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Effect Of Removal Of Tongue Coating And Evaluation Of Salivary Thiol Levels In Patients With Halitosis: A Randomised Controlled Study

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Abstract
Background: *Halitosis refers to bad breath from a patient's oral cavity. Intra oral causes such as periodontal disease, abscess, poor oral hygiene, pericoronitis, xerostomia, tongue coating and extra oral causes associated with systemic conditions have been attributed with halitosis. Volatile sulphur compounds and oxidative stress have been commonly associated with the pathophysiology of halitosis. Saliva, commonly used as a non-invasive diagnostic tool can aid in the diagnosis of various oral disease.*
Materials & Methods: *45 subjects with genuine halitosis were included in the study after being subjected to an Oral Malodor Assessment by Organoleptic Score(OLP). The subjects were then divided into 3 groups (n=15). Group 1 received oral prophylaxis but no instruction on removal of tongue coating. Group 2 received instructions on removal of tongue coating but no oral prophylaxis. Group 3 received oral prophylaxis and instruction of removal of tongue coating. Oral Hygiene Index (OHI) and Winkle Tongue Coating (WTC) was recorded at baseline and 6 weeks. Saliva samples were collected at baseline and 6 weeks for estimation of Salivary Thiol levels.*
Results: *Intra-group comparison of mean Organoleptic score (OLP) at baseline to 6 weeks showed significant reduction in all groups Intragroup comparison of mean OHI score at baseline to 6 weeks showed significant reduction only in group 1 and group 3. The mean difference of Winkle Tongue Coating score from baseline to 6 weeks showed a significantly greater difference in Group 2. There was a significant increase in the thiol scores at baseline to 6 weeks in all groups with a significant increase in Group 3 compared to Group 2 and Group 1.*
Conclusion: *Non-surgical periodontal therapy and removal of tongue coating does reduce the severity of halitosis. Salivary thiol levels showed a significant increase in all three groups.*
Keywords: *Halitosis, Volatile Sulphur Compound, Tongue Coating, Organoleptic, Saliva, Thiol*

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I. Introduction

The WHO defines Health as "a state of complete physical, mental and social well- being and not merely the absence of disease or infirmity." Conditions that affect our social well-being also render us unhealthy. Halitosis (In Latin, halitus meaning "breath"and oris meaning "mouth") is a problem analogous to body odour and is a cause of concern among many sufferers. For most patients suffering from bad breath, it causes embarrassment and affects their social life. Moreover, halitosis can be an indication of underlying diseases. Halitosis is one of society's oldest and most troublesome social maladies.[1] It is a relatively common condition in which a person either suffers from bad breath or perceives himself/herself as having offensive mouth odour (halitophobia).[2]

It can have multiple causes, but in most cases, it originates from the oral cavity. Bad breath originating from the oral cavity is due to by-products generated through bacterial metabolic degradation. An important site for accumulation of bacteria associated with halitosis is the dorsum of the tongue. The tongue is one of the most important anatomical structures in the mouth harbouring one of the most complex microbiological niches. With its papillary structure and the fissures on the dorsum, the tongue offers a large surface for the accumulation of oral debris and microorganisms, especially its posterior portion, which provides an environment conducive to the growth of anaerobes.

During swallowing, soft food does not abrade this coating significantly, and the resultant whitish-grey layer of debris and microorganisms remains intact. During the putrefaction of debris on the tongue, hydrogen sulphide and methyl mercaptan are produced, both of which have been related directly to oral malodour. Poor oral hygiene results in accumulation of a bacterial biofilm in subgingival areas that may lead to periodontal disease and hence it has also been associated with halitosis.[3] Bacteria, during metabolic and catabolic processes, emit volatile organic compounds (VOCs). Saliva as a matrix represents a source of VOCs with less complex composition, compared with other matrices. It is easy to collect and store, becoming ideal for early disease detection studies, since it may contain specific biomarkers[4,5] Consequently, saliva can be useful in the development of non-invasive diagnostic tools, which can provide monitoring of both disease progression and effects of treatments. [5] Saliva is the first line of defense against oxidative stress (OS), the primary source of several systemic and oral illnesses as it contains antioxidants: albumin, ascorbate, uric acid and enzymes that establish the antioxidant capacity of saliva. Reduced antioxidant protection and elevated oxidative stress can play a significant part in the etiology of several diseases. [6] Total thiol groups are highly vulnerable to oxidative stress. Once cells are exposed to oxidative stress, thiol groups are the first antioxidants that are affected and hence, may act as a sensitive marker of oxidative stress. [7]

Hence, the aim of this study was to evaluate the effect of non-surgical periodontal therapy and removal of tongue coating in patients with halitosis and to measure the thiol level in saliva and test in validity as a marker for halitosis.

II. Material And Methods

This study involved 45 subjects who visited the Department of Periodontology, Manipal College of Dental Sciences, Manipal with a chief complaint of "bad breath". All the patients were informed about the study and only those willing to participate signed an informed consent. The present study protocol was reviewed and approved by the Institutional Ethics Committee, Manipal University, Manipal.

Subjects with systemic diseases that could be an extra-oral cause of halitosis, pregnant and lactating mothers, smokers, subjects with oral prophylaxis done in the previous 6 months, subjects with antibiotic usage in the previous month, and subjects on chronic medication, particularly drugs that cause dry mouth were excluded from the study.

The patients were then subjected to an Oral Malodor Assessment, so as to delineate subjects with genuine and pseudo halitosis. This was evaluated by a single examiner using Organoleptic (OLP) rating. Those subjects who were diagnosed positive for halitosis, were included in this study and a clinical examination was performed, in which, parameters of Oral Hygiene Index(OHI) (Greene & Vermillion,1960) and Tongue Coating (Winkel,2003) were recorded at baseline and at 6 weeks.

The subjects were randomly divided into 3 groups:

Group 1: These patients received oral prophylaxis but no instruction on the technique of removal of tongue coating.

Group 2: These patients were instructed on the technique of removal of tongue- coating. No oral prophylaxis was performed on these subjects.

Group 3: These patients received both, oral prophylaxis and instructions on the technique of removal of tongue-coating.

Saliva Collection & Estimation of Thiol Level

5 ml of whole unstimulated saliva was collected from subjects in plastic beakers which was then transferred to Eppendorf tubes using a syringe as the samples had to be centrifuged. The saliva samples were analysed and the thiol levels were estimated by the Spectrophotometric method using dinitrobenzene (DTNB)-Ellman's method. These samples were evaluated by the Department of Biochemistry, Kasturba Medical College, Manipal for estimation of Thiol levels.

Statistical Analysis

All the analysis was done using SPSS version 18 and MedCalc Version 14. A p-value of <0.05 was considered statistically significant. Inter-group comparison of continuous variables was done using ANOVA with post-hoc Tukey's test, ANOVA with post-hoc Games Howell test and Kruskal Wallis ANOVA with Post-hoc Conover test as applicable. Intra-group comparisons were done by Wilcoxon signed rank test and paired t test. Categorical variables were compared by Chi-square test.

III. Results

45 patients were included in the study and 1 was lost to recall. The percentage of males and females in Group 1, Group 2 and Group 3 were 93.3% and 6.7%, 40% and 60%, 57.1 % and 42.9% respectively. The mean age of patients in Group 1 was 36.67 years, in Group 2 was 34.27 years and in Group 3 was 39.64 years. (Table 1)

		Group 1		Group 2		Group 3		p-value
		N	%	N	%	N	%	
Sex	Male	14	93.3%	6	40.0%	8	57.1%	0.008; Sig
	Female	1	6.7%	9	60.0%	6	42.9%	
Age		36.67 ± 15.76		34.27 ± 11.07		39.64 ± 13.30		0.568; NS

Table 1: Demographic data: Gender and Age Distribution.

Intra-group comparison of mean Organoleptic score (OLP) at baseline to 6 weeks showed significant reduction in Group 1, Group 2 and Group 3. (Table 2) Intragroup comparison of mean OHI score at baseline to 6 weeks showed significant reduction only in group 1 and group 3. There was no significant reduction in Group 2. The mean difference of Winkle Tongue Coating score from baseline to 6 weeks in Groups 1, 2 and 3 was significant. There was a significantly greater difference in Group 2 than in Group 3 and Group 3 than in Group 1. (Table 3)

OLP	Baseline		6 weeks		p-value	% reduction
	Mean	SD	Mean	SD		
Group 1	2.13	.35	1.07	.26	<0.001	48.10
Group 2	1.87	.52	1.00	.00	0.001	51.6
Group 3	2.00	.00	1.21	.43	0.001	58.5

Table 2: Intra-group comparison of mean Organoleptic scores at baseline and 6 weeks

	Group 1		Group 2		Group 3		p-value	Post-hoc test
	Mean	SD	Mean	SD	Mean	SD		
OHI (baseline)	5.33	2.00	3.51	1.95	3.35	1.86	0.014†	1>2,3
OHI (6weeks)	1.53	.69	3.41	1.77	1.41	.42	0.001‡	2>1,3
WTC (baseline)	.36	.29	1.18	.20	.87	.23	<0.001†	2>3>1
WTC (6weeks)	.36	.30	.39	.15	.40	.14	0.86	-

†ANOVA with post-hoc Tukey's test

‡ ANOVA with post-hoc Games Howell test

Table 3: Inter-group comparison of OHI and WTC scores at baseline and 6 weeks

There was a significant increase in the thiol scores at baseline to 6 weeks in all groups with a significant increase in Group 3 compared to Group 2 and Group 1.(Table 4)

	Group 1		Group 2		Group 3		p-value	Post-hoc test
	Mean	SD	Mean	SD	Mean	SD		
Thiol (baseline)	70.35	4.72	69.95	5.09	70.71	4.59	0.913	-
Thiol (6weeks)	73.95	4.78	73.65	4.75	85.18	2.83	<0.001‡	3>1,2

Table 4: Intergroup Comparison of Thiol levels

IV. Discussion

The measurement of halitosis is largely complicated by a variety of factors. It is necessary to differentiate between patients with genuine halitosis and those having pseudo-halitosis or halitophobia. Patients with pseudo halitosis complain of bad breath in the absence of any objective signs. Halitophobic patients are those who have been treated for halitosis but still believe they suffer from the condition even after the resolution of the condition. If these psychosomatic patients are incorrectly managed, the psychological condition may worsen.[8] Hence, a proper diagnosis and classification is a prerequisite for treatment.

In the present study, out of 45 subjects diagnosed with genuine halitosis, only 44 reported after 6 weeks for the scheduled follow-up. One patient dropped out from the study. Oral malodour was evaluated using organoleptic rating for a clear distinction of patients with genuine halitosis. Organoleptic rating is considered to be the gold standard for measurement of oral malodour. The low cost and ease of performance contribute to its wide use.[9]

Considering the organoleptic scores taken at baseline and the 6 week follow- up, it was observed that there was a statistical difference in the 3 groups, owing to the reduction in elimination of local factors which contribute to bad breath (Table 2). In a similar study by Seemann et al, a reduction in VSC levels were seen immediately after, 1 week after, and 4 weeks after professional tooth cleaning.[10] The main factor of periodontal disease is deposits and bacterial dental plaque accumulated on the tooth surfaces. Hence, evaluation of these factors is important. In the present study, the Oral Hygiene Index developed in 1960 by John C. Greene and Jack R. Vermillion was used to classify and assess oral hygiene status. In the present study, there was a statistically significant decrease in the OHI score in Group 1 and Group 3 from baseline to the 6 weeks recall. This difference was observed most in Group 1 compared to Group 3 and was the least in Group 2. The decrease in these 2 groups could be due to the oral prophylaxis that was performed on these patients. The difference between group 1 and 3 could be due to patient factors like lack of compliance or motivation. This is similar to a study by Kara et al in 2006[11] where they studied the effect of oral hygiene instruction and scaling on oral malodour in a population of Turkish children and a significant improvement in the oral health was seen.

It has been seen that over production of lipid peroxidation by-products and disturbances in antioxidant defence system have been implicated in the pathogenesis of several diseases including periodontitis. Oxidatively modified thiol groups of cysteine residues are known to modulate the activity of a growing number of proteins.

In the present study, the thiol levels showed a significant increase in all three groups, demonstrating that the interventions helped to decrease the inflammation that was seen in the diseased state at baseline. There was a significant increase in the thiol scores at baseline to 6 weeks in all the groups with the maximum increase seen in Group 3. This could be attributed to the combined therapeutic intervention through oral prophylaxis and tongue brushing. This is in accordance with a study done by Ghezzi P (2005).[12] Thiol levels estimated pre- and post-nonsurgical therapy have provided information regarding the inflammatory status. Hence, it can be used as a diagnostic criteria for assessment of oral malodour.

V. Conclusion

Within the limits of this study, the presented data indicates that non-surgical periodontal therapy and removal of tongue coating does reduce the severity of halitosis. The thiol levels showed a significant increase in all three groups, demonstrating that the interventions helped to decrease the inflammation that was seen in the diseased state at baseline.

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