patients are at a high risk of systemic inflammation. Hence, management of oral disease is essential for the maintenance of systemic health.

The modern dentistry is sound enough and is coming out with emerging trends in early diagnosis and treatment of periodontitis. Nevertheless, integrative medicinal approach that combines the conventional medicinal practices and traditional medicines is gaining interest among population worldwide. One of the reasons could be that it is cost-effective and also emphasis on prevention of disease.<sup>[2]</sup> Till date, few researches in the past have been attempted to evaluate the effect of complementary and alternative medicine (CAM) in the management of periodontitis.

Defensins are cationic peptides produced by epithelia at mucosal surfaces. The role of salivary human beta-defensin-2 (HBD-2) in various oral pathologies such as microbial infections, periodontal inflammation, and oral cancers are being scientifically documented.<sup>[3-5]</sup> Therapeutic effect of synthetic forms of HBD-2 is stated in literature.<sup>[6]</sup> After numerous research works on HBD-2 in periodontal health, it is considered that severity of periodontal inflammation could influence the salivary concentration of HBD-2.

Yoga is a time-tested mind-body medicine which originated from India 5000 years ago.<sup>[7]</sup> This traditional medicine has been a nondrug treatment in preventing and curing various psychological and physiologic diseases involving various systems such as cardiovascular system, respiratory system, and central nervous system. Literature review shows the efficiency of SKP, a form of yoga in improving the quality of life in cancer patients.<sup>[8]</sup> In addition, SKP has a beneficiary effect on the modifiable and nonmodifiable risk factors of periodontitis.<sup>[9-11]</sup> To our knowledge, there is no work done so far in exploring the effect of SKP on periodontal health. Hence, we aim to assess the periodontal parameters such as plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL), and salivary HBD-2 in periodontitis patients at baseline and at 90<sup>th</sup> day following Sudarshan Kriya Pranayama (SKP) practice.

## MATERIALS AND METHODS

For this interventional study, one hundred and ten males of age between 18 and 35 years with the presence of at least

24 teeth were recruited for the study from the Art of Living Centers in Chennai, Tamil Nadu, India. Of 110 individuals, 10 refused to participate in the study, two 2 were diabetic, 1 was hypertensive, 4 were smokers, 2 underwent periodontal therapy in the last 6 months, and 1 individual was currently under antibiotics and hence twenty males were excluded from the study. Females were also excluded from the study since their periodontal health maybe influenced by hormones. Finally, inclusion criteria included 90 males who were systemically healthy. They were also screened for periodontal disease and divided into three groups: Group I – 30 individuals (volunteers) who were systemically and periodontally healthy, Group II – 30 individuals with chronic gingivitis, and Group III – 30 individuals with chronic periodontitis. Patients who showed the GI index score of 2 and 3 were considered as gingivitis patients. Patients with >30% of sites showing >5 mm of PPD or 3–4 mm of CAL were considered as chronic periodontitis patients. The study was approved by the Institutional Ethics Committee and Institutional Review Board, MAHER University, Chennai (MAHER-MU-002-IEC/2016), following the Declaration of Helsinki. All individuals were explained about the study, and written informed consent was obtained.

### Clinical data collection

All individuals were seated comfortably in an upright position and underwent a full-mouth periodontal examination.<sup>[12]</sup> The periodontal examination included PI, GI, PPD, and CAL. PI (Silness and Loe 1964) was recorded at 4 sites (mesiobuccal, midbuccal, distobuccal, and midpalatal sites) around each tooth: 0 = no plaque, 1 = a film of plaque, 2 = moderate accumulation of deposit, and 3 = abundance of soft matter.<sup>[13]</sup> GI (Silness and Loe 1963) was recorded at (facial, mesial, distal, and lingual) four gingival areas: 0 = no inflammation, 1 = mild inflammation, 2 = moderate inflammation, and 3 = severe inflammation (0.1–1 = mild gingivitis, 1.1–2 = moderate gingivitis, and 2.1–3 = severe gingivitis). PPD and CAL were recorded at six sites of each tooth and measured in millimeters. PPD was measured from gingival margin to the base of periodontal pocket, and CAL was measured as the distance from the cemento-enamel junction to the base of the periodontal pocket.<sup>[14]</sup>

### Sample collection

The participants were requested to take only water 2 h before pranayama session. 5 ml of unstimulated saliva was collected at baseline before SKP

session and at 90<sup>th</sup> day. Saliva was processed for HBD-2 by enzyme-linked immunosorbent assay kit (Qayee bio-technology Cat.No. 100–250-BD2).<sup>[15]</sup>

**Intervention tool**

SKP was used as the intervention tool.<sup>[16]</sup> Pranayama was taught and monitored by a certified yoga teacher for 90 days. SKP is a unique yogic breathing, with several types of cyclical breathing pattern ranging from slow and calming to rapid and stimulating breath.<sup>[16]</sup> The 4 stages of SKY are as follows: (1) ujjayi/victorious breath – 2–4 breaths/min, (2) bhastrika/bellows breath – 30 breaths/min, (3) Om chanting – three times, and (4) Sudarshan Kriya – normal breathing with slow, medium, and fast cycles followed by rest.

**Statistical analysis**

Statistical analysis was performed using SPSS software version 16 (IBM Corporation, Chicago IL USA). McNemar’s Chi-square test was used to compare the clinical parameters at baseline and 90<sup>th</sup> day in all the groups following SKP. Paired samples *t*-test was used to compare the pre- and postintervention mean values of salivary HBD-2. One-way ANOVA was used to compare the mean salivary HBD-2 values between groups at 90<sup>th</sup> day. *P* <0.05 was considered statistically significant.

**RESULTS**

On comparing the effect of SKP on PI from baseline to 90<sup>th</sup> day within the groups, 88.9% of Group I individuals were improved in PI score from score 1

to score 0. Similarly, 77.8% individuals in Group II had improved in PI score from score 3 and 2 to score 1 and 0, respectively, after intervention. In Group III, 87.5% individuals improved in PI score from score 3 to score 2 and 1 (*P* < 0.001) [Table 1].

On comparing the GI score from baseline to 90<sup>th</sup> day in Group II and Group III, 88.8% of Group II individuals showed improvement in GI score from score 3 and 2 to score 1 and 0, respectively. Similarly, GI in the Group III individuals had a significant change from score 3 to score 2 and 1 (*P* < 0.001). Group I individuals had the same GI score 0 before and at 90<sup>th</sup> day following SKP [Table 2].

When the clinical parameters such as PPD and CAL were compared from baseline to 90<sup>th</sup> day following SKP, Group III had no significant changes stating that there was no improvement in PPD and CAL (*P* < 0.001) [Tables 3 and 4].

The mean salivary HBD-2 levels in Group I, Group II, and Group III were found to be 91.78 ng/μl, 110.22 ng/μl, and 157.63 ng/μl which decreased to 95.22 ng/μl, 98.22 ng/μl, and 132.88 ng/μl, respectively, following SKP. HBD-2 level when compared at 90<sup>th</sup> day among three groups was statistically significant (*P* < 0.001) [Table 5].

Group III had a higher HBD-2 level when compared to Group II and Group I. Although the HBD-2 levels were reduced following SKP in all three groups, Group III showed higher level at 90<sup>th</sup> day as compared to other groups [Table 6].

**Table 1: Group wise comparison of plaque index before and after Sudarshan Kriya Pranayama**

Group	PI (baseline)	PI (follow-up)				
		0, n (%)	1, n (%)	2, n (%)	3, n (%)	Total, n (%)
I (healthy)	0	12 (80.0)	3 (20.0)	0	0	15 (100.0)
	1	12 (100.0)	0	0	0	12 (100.0)
	2	0	0	0	0	0
	3	0	0	0	0	0
	Total	24 (88.9)	3 (11.1)	0	0	27 (100.0)
II (gingivitis)	0	0	0	0	0	0
	1	6 (50.0)	6 (50.0)	0	0	12 (100.0)
	2	0	9 (75.0)	3 (25.0)	0	12 (100.0)
	3	0	0	0	3 (100.0)	3 (100.0)
	Total	6 (22.2)	15 (55.6)	3 (11.1)	3 (11.1)	27 (100.0)
III (periodontitis)	0	0	0	0	0	0
	1	0	3 (100.0)	0	0	3 (100.0)
	2	0	12 (80.0)	0	0	15 (100.0)
	3	0	0	3 (50.0)	3 (50.0)	6 (100.0)
	Total	0	15 (62.5)	6 (25.0)	3 (12.5)	24 (100.0)
Chi-square test	34.714			<i>P</i> <0.001*		

*P*<0.05. \*Significant. PI: Plaque index

**Table 2: Group wise comparison of gingival index before and after Sudarshan Kriya Pranayama**

Group	GI (baseline)	GI (follow-up)				
		0, n (%)	1, n (%)	2, n (%)	3, n (%)	Total, n (%)
I (healthy)	0	27 (100.0)	0	0	0	27 (100.0)
	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	Total	27 (100.0)	0	0	0	27 (100.0)
II (gingivitis)	0	0	0	0	0	0
	1	12 (100.0)	0	0	0	12 (100.0)
	2	0	12 (100.0)	0	0	12 (100.0)
	3	0	0	3 (100.0)	0	3 (100.0)
	Total	12 (44.4)	12 (44.4)	3 (11.1)	0	27 (100.0)
III (periodontitis)	0	0	0	0	0	0
	1	0	0	0	0	0
	2	0	12 (80.0)	3 (20.0)	0	15 (100.0)
	3	0	0	9 (100.0)	0	9 (100.0)
	Total	0	12 (50.0)	12 (50.0)	0	24 (100.0)
Chi-square test		47.00				P<0.001*

P<0.05. \*Significant, GI: Gingival index

**Table 3: Comparison of probing pocket depth before and after Sudarshan Kriya Pranayama in Group III**

PPD (baseline)	PPD (follow-up)		
	<3 mm, n (%)	>3 mm, n (%)	Total, n (%)
<3 mm	54 (100.0)	0	54 (100.0)
>3 mm	0	24 (100.0)	24 (100.0)
Total	54 (69.2)	24 (30.80)	78 (100.0)
Chi-square test	90.000		p<0.001*

P<0.05. \*Significant, PPD: Probing pocket depth

## DISCUSSION

Periodontal disease is the most common chronic bacterial infection of the supporting structures of the teeth which are predominantly associated with Gram-negative microorganisms that exist in subgingival biofilm. To identify individuals with periodontitis, new diagnostic tools have been developed. However, saliva is one of the most common tools to identify the various markers of periodontal inflammation. HBD-2 is one such marker which shows high sensitivity and specificity in saliva. Salivary HBD-2, an inflammatory marker of periodontal disease, is inducible in response to physical exercise.<sup>[17]</sup>

One of the treatment modalities for periodontal disease is scaling and root planing followed by complex surgical procedures. The use of CAM and practices, such as pranayama and yoga, can further reduce the risk of periodontitis and improve the

immune system. SKP is one novel type of yoga that influences the mind-body system, by extending its positive effects on humoral factors, nervous system, and immune system. Unlike medicines, pranayama strengthens the healing ability of an individual from inside. In addition, yoga is also an anti-inflammatory tool.<sup>[18]</sup> Low-intensity yogasana has also been proven to affect salivary HBD-2 with salivary flow rate which is essential for oral hygiene.<sup>[19]</sup> Various studies in the past have evaluated HBD-2 level in periodontal disease. However, to the best of our knowledge, this is the first study to evaluate the level of HBD-2 in individuals with periodontal disease following SKP intervention. Hence, we aimed to assess the clinical parameters such as PI, GI, PPD, CAL, and salivary HBD-2 level in individuals with gingivitis and periodontitis, compared with healthy controls with SKP as intervention.

In our study, 88.9% of Group I, 77.8% Group II, and 87.5% of Group III individuals showed improvement in PI score from poor to better. This is in accordance with Farsi *et al.* who compared saliva and periodontal health and stated that plaque score is directly proportional to the severity of periodontal disease.<sup>[20]</sup> The statistically significant improvement of PI in our study could be attributed to the fact that there would have been an increase in salivary flow rate following SKP intervention. In healthy gingiva where there is no periodontal disease, there is an increase in saliva flow rate, thereby preventing the accumulation of plaque. Hence, our observation is also in accordance with



**Table 4: Comparison of clinical attachment level before and after Sudarshan Kriya Pranayama in Group III**

CAL (baseline)	CAL (follow-up)		
	Absence, <i>n</i> (%)	Presence, <i>n</i> (%)	Total, <i>n</i> (%)
Absence of clinical attachment loss	54 (100.0)	0	54 (100.0)
Presence of clinical attachment loss	0	24 (100.0)	24 (100.0)
Total	54 (69.2)	24 (30.8)	78 (100.0)
Chi-square test	90.000		<i>P</i> <0.001*

*P*<0.05. \*Significant, CAL: Clinical attachment level

**Table 5: Group wise comparison of salivary human beta-defensin-2 level before and after Sudarshan Kriya Pranayama**

Group	Salivary HBD-2	<i>n</i>	Mean ng/μl,	SD	<i>t</i>	<i>P</i>
I (healthy)	Baseline	27	91.78	6.129	2.674	0.013
	90 <sup>th</sup> day	27	95.22	5.257		
II (gingivitis)	Baseline	27	110.22	8.331	11.130	<0.001*
	90 <sup>th</sup> day	27	98.22	6.259		
III (periodontitis)	Baseline	24	157.63	11.724	6.582	<0.001*
	90 <sup>th</sup> day	24	132.88	13.056		

*P*<0.05. \*Significant, SD: Standard deviation; HBD-2: Human beta-defensin-2

**Table 6: Intergroup comparison of salivary human beta-defensin-2 level**

Variables	Groups	<i>n</i>	Mean ng/μl,	SD	<i>F</i>	<i>P</i>
Salivary HBD-2 (baseline)	Healthy	30	91.60	5.829	455.329	<0.001*
	Gingivitis	30	110.10	7.897		
	Periodontitis	30	159.30	12.069		
	Total	90	120.33	30.071		
Salivary HBD-2 (90 <sup>th</sup> day)	Healthy	27	95.22	5.257	144.740	<0.001*
	Gingivitis	27	98.22	6.259		
	Periodontitis	24	132.88	13.056		
	Total	78	107.85	18.897		

*P*<0.05. \*Significant, SD: Standard deviation; HBD-2: Human beta-defensin-2

previous work done by Eda *et al.* where there was an increase in the salivary flow rate following yogic stretching (asana).<sup>[19]</sup> This observation is irrespective of the patients oral hygiene and food habits. Although food and brushing habit is said to have an impact on plaque deposition, physical and chemical properties of saliva play an incredible role. Hence, it is suggested that SKP can improve the salivary flow, which in turn can decrease plaque deposition and enhance oral hygiene.

Group II individuals had a significant decrease in gingival inflammation. 88.8% of individuals of Group II were recovered from severe and moderate gingivitis to mild gingivitis after SKP intervention, which was statistically significant. Similarly, individuals in Group III had 100% improvement

from moderate and severe gingivitis to mild and moderate gingivitis, respectively. When PPD and CAL were measured in Group II and Group III individuals, from baseline to 90<sup>th</sup> day, no change was seen in their scores. The increases in pocket depth and clinical attachment loss are the burden of past gingival inflammation that remains irreversible unless intervened surgically. The normal depth of gingival sulcus is 0–3 mm and worsens when PPD progresses.<sup>[21]</sup> In our study, the PPD and clinical attachment loss in Group III individuals did not show any clinically significant difference. There was neither improvement nor worsening. To our knowledge, there are no similar studies to compare the results. Nevertheless, it is shown that the practice of SKP increases antioxidant defense and cytotoxic function of natural killer cells and inhibits apoptosis.<sup>[22,23]</sup> It is suggested that this cumulative effect of SKP would have modulated the immune response, thereby reducing the inflammation and further preventing the disease progression.

In our study, the salivary HBD-2 level decreased significantly from baseline to 90<sup>th</sup> day following SKP in all the three groups. This finding is in concurrence with Ebrahim who also documented decreased level of gingival crevicular fluid-HBD-2 in the periodontitis patients with nonsurgical periodontal therapy, indicating the downregulation of inflammation.<sup>[24]</sup> In our study, SKP was used as an intervention. SKP directly influences the autonomic nervous system, stimulates vagus nerve, and energizes the endocrine and immune system which in turn balances inflammatory response.<sup>[25]</sup> It is likely that the SKP practice would have shown a direct impact on immune mechanism in all individuals.

In our study, the salivary HBD-2 level was higher in Group III than Group II and Group I at 90<sup>th</sup> day practice of SKP. Based on this observation, it can be suggested that salivary HBD-2 level increases with inflammation. This observation is in accordance with the work of Pereira *et al.*<sup>[26]</sup> and Aas *et al.*<sup>[27]</sup> who

also found that HBD-2 levels were similar in healthy and gingivitis individuals but higher in periodontitis. Kuula *et al.* in their study found an increased level of HBD-2 in inflamed periodontal individuals, suggesting that the response was due to increased bacterial burden in periodontitis patients than healthy controls.<sup>[28]</sup> Similarly, Hosokawa *et al.* recorded higher HBD-2 concentration in chronic periodontitis group followed by gingivitis patients and healthy controls.<sup>[29]</sup> Studies on chronic periodontitis and periopathogens such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* suggest that bacteria can induce the upregulation of HBD-2.<sup>[30]</sup> This observation abides with our study, suggesting that Group III individuals have deeper periodontal pockets with more bacterial colonization and hence have higher levels of salivary HBD-2.

Davison *et al.* and Usui *et al.* worked on the effect of strenuous physical exercise and sports activity on salivary antimicrobial peptides and recorded increased levels of cathelicidin, human alpha-defensin, and cortisol with a decrease in salivary flow and IgA level.<sup>[17,31]</sup> Yogic practice is considered superior to traditional exercise for the fact that results obtained after high-intensity workouts are quantitative at the muscular level when compared with qualitative output of yoga practice. The oxygen and nutrition consumption is at the organ level in yoga practice which energizes the chakras (energy centers) of the body.<sup>[32]</sup>

Yoga plays a positive effect on periodontitis by decreasing the stress level. It is known that stress can influence factors such as endocrine system. It has also shown an impact on poor oral hygiene, dietary intake, smoking, change in salivary flow, and components, thereby decreasing the risk for periodontal disease.<sup>[33]</sup> Yoga has proven to have a beneficial effect on stress by decreasing the cortisol level, a stress marker.<sup>[34]</sup> Yoga stimulates phagocytosis and regulates the formation of fibroblasts and epithelial cells, thereby enhancing the periodontal health.<sup>[35]</sup>

The limitation of the study was that salivary HBD-2 was only quantitatively analyzed. The mechanism and role of HBD-2 in periodontal disease is yet to be explored. This study provides a base for the CAM, mainly SKP and yoga in dentistry. Further, extensive evidence-based research should be performed with larger sample size and long-term follow-up to power the current study. Research in combination with

the mainstream periotherapy must be performed to highlight the complementary and alternative effect of SKP on periodontal diseases.

## CONCLUSION

SKP effectively decreased the periodontal inflammation in periodontitis individuals. This study paves a way for future research in the field of pranayama against various oral pathogenic bacteria.

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## Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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