

# Effect of Pranayama on Ppar- $\gamma$ , Nf- $\kappa$ B Expressions and Red Complex Microorganisms in Patients with Chronic Periodontitis – A Clinical Trial

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## ABSTRACT

**Introduction:** Sudarshankriya pranayama is the control of breathing movements to regulate the energy flow throughout the body, which has shown to positively affect immune function, autonomic nervous system and psychologic-neuro pathways. The practice of pranayama has been proven to show several benefits such as reduction in stress levels, relieves anxiety and depression, increase in antioxidant levels, reduces insulin resistance and improves respiratory function.

**Aim:** The objective of the study was to evaluate the levels of Peroxisome Proliferator-Activated Receptor Gamma (PPAR- $\gamma$ ), Nuclear Factor-Kappa B (NF- $\kappa$ B) and the presence of Red Complex Microorganisms (RCM) such as *Treponema denticola*, *Porphyromonas gingivalis* and *Tannerella forsythia* in the subgingival plaque samples of chronic periodontitis subjects before and after intervention with pranayama as an adjunct to Scaling and Root Planing (SRP).

**Materials and Methods:** A total of 30 subjects (control group) were treated with SRP and 30 subjects (pranayama group) underwent SRP and pranayama for three months. Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), Bleeding Index (BI) and Plaque Index (PI) were recorded and the presence

of PPAR- $\gamma$ , NF- $\kappa$ B and RCM were assessed at baseline and after three months using polymerase chain reaction. ANCOVA test was done to compare the clinical parameters between the groups. Fisher's Exact test was done to identify RCM and Mann-Whitney and Wilcoxon-signed test was used to identify the expression of NF- $\kappa$ B and PPAR- $\gamma$  in the plaque samples.

**Results:** The change in the mean CAL from baseline to third month was significantly higher in pranayama group compared to control group ( $p \leq 0.05$ ). There was a statistically significant reduction in the expression of NF- $\kappa$ B and increase in PPAR- $\gamma$  expression levels in pranayama group on comparison with the control group ( $p < 0.001$ ). The reduction in number of positive samples with *T.denticola*, *P.gingivalis* and *T.forsythia* at third month post-intervention did not affect the change in the expression levels of NF- $\kappa$ B and PPAR- $\gamma$ .

**Conclusion:** The CAL showed significant improvement with reduction in the RCM, NF- $\kappa$ B and increase in PPAR- $\gamma$  levels in subjects who underwent pranayama as an adjunct to SRP. In future, pranayama can be used as an additional treatment modality to provide a new dimension in treatment of periodontitis.

**Keywords:** Inflammatory mediators, Periodontal disease, Polymerase chain reaction, Putative pathogenic microorganisms

## INTRODUCTION

Periodontitis is the most common chronic bacterial infection of the supporting structure of the teeth which is predominantly associated with the gram negative microorganisms that exist in subgingival biofilm [1]. The most common etiology of periodontal disease is dental plaque, which consists of more than 700 distinct microbial species [2]. The inflammation in periodontitis is based on the effects of systemic dissemination of pro-inflammatory cytokines such as C-Reactive Protein (CRP), Interleukin-1 Beta (IL-1 $\beta$ ), IL-6, Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) through NF- $\kappa$ B signalling, modified by other local, environmental and genetic factors. A transcriptional factor in immune and inflammatory response is the NF- $\kappa$ B that controls the expression of various cytokines [3]. It is associated with an inhibitory protein known as Inhibitors of Kappa B (I $\kappa$ B) which is found in the cytoplasm. In chronic periodontitis, stimulation of cells with various inflammatory stimuli such as Lipopolysaccharides (LPS), TNF- $\alpha$ , IL-1 $\beta$  activates NF- $\kappa$ B and induces a local and systemic inflammatory response [4]. PPAR- $\gamma$  is a nuclear hormone receptor [5]. Endogenous PPAR- $\gamma$  has shown anti-inflammatory effect by causing down-regulation of NF- $\kappa$ B [6]. Also, PPAR- $\gamma$  agonists have been found to: 1) strongly up-regulate PPAR- $\gamma$  expression and activity; 2) suppress secretion of inflammatory cytokines; and 3) reverse activation of NF- $\kappa$ B by inhibiting the I $\kappa$ -B kinase (IKK) pathway and by promoting direct inhibitory binding of PPAR- $\gamma$  [7].

It is predominantly expressed in adipocytes and plays an extensive role in metabolism [8].

The body's nature is classified based on the dominance of one or more of three physical humors called doshas. They are the wind (vata), bile (pitta) and phlegm (kapha) [9]. The presence of specific dosha in an individual and in nature determines healthcare in ayurveda, including oral health. Yoga involves disciplining the mind and body through exercise and meditation. Numerous researches have been conducted to look at the health benefits of yoga including yoga postures (asanas), yoga breathing (pranayama) and meditation [10,11].

Pranayama is the control of prana through the breath. 'Prana' refers to the universal life force and 'ayama' means to regulate or lengthen. This technique relies on breathing through the nostrils [12]. The regular practice of yoga by an individual enhances the systemic immunity which in turn enhances the overall defense mechanism. Though, earlier studies have shown the stress reduction among the dental professionals, however to the best of our knowledge this is the first study to assess the effect of pranayama on periodontitis subjects [13,14]. Hence, we aimed to assess the effect of pranayama as an adjunct to SRP on the periodontal status, PPAR- $\gamma$ , NF- $\kappa$ B levels and presence of RCM such as *Treponema denticola*, *Porphyromonas gingivalis* and *Tannerella forsythia* at baseline and after three months.

## MATERIALS AND METHODS

### Study Population and Selection Criteria

In a three month follow up clinical trial, 60 subjects with age range between 30 to 65 years were recruited from the outpatient pool of Department of Periodontology, Meenakshi Ammal Dental College and Research Institute, Chennai, India. Patients with aggressive periodontitis, systemic disorders, pregnant and lactating females, long term steroid medication, smokers, alcoholics, immunocompromised individuals and those with history of antibiotic or periodontal therapy in preceding six months were excluded from the study. Sixty systemically healthy subjects with generalized chronic periodontitis, with probing pocket depth  $\geq 5$  mm and those who had the ability to maintain optimum oral hygiene, after the initial phase of treatment were included in the present investigation. The power of the study was calculated as 85% based on the number of chronic periodontitis subjects undergoing pranayama from the previous data.

The participants were randomly assigned by a computer generated system into two groups. Control group consisting of 30 subjects, was treated with SRP. Pranayama group consisting of 30 subjects was also treated with SRP and underwent pranayama as an intervention for three months. The "Meenakshi Institutional Review Board" [MAHER-MU-002-IEC/2016] approved this study following the Declarations of Helsinki[15]. All participants were verbally informed and written consent was obtained. The study was conducted from January 2016 to July 2016 (Clinical trials.gov identifier: NCT02967861).

For all 60 subjects in both the groups, the clinical parameters such as PPD, CAL, BI [16] and PI [17] were assessed at baseline (zero day), before SRP. A custom-made acrylic stent was used to standardize the measurements of clinical parameters in order to reach the same position before and after the treatment and to avoid error or bias during treatment. After the clinical parameters were recorded, SRP was done in both the groups and pranayama-sudarshankriya was intervened in pranayama group for 20 minutes daily for a period of three months. Patients were recalled after three months and clinical parameters were recorded again. Williams periodontal probe was used for periodontal examination.

### Sample Collection and Pranayama Intervention

In both the groups, once the periodontal parameters were recorded, thorough SRP was done. The teeth were then isolated with cotton rolls and subgingival plaque samples were taken from the deepest periodontal sites at baseline to assess the presence of *T.denticola*, *P.gingivalis*, *T.forsythia*, PPAR- $\gamma$  and NF- $\kappa$ B were analysed from subgingival plaque samples using polymerase chain reaction (PCR). In the pranayama group 30 subjects were intervened with pranayama session daily for three months.

Pranayama was performed according to Sri Swami Sivananda by deep breathing in and out [12]. The participants were seated comfortably on the ground in cross-legged position. Pranayama was taught and monitored by a certified yoga teacher in the yoga centre daily for three months. The procedure was done as follows - Close the index finger and the middle finger of your right hand. Then, close the right nostril with the thumb and slowly exhale from the left nostril. After exhaling, slowly inhale through the same nostril. Withhold the breath for two seconds. Then close the left nostril with ring finger and exhale through the right nostril. Now, inhale from the right one, hold it for two seconds and exhale from the left one closing the right nostril with the right thumb. This process is repeated for two-five minutes for 20 minutes. The participants were requested to take only water two hours before pranayama session.

### Identification of Red Complex Microorganisms

Subgingival plaque samples, collected before and after the treatment protocol in both the groups, were homogenised and centrifuged for

10 minutes at 10,000 rpm. The supernatant was discarded and the resulting pellet was resuspended in 200  $\mu$ l of lysis solution (100 mM Tris, 1.0 mM ethylenediaminetetraacetic acid, 1.0% Triton X-100, pH 7.8). The samples were kept in a boiling water bath for 10 minutes, allowed to cool and again centrifuged for 5 minutes at 10,000 rpm. The supernatant was collected from the centrifugation product and stored at  $-70^{\circ}\text{C}$  which was later used as DNA template. The RCM was detected by using 16S rRNA PCR amplification. As per the protocol of Larsen N et al., [18] PCR primers were designed for the study [Table/Fig-1]. The upstream and downstream sequence primers were then verified for their species specificity by comparing the sequences with all the available 16S rRNA sequences in the RDP database. PCR was performed as described by Saiki RK et al., [19].

### Expression of PPAR- $\gamma$ and NF- $\kappa$ B

The expression of PPAR- $\gamma$  and NF- $\kappa$ B through RT-PCR was performed according to Mahendra J et al., [6]. Forward and reverse primers are shown in [Table/Fig-1]. The results were expressed as fold increase in mRNA expression with respect to expression in control. Relative expression level was calculated using the comparative threshold cycle method ( $2^{-\Delta\Delta\text{CT}}$ )[20].

### STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software version 16.0. The mean and standard deviation was calculated for the continuous variables (PPD, CAL, BI, PI, expression of NF- $\kappa$ B and PPAR- $\gamma$ ). To compare the clinical parameters (PPD, CAL, BI and PI) between control and pranayama groups and the time of evaluation, ANCOVA test was used with their mean values adjusted to baseline values (constant). The number of positive samples for *T.denticola*, *P.gingivalis* and *T.forsythia* were calculated as categorical data and Fisher's Exact test was used to perform an intragroup and intergroup comparison. Non-parametric tests were implemented to analyze the mean fold change in the expression of NF- $\kappa$ B and PPAR- $\gamma$  at baseline and third month between the pranayama and control groups using Mann-whitney test and Wilcoxon-signed test. To assess the effect of reduction in the presence of subgingival microorganisms on the change expression levels of NF- $\kappa$ B and PPAR- $\gamma$  from baseline to third month, non-parametric test - mann-whitney was performed. In the present study,  $p < 0.05$  was considered statistically significant.

### RESULTS

There was no statistically significant difference observed in the post interventional (third month) mean PPD, BI and PI scores. However, the change in the mean CAL from baseline to third month was significantly higher in pranayama group compared to control group

Microorganisms	Product size (bp)	
<i>Treponema denticola</i> TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	316	
<i>Porphyromonas gingivalis</i> AGG CAG CTT GCC ATA CTG C ACT GTT AGC AAC TAC CGA TGT	729-1132 (404)	
<i>Tannerella forsythia</i> GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC	120-760 (641)	
Primers used for Real-Time PCR* Analysis		
Genes	Primers	Sequence 5'-3'
PPAR- $\gamma$	Forward Reverse	CAG GAG CAG AGC AAA GAG GTA CAA ACT CAA ACT TGG GCT CCA
NF- $\kappa$ B	Forward Reverse	GTG AGG ATG GGA TCT GCA CT CCTTCTGCTTCAATAGGC

[Table/Fig-1]: List of the PCR\* primers for analysis of red complex microorganisms.

\*PCR - Polymerase chain reaction

Clinical Parameters	Groups	No. of participants	Zero day		Third month		F value	p-value
			Mean	S.D	Mean	S.D		
Probing pocket depth(mm)	Control	30	6.46	1.17	4.00	0.63	0.544	0.464
	Pranayama	30	6.050	1.307	3.00	0.51		
Clinical attachment level(mm)	Control	30	6.59	1.33	4.21	1.03	4.149	0.046*
	Pranayama	30	6.49	1.35	3.10	0.88		
Bleeding index	Control	30	92.10	10.32	55.66	10.68	0.068	0.795
	Pranayama	30	91.00	10.05	34.30	7.99		
Plaque index	Control	30	2.00	0.42	1.07	0.22	2.152	0.148
	Pranayama	30	2.099	0.35	1.02	0.32		

**[Table/Fig-2]:** Intergroup comparison of clinical parameters of control and pranayama group after three months using ANCOVA.

\* Statistically significant at  $p \leq 0.05$ .

Groups	Subgingival microorganisms	Number of positive samples		$\chi^2$ value	p-value
		Baseline	Third month		
Pranayama group	<i>T. denticola</i>	27	6	29.69	<0.001*
	<i>P.gingivalis</i>	30	4	45.88	<0.001*
	<i>T.forsythia</i>	24	8	17.14	<0.001*
Control group	<i>T. denticola</i>	25	20	2.22	0.23
	<i>P.gingivalis</i>	30	26	4.29	0.11
	<i>T.forsythia</i>	28	23	3.27	0.14

**[Table/Fig-3]:** Fisher's Exact test for Intragroup comparison of *T.denticola*, *P.gingivalis*, *T.forsythia* of Control and pranayama group at third month.

\* Statistically significant at  $p \leq 0.05$ .

Subgingival microorganisms	Number of positive samples at third month		$\chi^2$ value	p-value
	Pranayama group	Control group		
<i>T. denticola</i>	6	20	13.30	<0.001*
<i>P.gingivalis</i>	4	26	32.26	<0.001*
<i>T.forsythia</i>	8	23	15.01	<0.001*

**[Table/Fig-4]:** Fisher's Exact test for Intergroup comparison of *T.denticola*, *P.gingivalis*, *T.forsythia* of Control and pranayama group at third month.

\* Statistically significant at  $p \leq 0.05$ .

Groups	Mean fold change in Expression	Baseline	Third Month	Wilcoxon-signed rank test	Mann-Whitney test
Control group	NF- $\kappa$ B	2.59	2.18	$p = 0.766$ $z = -0.298$	$p = 0.355$
	PPAR- $\gamma$	-1.23	-0.445	$p = 0.128$ $z = -1.520$	$p = 0.848$
Pranayama group	NF- $\kappa$ B	2.29	-0.29	$p = 0.004^*$ $z = -2.900$	$p < 0.001^*$
	PPAR- $\gamma$	-1.55	1.88	$p = 0.00^*$ $z = -4.209$	$p < 0.001^*$

**[Table/Fig-5]:** Comparison mean fold change in the expression of NF- $\kappa$ B and PPAR- $\gamma$  among the pranayama group and control group from baseline to third month.

\* Statistically significant at  $p \leq 0.05$ .

( $p \leq 0.05$ ) showing gain in the CAL post pranayama intervention [Table/Fig-2].

The number of positive samples for the presence of *T.denticola*, *P.gingivalis* and *T.forsythia* in baseline and third month in the both the groups were compared. The intragroup comparison from baseline to third month did not show reduction for the presence of microorganisms in control group. Nevertheless, there was

Difference in expression levels of	Groups	Change in number <i>T.denticola</i> positive samples (p-value)	Change in number <i>P.gingivalis</i> positive samples (p-value)	Change in number <i>T.forsythia</i> positive samples (p-value)
NF- $\kappa$ B	Control group	0.388	0.464	0.522
	Pranayama group	0.455	0.583	0.360
PPAR- $\gamma$	Control group	0.521	0.445	0.289
	Pranayama group	0.243	0.758	0.528

**[Table/Fig-6]:** Association between change in *T.denticola*, *P.gingivalis*, *T.forsythia* positive samples from baseline to third month with the difference in the expression levels of NF- $\kappa$ B and PPAR- $\gamma$  from baseline to third month using Mann-whitney test.

\* Statistically significant at  $p \leq 0.05$ .

statistically significant reduction in number of *T.denticola*, *P.gingivalis* and *T.forsythia* from baseline to third month in the pranayama group [Table/Fig-3]. The intergroup comparison of number of positive samples for *T.denticola*, *P.gingivalis*, and *T.forsythia* between control and pranayama group at third month showed statistically significant reduction in pranayama group compared to control group [Table/Fig-4].

The mean fold change in the expression of NF- $\kappa$ B in both control and pranayama group at baseline and at third month is shown in [Table/Fig-5]. The non-parametric comparison showed statistically significant reduction in the expression of NF- $\kappa$ B from baseline to third month in the pranayama group ( $p=0.004$ ), whereas the expression of NF- $\kappa$ B in the control group did not show statistically significant difference at third month ( $p=0.766$ ). Furthermore, there was a significant reduction seen in the expression of NF- $\kappa$ B in pranayama group on comparison with the control group ( $p < 0.001$  intergroup comparison), [Table/Fig-5].

The mean fold change in the expression of PPAR- $\gamma$  in both control and pranayama group at baseline and at third month is shown in [Table/Fig-5]. On comparison, there was a statistically significant increase in the expression of PPAR- $\gamma$  from baseline to third month in the pranayama group ( $p < 0.001$ ), whereas the expression of PPAR- $\gamma$  from baseline to third month in the control group did not show significant difference ( $p = 0.128$ ). Intergroup comparison showed statistically significant increase in the expression of PPAR- $\gamma$  in pranayama group than the control group ( $p < 0.001$ ) [Table/Fig-5].

Mann-Whitney test was performed to determine the association between the difference in the expression levels of NF- $\kappa$ B and PPAR- $\gamma$  from baseline to third month and the change in the number of *T.denticola*, *P.gingivalis* and *T.forsythia* positive samples in both the groups [Table/Fig-6]. The reduction in number of *T.denticola*, *P.gingivalis* and *T.forsythia* at third month post-intervention did not affect the change in the expression levels of NF- $\kappa$ B and PPAR- $\gamma$ . Alternatively, this can be interpreted that the pranayama itself has

affected the expression levels of NF- $\kappa$ B and PPAR- $\gamma$ , irrespective of the reduction of subgingival microorganisms (RCM) at third month.

## DISCUSSION

To the best of our knowledge, this is the first study to assess the effect of pranayama on the periodontal clinical parameters, RCM and the expression of inflammation related gene NF- $\kappa$ B and PPAR- $\gamma$  levels in subgingival plaque samples. The present study showed reduction in the PPD, CAL, BI, and PI at third month in both the groups compared to baseline. However, CAL showed a significant gain in pranayama group from baseline to third month when compared to control group. CAL is a best risk indicator for disease progression, which showed a significant difference at third month after intervention with pranayama. Similarly, the tested *T.denticola*, *P.gingivalis* and *T.forsythia* were reduced in both pranayama and control group from baseline to third month, however a significant reduction was found in pranayama group as compared to control group [Table/Fig-3]. Furthermore, intergroup comparison showed a significant reduction in number of *T.denticola*, *P.gingivalis* and *T.forsythia* positive samples in the pranayama group compared to the control group at third month [Table/Fig-4]. It is suggested that pranayama improves the immune defense mechanism whereby lowering the risk for inflammatory diseases [21].

In order to investigate the effect of pranayama on periodontitis at a molecular level, the changes in the expression of NF- $\kappa$ B and PPAR- $\gamma$  were evaluated. The LPS of RCM activate the NF- $\kappa$ B through transmembrane receptors, leading to production of pro-inflammatory cytokines, which in turn recruits the immune cells evoking an inflammatory process in the periodontal tissues. Increased activation of NF- $\kappa$ B in periodontally diseased tissues has been reported earlier, which links the molecular signaling of the immune system to cause the progression of inflammation. Systemic disorders such as cardiovascular diseases and diabetes mellitus which have reportedly shown enhanced NF- $\kappa$ B activation [22,23], have been treated with sudarshankriya practice [24,25]. In our study there was significant decrease in the expressions of NF- $\kappa$ B in subgingival plaque samples from baseline to third month in both the groups. However the pranayama group showed significant reduction in the NF- $\kappa$ B levels with pranayama as an intervention for three months ( $p$ -value<0.001, [Table/Fig-5]), thereby reducing the inflammation itself. It is suggested that reduction in NF- $\kappa$ B inhibits the cytokine production leading to reduction in the periodontal inflammatory process that result in improvement in the clinical parameters [26].

Anti-inflammatory properties of PPAR- $\gamma$  include the potential to interfere with transcription pathways involved in inflammatory response, such as modulation of NF- $\kappa$ B signalling [6,7]. The synthesis and release of immunomodulatory cytokines has been found to be down-regulated by PPAR- $\gamma$  activation [27]. In the present study, PPAR- $\gamma$  expression levels in the subgingival plaque samples were increased in both the groups, however pranayama group showed a significant difference when compared to control group which is suggestive of its anti-inflammatory effect ( $p$ -value<0.001, [Table/Fig-5]). This is the first study attempted to investigate the expression of PPAR- $\gamma$  in chronic periodontitis subjects with pranayama as an intervention and the results of this study has exhibited the possible anti-inflammatory action of PPAR- $\gamma$  in maintaining the periodontal health.

When RCM were associated with the expression of NF- $\kappa$ B and PPAR- $\gamma$  in both the groups, it showed an interesting outcome. The change in the number of *T.denticola*, *P.gingivalis* and *T.forsythia* from baseline to third month in both the groups did not affect the change in the expression levels of NF- $\kappa$ B and PPAR- $\gamma$ . Hence, we can infer that the reduction in NF- $\kappa$ B and increase in anti-inflammatory PPAR- $\gamma$  is attributed to the effect of intervention.

## LIMITATION

The limitation of the study was the duration of pranayama intervention which was only for three months. Within the limitations of the study, more randomized controlled trials for longer duration are needed to evaluate the efficacy and efficiency of pranayama on periodontal health. This is the first study to investigate the effect of pranayama on periodontitis as a treatment modality.

## CONCLUSION

The periodontal CAL showed significant improvement with reduction in the RCM, NF- $\kappa$ B and increase in PPAR- $\gamma$  levels in subjects who underwent pranayama as an adjunct to SRP. Also, the change in the expression levels of NF- $\kappa$ B and PPAR- $\gamma$  and the reduction in the RCM positive samples were independently effected by the pranayama intervention. In future, pranayama can be used as an additional treatment modality to provide a new dimension in treatment of periodontal diseases.

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