ORIGINAL ARTICLE

Effect of *Nigella Sativa* (Kalonji) Oil Based Mouth Wash on Salivary Matrix Metalloproteinase-8 Levels in Patients with Chronic Periodontitis. A Randomized Controlled Trial

Ghazala Hassan[1], Sarah Ghafoor[2], Saima Chaudhry[3], Zubair Ahmed Khan[4]

ABSTRACT

Background and Objective: Salivary level of Matrix metalloproteinase-8 (MMP-8), a collagen degradation enzyme, is known to increase during chronic periodontitis. *Nigella sativa* is one of the potent anti-inflammatory compounds that has a promising role in oral inflammatory conditions. Present study aims to explore the salivary MMP-8 levels after *Nigella sativa* oil-based mouth wash as compared to placebo (normal saline) after non-surgical periodontal therapy in patients with chronic periodontitis.

Methods: This parallel arm randomized controlled trial was conducted at FMH College of Medicine & Dentistry, Lahore Pakistan from January 2018 to June 2019. Fifty patients suffering from chronic periodontitis were recruited after institutional ethical approval and written informed consent by the participants. The study participants were randomized into the treatment group that received *Nigella sativa* oil and the control group that received simple normal saline. Salivary samples for MMP-8 levels were collected from all patients, once before non-surgical periodontal therapy and secondly after two weeks of rinsing with *Nigella sativa* oil-based mouthwash in one group and normal saline in the second. Salivary MMP-8 levels were determined through ELISA. The comparison within groups was done through paired t-test and between groups through independent sample t-test.

Results: Mean pre-treatment concentration of salivary MMP-8 of the Nigella Sativa group and normal saline group were 199.37 ± 300.75 ng/l and 195.29 ± 175.89 ng/l that decreased post-treatment to 147.35 ± 144.9ng/l and 114.17 ± 129.21ng/l respectively. Statistical analysis revealed no difference in the salivary MMP-8 levels between the chronic periodontitis patients using *Nigella sativa* or normal saline mouth wash twice daily for two weeks.

Conclusion: The salivary levels of MMP-8 reduced in patients with chronic periodontitis after scaling and root planning with the use of *Nigella sativa* oil-based mouthwash as well as placebo (normal saline). However, this reduction was statistically non-significant both within and between the study groups.

KEYWORDS: *Nigella sativa*, Chronic periodontitis, Matrix metalloproteinase-8, Saliva.

1. Ghazala Hassan
    M.Phil (Trainee-II), Department of Oral Biology, University of Health Sciences, Lahore – Pakistan.
2. Sarah Ghafoor
    Assistant Professor, Department of Oral Biology, University of Health Sciences, Lahore – Pakistan.
3   Saima Chaudhry
    Assistant Professor, Department of Oral Pathology, University of Health Sciences, Lahore – Pakistan.

4. Zubair Ahmed Khan
    Associate Professor, Department of Periodontology, Fatima Memorial College of Medicine and Dentistry, Lahore – Pakistan.

Corresponding Author:
Dr. Sarah Ghafoor
Assistant Professor of Oral Biology
University of Health Sciences, Lahore – Pakistan.
Email: sarahghafoor@uhs.edu.pk

INTRODUCTION

Periodontitis is the second most prevalent cause of tooth loss across the globe. People with periodontitis present with inflamed gingivae, mobile teeth, and oral malodor that may have a serious impact on the overall systemic health of these patients. Half of the world's population suffers from periodontitis which is, in fact, a preventable disease.[1] The most important factor in the pathogenesis of the periodontal disease is the host response which is discernible through several robust inflammatory markers present in saliva. Among these, matrix metalloproteinase-8 (MMP-8) is a key enzyme which is released during collagen degradation during the acute phase of periodontal disease.[2] Salivary MMP-8 is regarded as an indicator of the inflammatory process going on in the periodontal disease as its levels are reported to be consistently high among studies in patients suffering from chronic periodontitis.[3,4] Phytomedicines (medicines derived from plants) have evolved as emerging substances worldwide for the treatment of a wide array of inflammatory conditions. Nigella sativa, commonly called "Kalonji" (Urdu) or black cumin is an aromatic plant that has been labelled as having a potent anti-inflammatory effect.[5] Nigella sativa oil has also been used in oro-dental disease in the form of local application of its oil. The role of its active ingredient "thymoquinone" has also been investigated in periodontal therapy. Studies suggest that Nigella sativa has an effect on reducing symptoms of chronic periodontitis, yet the mechanism of action is still to be elucidated.[5,6] Although it is known that salivary MMP-8 levels are increased in chronic periodontitis and that Nigella sativa has anti-inflammatory effects on the periodontal disease, however the association between the two needs further inquiry.[3-4,6] Therefore, the objective of the present study was to investigate changes if any that may occur in salivary MMP-8 levels after local application of Nigella sativa oil as compared to application of placebo in patients suffering from chronic periodontitis.

METHODS

This parallel arm randomized controlled trial was conducted at Department of Periodontology, FMH College of Medicine & Dentistry, Lahore Pakistan from January 2018 to June 2019, after ethical approval from the institutional ethical committee vide letter number FMH-07-2017-IRB-268-F. The trial was also registered with ClinicalTrials.gov (NCT03270280).

Eligibility criteria of participants were, the adult individuals of both genders reporting to periodontology department with chronic periodontitis as determined through bleeding on probing and clinical attachment loss. Participants with a history of smoking, systemic illnesses, allergies, pregnancy, any drug intake in the last fifteen days and receiving periodontal treatment in the past six months were excluded from the study. Ninety-three individuals having chronic periodontitis were evaluated for eligibility. Out of those fifty individuals were enrolled for the trial. The sample size was calculated to be 18 in each group with 90% power of study and 5% margin of error based on the difference between means and standard deviations of salivary MMP levels between patients with and without chronic periodontitis[3] however trial was started on a total of 50 patients to cater for loss to follow-up as shown in flow diagram of the trial (Fig.1).
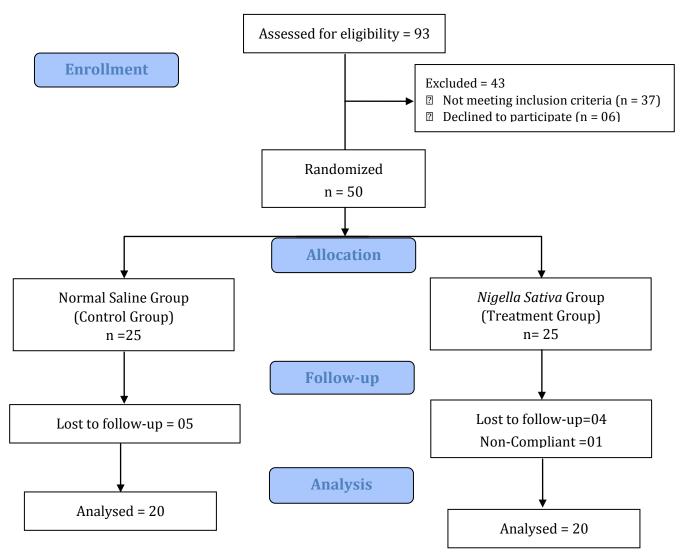
Before the commencement of the clinical trial, all the patients were given information sheets to explain the research procedures in detail and a consent form was signed by each patient. The nigella sativa oil and the normal saline applications were dispensed in same bottles which were sequentially numbered with *Nigella Sativa* mouthwash (Kalonji Oil-Marbaha) numbered 1-25 (Batch 1) and the normal saline mouth wash (UNISOL) numbered 26-50 (Batch 2). To ensure allocation concealment the prescribing periodontist did not know that the test drug was in the first batch or the second. The enrolled participants were randomized to two groups of twenty-five patients each through a random number table and each participant received the bottle that matched his number. Blinding was ensured at the level of the
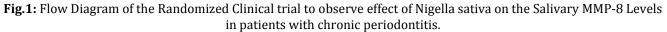
patient, the periodontist, and the data analyst to minimize bias during the study.

At the first visit the unstimulated saliva was collected from fifty patients. All patients were instructed not to eat, drink, or perform any oral hygiene measures for at least 1 hour before reporting for checkup. Distilled water was given to the patients to rinse their oral cavity for 1 minute to remove any debris. The patients were instructed to drool the saliva passively into a 50 ml sterile tube. Unstimulated salivary samples collected from patients were transported on ice and stored at-80°C before further analysis. All patients underwent scaling and root planning therapy. Patients were instructed to rinse their oral cavities

with the prescribed mouth wash twice daily for two weeks. After two weeks of drug use patients were asked to report to the department for follow-up and saliva was collected and stored following the same protocol as was followed pre-intervention.

Analysis of salivary MMP-8 was carried out using the Enzyme-Linked Immunosorbent Assay kit (Sino Gene Clon Biotech Co., Ltd SG-10411) in the Oral Biology Department, University of Health Sciences, Lahore, Pakistan according to manufacturer's instructions. Salivary samples were added to wells coated with MMP-8 specific antibodies and the absorbance values were determined by spectrophotometric ELISA-Reader (Biochrom *EZ* read 400) using Galapagos Software.

Fig.1: Flow Diagram of the Randomized Clinical trial to observe effect of Nigella sativa on the Salivary MMP-8 Levels in patients with chronic periodontitis.

STATISTICAL ANALYSIS

Statistical analysis was done using IBM Statistical Package for the Social Sciences (SPSS, Version 25.0). Gender is presented as frequency and percentage while the variables of age and concentration of MMP-8 are presented as means and standard deviations. Paired sample t-test was used to compare the pre-treatment and post-treatment salivary MMP-8 levels in both the groups individually. The comparison of MMP-8 levels among groups was done using independent sample t-test. With the confidence level of 95% the *p*-value of ≤ 0.05 is taken as significant for all analysis.

Table-1: Comparison of MMP-8 levels at baseline and after two weeks using mouthwash within Nigella sativa oil group and normal saline group.

Groups		Matrix Metalloproteinase 8 Levels (Mean ± Standard Deviation)		Level of Significance
	Measure	*Pre-Treatment*	*Post-Treatment*	*(p-value)*
Normal Saline Group	Optical Density	1.165 ± 0.57	1.008 ± 0.54	0.37
	Concentration	195.29 ± 175.89	147.35 ± 144.9	0.33
Nigella Sativa Group	Optical Density	1.06 ± 0.57	0.89 ± 0.49	0.35
	Concentration	199.37 ± 300.75	114.17 ± 129.21	0.24

Table-2: Comparison of MMP-8 levels at baseline and after two weeks using mouthwash between Nigella sativa oil group and normal saline group.

Treatment Time	Measures	Matrix Metalloproteinase 8 Levels (Mean ± Standard Deviation)		Level of Significance (p-value)
		Normal Saline Group	Nigella Sativa Group	
Pre-Treatment	Optical Density	1.165 ± 0.57	1.06 ± 0.57	0.50
	Concentration	195.29 ± 175.89	199.37 ± 300.75	0.45
Post-Treatment	Optical Density	1.008 ± 0.54	0.89 ± 0.49	0.57
	Concentration	147.35 ± 144.9	114.17 ± 129.21	0.96

RESULTS

Twenty patients completed the study in each group out of twenty-five individuals. There was 20% loss to follow-up, but it was already catered for as the calculated sample size for the study. Interestingly, number of males and females in both the groups was the same that is 11 (55%) and 9 (45%) respectively. The mean age of nigella sativa group was 38.27 ± 7.446 years while the mean age of the normal saline group was 36.39 ± 8.147 years. Mean optical density (OD) and concentration of salivary MMP-8 for the two groups are given in Tables-1&2. There was a decrease in the mean salivary MMP-8 levels through ELISA in both the groups however this decrease was statistically non-significant both within and among groups in the present trial two weeks after non-surgical periodontal therapy along with administration of nigella sativa mouthwash.

DISCUSSION

Salivary enzymes can be secreted from salivary glands, epithelial cells, microbes, neutrophils and gingival crevicular fluid and regulate essential functions in the destruction of periodontal tissues. Increased enzymatic activity has been reported in chronic periodontitis patients as compared to healthy individuals. MMP-8 is one of the enzyme released from neutrophils and is considered to be an essential marker for the periodontal disease as it can decompose types I and III collagens that perform essential roles during the destructive process of periodontal disease. Hence the levels of MMP-8 are raised during periodontitis as shown by previous studies on the subject.[3,4]

MMP-8 has been regarded as a biomarker for the chronic periodontitis disease indicating that higher MMP-8 levels are associated with the disease and the levels decrease after periodontal therapy such as scaling and root planning.[7-9] Previous study that analyzed salivary MMP-8 through ELISA reported presence of higher levels of salivary MMP-8 in chronic periodontitis patients at baseline as compared to the healthy periodontal group. These salivary MMP-8 levels however decreased after twelve weeks following periodontal therapy in the chronic periodontal group and remained static in the periodontally healthy group. However, to our knowledge, no study has investigated the change in salivary MMP-8 levels after use with *Nigella sativa* oil in patients with chronic periodontitis.[7]

It has been found in current study that salivary MMP-8 levels decreased in patients after treatment

with *Nigella sativa* oil in these patients as compared to patients that were using normal saline. In current study *Nigella sativa* for two weeks and observed that in patients with chronic periodontitis the mean pre-treatment concentration of salivary MMP-8 was higher and after using *Nigella sativa* the value of post-treatment concentration of salivary MMP-8 decreased. This decrease was more than the reduction of MMP-8 levels in the control group that had used normal saline after periodontal therapy for the same duration, however the results were not statistically significant. A previous study has shown that biochemical changes as a result of periodontitis are reversed after 4 weeks use of thymoquinone.[10] As the duration of current study was lesser, that may be one reason of not finding a statistically significant decrease in MMP-8 levels. Another reason of difference can be the type of sample used as present study detected the MMP-8 levels in unstimulated saliva through ELISA while other studies have done their investigations on gingival crevicular fluid (GCF) or gingival tissues and analyzed MMP-8 levels through western blotting or co-immunoprecipitation techniques.[8,11,12] Gingival crevicular fluid exhibits various biomarkers that are also present in saliva, however, unlike salivary collection, the collection of gingival crevicular fluid is more difficult hence poses a constraint leading to its limited use in disease diagnostics.[13]

It is pertinent to note that while protective effects of TQ have been documented regarding its interaction with molecular targets in inflammation, including pro-inflammatory cytokines and enzymes, precise mechanism responsible for anti-inflammatory role of TQ or Nigella sativa still remain less-understood.[14-16] It is also possible that variations in the levels of MMP-8 can occur during periods of exacerbations and inactive phases of chronic periodontitis which will then be reflected in oral fluids.[2] Therefore, future studies on the subject need to explore the mechanism through which thymoquinone, the active ingredient in *Nigella sativa*, exhibits its documented anti-inflammatory effect in oral cavity with longer follow-up to observe the effects over time.

CONCLUSION

The administration of *Nigella sativa* oil-based mouth wash after non-surgical periodontal therapy in patients with chronic periodontitis resulted in slight decrease in the MMP-8 levels however this decrease was not statistically significant. There was no difference found between the two study arms in terms of MMP-8 levels after administration of nigella sativa mouthwash in one and normal saline in the other twice daily for two weeks.

LIMITATION OF THE STUDY

The duration of the follow-up to detect the effect of Nigella sativa (Kalonji oil) on MMP-8 levels in saliva was two weeks. As we did find a decrease in post-treatment MMP-8 concentration in the treatment group, there is a possibility that results could have a different presentation in case of longer clinical use of *Nigella sativa* oil. However, due to time constraints it was not possible in the current investigation.

CONFLICT OF INTEREST

None to declare.

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Author's Contribution

GH: Acquisition and analysis of data, Drafting article.

SG: Conception of study, drafting article, critical revision of the manuscript for intellectual content.

SC: Critical revision of the manuscript for intellectual content

ZAK: Acquisition of data.

ALL AUTHORS: Approval of the final version of the manuscript to be published.