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Estimation of Secretory Iga Levels in Saliva and Its Correlation with Tongue Coating and Oral Malodour in Periodontal Health and Disease- A Clinico-Biochemical Study

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Abstract:
Aims: *To investigate the relation of 1) S-IgA levels in saliva with tongue coating 2) S-IgA levels in saliva with oral malodour, in patients with health, gingivitis and chronic periodontitis.*
Materials and method: *90 systemically healthy subjects aged 18-60 years were included in this study. They were grouped into three groups after clinical examination. Each group comprised of 30 subjects. Group I- Healthy subjects, Group II- Subjects with Gingivitis, Group III – Subjects with severe chronic periodontitis. Modified Gingival Index, Plaque Index and the Russel's Periodontal index were recorded for all subjects. Tongue coating was scored as per the scoring criteria described by Kojima et al, 1985. A simplification of the Organoleptic assessment scale by Rosenberg and McCulloch (1992) was used for grading oral malodour. 2ml of unstimulated saliva was collected from all 90 subjects into sterile ependoff tubes for estimation of secretory IgA levels using ELISA procedure.*
Results: *This S-IgA level shows an inverse relationship with tongue coating and oral malodour as the disease activity progresses from gingivitis to severe chronic periodontitis*
Conclusions: *There was a statistically significant inverse correlation seen between the tongue coating and S-IgA levels in saliva in health, gingivitis and chronic severe periodontitis. 2) A statistically significant inverse relationship was noticed in the oral malodour and S-IgA levels in healthy subjects and patients with gingivitis and chronic severe periodontitis. Thus, S-IgA levels in unhealthy subjects may show potential in limiting periodontal disease activity and may prove to be a significant biomarker of disease alteration.*
Keywords: *oral malodour, tongue coating, saliva, IgA*

I. Introduction

Breath odour or halitosis denotes any type of disagreeable scent felt on a person's breath during exhalation and speech.[1] This odour is termed as 'oral malodour' or as " **Fetor oris**" or "**Feotor ex ora**" when it originates from the oral cavity.[1]

The potential loci for oral malodour production include the posterior dorsum of the tongue, periodontal pockets, faulty restorations, dentures and abscesses. Transient oral dryness brought about by a temporal reduction in the salivary flow promotes this condition. [1] The accumulation of food remnants intermingled with exfoliated cells and bacteria causes a coating on the irregular tongue dorsum. The dorsum of the tongue has therefore been considered as a primary source of oral malodour. [2]

The secretory immunoglobulin A (S-IgA) in saliva is the principal immune component contained in secretions of the salivary glands. It possesses the capacity to inhibit the bacterial adhesion to mucosal surfaces by agglutination and plays a role in the degree of tongue coating.[3] The secretory IgA antibodies prevent bacteria from colonising the mucosal surfaces, kill them directly or activate the complement or act in concert with innate defense mechanisms .[4] Higher secretory IgA activity against specific bacterium may be related to the fact that the subjects might have been infected by this bacterium and that the increase of these bacteria on the tongue dorsum might lead to further production of volatile sulphur compounds (VSC).[4] S-IgA plays a role in inhibiting and reducing the accumulation of tongue coating.[3]

1.1. Aims are to investigate the relation of
1) S-IgA levels in saliva with tongue coating
2) S-IgA levels in saliva with oral malodour, in healthy subjects and patients with gingivitis and chronic periodontitis.
1.2. Objectives: To find a possible correlation, if any, between tongue coating, oral malodour and S-IgA levels in saliva in health and disease.

II. Materials and Methods:

Ninety systemically healthy subjects aged between 18-60 years were recruited for this clinico-biochemical study from the outpatient department of Periodontics and Oral Implantology, SDM College of Dental Sciences and Hospital, Dharwad. The study was carried out from May 2012 – August 2012. Written informed consent was obtained from all the subjects before participating in the study. The ethical clearance was obtained from the institutional review board. The subjects were examined and grouped into three groups with each group comprising of 30 subjects.

Group I- Healthy subjects
Group II- Subjects with gingivitis
Group III – Subjects with 'severe' chronic periodontitis as per the American Academy of Periodontology (AAP) classification of periodontal diseases, 1999. [5]

The subjects in Group III (severe chronic periodontitis) satisfied the following criteria
1) Presence of minimum 2 sites with attachment loss of >=5mm on two separate teeth using the Hu-Friedy University of North Carolina (UNC) 15 probe with millimetre (mm) markings at every one millimetre from 1mm upto 15mm.
2) Bleeding on probing (BOP) positive at the deepest probing sites
3) Presence of radiographic evidence of alveolar bone loss.

Modified Gingival Index (MGI) (Lobene and Associates, 1986)[6], Plaque Index (Silness J, Loe H 1967)[7], Periodontal Index (Russell AL, 1956)[8] were recorded for each subject. Periodontal Index (Russell AL, 1956)[8] was recorded to establish the stage of periodontal disease activity and measurement of periodontitis taking the clinical and radiographic features into consideration.

Subjects with absence of gingival bleeding, deepest probing pocket < 3mm and Good/ Fair oral hygiene as per the Plaque Index (Silness and Loe, 1967)[7] were included in Group I (Healthy). Subjects with MGI (Lobene and associates, 1986)[6] score ≤ 3 and deepest probing pocket < 5mm were included in Group II (Gingivitis). Subjects with MGI (Lobene and Associates, 1986)[6] ≥ 3 and clinical attachment loss ≥5mm on minimum two sites on two different teeth and radiographic evidence of alveolar bone loss were included in Group III (Chronic Periodontitis)

2.1. Subject exclusion criteria:
1) Subjects who had undergone any type of nonsurgical or surgical periodontal therapy in the past 6 months.
2) Subjects suffering from any systemic diseases.
3) Females who were pregnant/lactating.
4) Subjects who were on any antibiotic treatment in the past six months or during the study period.
5) Subjects who were on any drugs causing xerostomia during the study period.
2.2. Recording of Modified Gingival Index (Lobene and associates, 1986)[6]
This being a non invasive index, the scores were recorded by only visualising the gingival tissue. The following scores were assigned based on the findings:
0 = absence of inflammation;
1 = mild inflammation or with slight changes in color and texture but not in all portions of gingival marginal or papillary;
2 = mild inflammation, such as the preceding criteria, in all portions of gingival marginal or papillary;
3 = moderate, bright surface inflammation, erythema, edema and/or hypertrophy of
gingival marginal or papillary;
4 = severe inflammation: erythema, edema and/or marginal gingival hypertrophy of the unit or spontaneous bleeding, papillary, congestion or ulceration

1.3. Recording of Plaque Index (Silness J, Loe H 1967)[7]

This was done by recording the soft debris and mineralised deposits on the index teeth namely 16, 12, 24, 36, 32, 44. Each of the tooth surfaces (buccal, lingual, mesial, distal) is examined and given a score from 0-3. All the four surface scores are added and divided by 4 to give the plaque score for the tooth.
The scoring criteria is as follows:

0 - No plaque
1 - A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface.
2 - Moderate accumulation of soft deposit s within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye
3 - Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.
1.4. Recording of Periodontal Index (Russell AL, 1956)[8]
This was used to estimate the presence or absence of periodontal disease and its severity based on gingival inflammation, pocket formation and masticatory function.
The scores are as follows:
Score 0: Negative. There is no overt inflammation in the investing tissues nor loss of function due to loss of supporting tissues.
Score 1: Mild Gingivitis. There is an area of overt inflammation in free gingival but does not circumscribe the tooth.
Score 2: Gingivitis. Inflammation circumscribing the entire tooth but there is no apparent break in epithelial attachment.
Score 4: Used when radiographs are available. There is notch like resorption of alveolar bone crest.
Score 6: Gingivitis with pocket formation. The tooth is firm in its socket and has not drifted. There is no loss of masticatory function.
Score 8: Advanced destruction with loss of masticatory function.

1.5. Assessment of Tongue Coating:

This was done by visual examination by a single trained operator for all 90 subjects. Scrapping of the tongue was performed to check the thickness of the coating.
Scores for tongue coating (Kojima 1985)[4]

0- No coating (visual)
1- A thin coating of less than one third of the back of the tongue
2- A thin coating of less than two thirds of the tongue or less than one third covered with a thick coating
3- More than two thirds covered with a thin tongue coating or less than two third covered with a thick tongue coating
4- More than two thirds of the tongue is covered with a thick tongue coating

1.6. Assessment of Oral Malodour:

Subjects were instructed not to eat or drink and not to perform any oral hygiene activity (brushing, rinsing,etc.) atleast one hour prior to the organoleptic assessment. A single trained examiner sniffed the subject's expired air and assessed it using a simplification of an intensity rating scale originally proposed by Rosenberg and McCulloch, 1992 as follows [9]

0- No odour present
1-barely noticeable odour
2-slight but clearly noticeable odour
3-moderate odour
4-strong, offensive odour
5-extremely foul odour

The above mentioned scores tend to overlap. A simplified adaptation of the above scoring was used inorder to distinctively categorize the subjects with absence of malodour , slight presence of malodour and extremely foul odour.

0- No odour present
1- Slight but clearly perceptible malodour present
2- Extremely foul odour present

1.7. Collection of Saliva samples:

The subjects were asked to collect/ pool unstimulated saliva into the mouth for a few minutes. 2ml of resting saliva was to be collected. The patient was asked to hold the orifice of the sterile collecting ependoff tube along his/her lips and look down and let the saliva drool into it till the required volume was collected. Estimation of secretory IgA levels in saliva was done using by Enzyme Linked Immunosorbent Assay (ELISA) according to the procedure given by Ishii et al in 1998.[10]

III. Statistical Analysis:

The data so collected was analysed statistically. The Kruskal wallis – ANOVA was used for comparison of the three groups with respect to their Modified Gingival Index, Plaque index, Russell's periodontal Index, the tongue coating scores. Pair wise comparison of the groups was done using the Mann Whitney-U- test. Correlation between tongue coating, oral malodour and salivary IgA was done using Spearman's Rank Correlation. Pair wise comparison of three groups with respect to salivary IgA scores was done using Tukeys multiple post hoc procedures.

IV. Results:

Each of the subject included in the three groups was a non smoker, systemically healthy, aged between 18-60 years. The tongue coating scores among the three groups increased with progression of inflammation and disease with the mean score in Group (Healthy) being 0.03, that of Group II(Gingivitis) was 1.73 and in Group III (Chronic severe periodontitis) was 2. There was a statistically significant relationship observed in the tongue coating scores of Group I and Group II, and, Group I and Group III.

The mean oral malodour scores of Groups I (Healthy), Group II (Gingivitis) and Group III (Chronic severe periodontitis) were 0.07, 0.63 and 0.80 respectively**.** The standard deviations of the three groups, namely, Group I, Group II and Group III were 0.25, 0.49, 0.61 respectively. There was a statistically significant correlation seen between the oral malodour scores of Group I and Group II, and, Group I and Group III. The oral malodour scores of Group II and Group III did not show a statistically significant increase.

The mean S-IgA levels in saliva of Group I , Group II and Group III are 225.42µg/ml, 587.97 µg/ml and 564.73 µg/ml respectively. There was a statistically significant correlation seen between Group I and Group II and Group I and Group III. The S-IgA levels showed a reduction from Group II to Group III with progression of disease activity.

V. Discussion:

Ratcliff and Johnson (1999) have reported the potential importance of Volatile Sulphur Compounds (VSC's) in the transition of oral tissues from clinical health to gingivitis and then to periodontitis.[11] Yaegaki and Sanada have reported that most VSC's originate from the surface of tongue dorsum indicating that tongue coating might be related to breath odour. It is likely that S-IgA also affects the accumulation of bacteria on the tongue surface due to its capacity to inhibit bacterial adhesion to mucosal surfaces by agglutination.[12,13] Therefore, there arises a need to include oral malodour, tongue coating and S-IgA levels in saliva, in an attempt to find a possible correlation between them in health, gingivitis and periodontitis, since the organisms responsible for producing malodour are also found in supra and sub gingival plaque.

In our study, subjects in the healthy group (Group I) showed no tongue coating except one subject. And relatively lower levels of S-IgA were noted in saliva. Similar results have been reported by Shah et al (2010). [8] Rashkova et al (2010) explained that the presence of biofilm is an important immunogenic factor for presence of antibodies.[14] Thus, lower plaque scores in healthy subjects may contribute to lower amounts of biofilm formation and therefore, lower S-IgA levels in the Healthy Group (Group I) in comparison to Group II and Group III groups as seen in our study. This may also explain the higher S-IgA level in saliva noted in the only healthy subject with presence of tongue coating.

In the gingivitis group (Group II), similar observations were noted. As the tongue coating increased from Group I to Group II, the S-IgA levels also showed a rise. Similar results were noted by Gupta et al (2011).[15] This could be because S-IgA which forms the major surface defense mechanism, increases and tries to limit the disease as explained by Gupta et al (2011).[15] Shah et al (2010) who observed similar results also concluded that the concentration of salivary IgA is directly and positively correlated to severity of inflammation and depends on the presence of plaque.[16]

Group III, showed an increase in tongue coating with respect to group I. S-IgA levels in Group III were higher than those in Group I. As presence of biofilm is important for presence of antibodies, lower plaque scores in healthy group contribute to lower S-IgA levels. Group III showed an increase of tongue coating with a corresponding decrease of S-IgA levels in saliva from Group II to Group III). Similar results were seen by Hinode et al (2003) who described reduced tongue coating scores as S-IgA levels increased in periodontitis group than in patients without periodontitis as the tongue coating increased. They explained this by stating that S-IgA levels might not be affected by periodontal condition and by explaining the role of S-IgA in inhibiting bacterial aggregation on mucosal surfaces.[2]

Our study demonstrates that the mean tongue coating scores were highest in the Group III, followed by Group II and the lowest in Group I i.e the healthy group. The S-IgA levels were seen to be the highest in Group II, followed by Group III and the least in Group I. Hinode et al (2003) explain that S-IgA antibodies prevent bacteria from colonizing the mucosal surfaces, kill them directly or activate the complement or act in concert with innate defense mechanisms.[2] These functions of S-IgA support their role in inhibiting or reducing

the accumulation of tongue coating. This may further explain the higher tongue coating scores in Group III than Group II and the lower value of S-IgA in Group III than Group II. Thus we may state that in our study, as S-IgA levels reduced from gingivitis to periodontitis, the tongue coating showed an increase as previously proved by Hinode et al (2003).[2] Also, IgA is the only immunoglobulin that can be selectively passed across the mucosal walls to reach the lumens of organs lined with mucosal cells. Combination of bacteria to IgA inhibits their sticking to epithelial cells and reduces colonization as mentioned by Shah et al (2010).[15] This also explains the increased tongue coating from health to gingivitis to periodontitis and the subsequent reduction of S-IgA from gingivitis to periodontitis.

The results of the study conducted by Moriya et al (2002) suggest that tongue coating is closely related to mal odour as seen in our study. This could be attributed to the accumulation process of tongue coating that is influenced by various factors. A low salivary flow rate relates to the positive accumulation of tongue coating. The microbes in the accumulated coating convert a part of proteins from foods into smelly gases called volatile sulphur compounds giving rise to malodour.[17]

In the Healthy group (Group I), malodour not detected except in one subject. The S-IgA levels in saliva of subjects from this group was relatively lower than the levels in gingivitis (Group II) and periodontitis (Group III) as mentioned earlier. The absence of oral malodour can be attributed to the absence of tongue coating observed in this group. Similar observation was noted by Moriya et al (2002) who found a linear correlation between tongue coating and oral malodour.[17]

In Group II, that is the Gingivitis Group, an increase in oral malodour led to an increase in the S-IgA levels in saliva in relation to Group I. Moriya et al (2002) mention the S-IgA activity to be strong for P.gingivalis and F.nucleatum.[17] Both these organisms play a significant role in the production of VSC's as mentioned by Hartley et al (1996).[18] Lower numbers of these organisms would be expected in Group II than Group III, and therefore, reduced oral malodour. Also the S-IgA levels in Group II were higher than that in Group III. This correlation could be explained by the role of S-IgA in inhibiting the accumulation of tongue coating with the progression of disease activity as described by Shah et al (2010).[8] Reduced tongue coating would lead to decreased production of volatile sulphur compounds and thus decreased malodour. Since Group II had lower oral malodour than Group III, it had higher levels of S-IgA in saliva as previously explained by Yaegaki and Sanada.[12,11]

Similar results were also noted by Yaegaki and Sanada.[12,13] Oral malodour is most often a consequence of oral bacterial activity. Saliva from individuals with periodontitis putrefies more rapidly and the odour is more disagreeable than the saliva from healthy individuals. This explains the lower concentration of S-IgA levels in saliva of periodontitis patients than of subjects with Group II. The uptake of volatile sulphurs by epithelial cells may play an important role in the pathogenesis of periodontal disease. As stated by Yaegaki and Sanada [12,13] and Kostelc et al (1984)[19] , the concentration of VSC precursors increases with severity of periodontal disease and VSC's are reduced in subjects with good oral hygiene compared to those with gingivitis. Bosy et al (1994) mention that the presence of periodontal disease and tooth bound plaque appear to be strongly related to detection of VSC's in mouth air.[20] Protected plaque in approximal sites produces substantial odours and is associated with overall VSC levels as stated by Tonzetich J (1977).[21] Oral gram negative micro-organisms causing periodontal disease have the capacity to produce oral malodour presumably through putrefaction of sulphur containing protein substrates such as cysteine, cystine and methionine.[21] This explains the increased malodour in this group Group III and the corresponding decrease in S-IgA levels in saliva as explained previously.

VI. Conclusions:

1) There was a statistically significant inverse correlation seen between the tongue coating and S-IgA levels in saliva in health, gingivitis and chronic severe periodontitis. Minimal tongue coating was observed in the healthy group with relatively higher levels of S-IgA. As the disease activity progressed, the tongue coating increased with reduced S-IgA levels.
2) A statistically significant inverse relationship was noticed in the oral malodour and S-IgA levels in healthy subjects and patients with gingivitis and chronic severe periodontitis. Oral malodour was relatively the highest in patients with periodontal disease with lower S-IgA levels. These levels were highest in healthy subjects with minimal oral malodour recorded.

Thus, based on our findings, we may state that a certain amount of S-IgA is found in the saliva of healthy subjects even in absence of tongue coating and oral malodour. This S-IgA level shows an inverse relationship with tongue coating and oral malodour as the disease activity progresses from gingivitis to severe chronic periodontitis.

In the future, S-IgA levels in unhealthy subjects may show potential in limiting periodontal disease activity and may prove to be a significant biomarker of disease alteration.

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Table 1:Comparison of three groups (I, II, III) with respect to Tongue coating scores by Kruskal Wallis ANOVA

Groups	Means	Std.Dev.	Median	Sum of ranks
Group I	0.03	0.18	0.00	580.50
Group II	1.73	1.23	2.00	1663.50
Group III	2.00	0.91	2.00	1851.00
H-value	50.3605			
P-value	0.0000*			
Pair wise comparison of three groups (I, II, III) with respect to Tongue coating score scores by Mann-Whitney U test				
Grp I vs Grp II	P=0.0000*			
Grp I vs Grp III	P=0.0000*			
Grp II vs Grp III	P=0.4161			

Table 2: Comparison of three groups (I, II, III) with respect to Oral Malodour scores by Kruskal Wallis ANOVA

Groups	Means	Std.Dev.	Median	Sum of ranks
Group I	0.07	0.25	0.00	822.00
Group II	0.63	0.49	1.00	1561.50
Group III	0.80	0.61	1.00	1711.50
H-value	28.8795			
P-value	0.0000*			
Pair wise comparison of three groups (I, II, III) with respect to Oral malodour scores by Mann-Whitney U test				
Grp I vs Grp II	P=0.0002*			
Grp I vs Grp III	P=0.0000*			
Grp II vs Grp III	P=0.3871			

Table 3: Pair wise comparison of three groups (I, II, III) with respect to Salivary Ig A (µg/ml) scores by Tukeys multiple post hoc procedures

Groups	Group I	Group II	Group III
Mean	225.42	587.97	564.73
SD	403.30	450.55	519.67
Group I	-		
Group II	0.0085*	-	
Group III	0.0149*	0.9792	-

Table 4: Comparison of three groups (I, II, III) with respect to Modified Gingival Index scores by Kruskal Wallis ANOVA

Groups	Means	Std.Dev.	Median	Sum of ranks
Group I	0.42	0.23	0.40	465.00
Group II	1.63	0.41	1.57	1365.00
Group III	2.95	0.21	2.90	2265.00
H-value	79.1280			
P-value	0.0000*			
Pair wise comparison of three groups (I, II, III) by Mann-Whitney U test				
Grp I vs Grp II	P=0.0000*			
Grp I vs Grp III	P=0.0000*			
Grp II vs Grp III	P=0.0000*			

Table 5: Comparison of three groups (I, II, III) with respect to Deepest Probing Pocket Depth scores by Kruskal Wallis ANOVA

Groups	Means	Std.Dev.	Median	Sum of ranks
Group I	2.13	0.35	2.00	594.00
Group II	2.93	0.52	3.00	1236.00
Group III	6.93	0.58	7.00	2265.00
H-value	75.1352			
P-value	0.0000*			
Pair wise comparison of three groups (I, II, III) by Mann-Whitney U test				
Grp I vs Grp II	P=0.0000*			
Grp I vs Grp III	P=0.0000*			
Grp II vs Grp III	P=0.0000*			

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