ORIGINAL RESEARCH PAPER

Dentistry

ESTIMATION OF MUCIN4 (MUC4) IN SALIVA OF HEALTH, PERIODONTITIS AND TYPE 2 DIABETES WITH PERIODONTITIS: A CROSS-SECTIONAL COMPARATIVE STUDY.

KEY WORDS: MUC4, chronic periodontitis, diabetes, saliva, ELISA.

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ABSTRACT

Background: MUC4, a member of transmembrane mucin is expressed in body fluids like saliva, providing lubrication and protection of epithelial surfaces. Altered expression of MUC4 in saliva and GCF is noted in subjects with periodontitis. In diabetes there is decreased salivary flow causing oral alterations such as an increase in the concentration of mucin and glucose. **Aim:** To detect, estimate, compare and co-relate the levels of MUC4 in health, periodontitis and type 2 diabetes with periodontitis. **Materials And Methods:** Eighty subjects aged 25-55 years participated in this study and were divided into four groups with 20 subjects in each group. Group I: Systemically and periodontally healthy subjects Group II: Systemically healthy individuals with chronic periodontitis Group III : Diabetes with chronic periodontitis Group IV: Diabetes with healthy periodontium **Results:** A statistically significant difference were observed when saliva levels of MUC4 from group I was compared to group II and group III, when group II was compared to group IV and also when group III was compared to group IV. **Conclusion:** MUC4 levels were increased in diseased subjects compared to healthy subjects. MUC4 levels were increased in diabetic subjects with periodontitis suggesting that diabetes may act as a risk factor in altering the levels of MUC4.

INTRODUCTION:

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterised by the progressive destruction of the tooth-supporting apparatus. While the formation of bacterial biofilm initiates gingival inflammation, the disease of periodontitis is characterised by three factors namely,

1. The loss of periodontal-tissue support, manifested through clinical attachment loss (CAL) and radiographically assessed alveolar bone loss
2. The presence of periodontal pocketing and
3. Gingival bleeding.[1]

Diabetes is one of the major systemic risk factor associated with Periodontitis and Periodontitis is considered as the sixth complication of diabetes, increasing approximately threefold in people with diabetes.[2]

Mucins are the high molecular weight glycoproteins that gives viscous property to mucus, encoded by mucin genes, represented as MUC in humans.[3] Mucins play a central role in innate immunity by promoting aggregation and clearance of bacteria from the oral cavity.

MUC4 expression has been detected in bronchus, gastric epithelium, small intestine etc and also in the epithelial surface of the oral cavity and salivary glands.[4] These provide lubrication and protection from infections and injuries. It is known that in diabetes there is decreased salivary flow leading to xerostomia, affecting the periodontium by reducing the amount of saliva.[5] This decreased salivary flow can cause oral alterations such as an increase in the concentration of mucin and glucose. In a recent study it was demonstrated that MUC4 levels were significantly lower in saliva and GCF from periodontitis patients relative to healthy controls.[6] Therefore, as mucin is one of the component of saliva, there may be an alteration in the levels of MUC4 in saliva of systemically healthy subjects and periodontitis subjects with or without diabetes.

With this background, this study was designed to detect, estimate, compare and co-relate the levels of MUC4 in health, periodontitis and type 2 diabetes with periodontitis.

MATERIALS AND METHODS

This study was conducted at S.D.M College of Dental Sciences and Hospital, Dharwad in the department of Periodontics and Implantology.

A total of 80 participants, within the age range of 25-55 years were recruited in this study. An ethical clearance was procured from the institutional ethical committee. A signed written informed consent was obtained from all recruits. Subjects with at least 20 natural teeth present aged 22-55 years with chronic periodontitis (probing pocket depth ≥ 5mm), radiographic evidence of bone loss and clinical attachment loss (according to AAP classification 1999), subjects with Type 2 Diabetes (history of Diabetes at least 3 years with HbA1c levels between 7-8%) with healthy periodontium and subjects with Type 2 Diabetes Mellitus having periodontitis were included in the study. Pregnant and lactating women, smokers, patients with salivary gland disorders and any other systemic disorders, individuals who are currently on Antibiotics and any other drugs other than the medication for diabetes and those who have undergone periodontal treatment for the past 3 months were excluded from the study.

The participants were categorized into four groups:

Group I: 20 systemically and periodontally healthy subjects.

Group II: 20 patients with periodontitis affecting more than 30 percent of teeth with pocket depths ≥ 5mm and clinical attachment loss.

Group III: 20 periodontitis participants with Diabetes Mellitus.

Group IV: 20 participants with Diabetes Mellitus with healthy periodontium.

Parameters like plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment loss (CAL) and glycated hemoglobin levels (HbA1c) were recorded.

METHOD OF COLLECTION OF SALIVA :

The participants were seated comfortably and allowed to rest for few minutes and then asked to rinse their mouth with water. They were then asked to slightly lean forward and to swallow or speak. After about 5 minutes, unstimulated saliva

sample collection was done by passively drooling into airtight, sterilized containers which were then stored immediately at -80^0 C until they were transported to the laboratory for the assessment of MUC4.

STATISTICAL ANALYSIS:

All characteristics were summarized descriptively. For continuous variables, the summary statistics of N, mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries and data was analyzed by non-parametric tests since the distribution was not normal. Kolmogorov-Smirnov test was applied for intergroup comparison of study parameters. Multiple linear regression analysis was carried out to know the association between MUC4 for all variables considered.

RESULTS :

The parameters which were assessed at baseline in all the four groups include HbA1c, PI, GI, PPD, CAL. Then the MUC4 levels were assessed. The results of the individual parameters obtained in various groups are as follows.

The mean age of participants in group I, group II, group III and group IV were 27±2.9, 41±7.4, 45.9±6.3 and 49.2±4.7 respectively. A statistically significant difference was observed among the study groups. **(Table 1)**

Group II & Group III compared to group IV & Group I showed higher plaque levels and was statistically significant. The mean gingival index levels were higher in diseased groups as compared to periodontally healthy groups and was statistically significant. **(Table 2,3)**

Intergroup comparison showed that the mean probing pocket depth and CAL levels were higher in diseased groups as compared to periodontally healthy groups and was statistically significant. HbA1c levels were increased in diseased group as compared to systemically and periodontally healthy individuals. MUC4 levels were higher in periodontitis subjects with or without diabetes compared to diabetics without periodontitis. **(Table 2, 3)** When the clinical parameters were compared among the groups there was a statistically significant difference in PI, GI, PPD, CAL, HbA1c levels and MUC4 levels. **(Table 2)**

Multiple linear regression analysis was carried out to know the association between MUC4 for all variables considered. MUC4 levels were found to be significantly ($p<0.05$) higher for group II and group III compared to group I and group II. **(Table 4)**

DISCUSSION :

The mucins play a role in the lubrication of epithelial surfaces and their protection from infections and injuries.[3] Studies have shown higher expression of MUC4 in patients with periodontitis as compared to healthy controls.[7]

Diabetes is a metabolic disorder of multiple etiologies. Any acute bacterial or viral infections have shown to increase the insulin resistance and aggravate glycaemic control. Thus glycaemic control is an important variable in the relationship between diabetes and periodontal diseases, with a higher prevalence and severity of gingival inflammation and periodontal destruction seen in those with poor glycaemic control.[10] The decreased salivary flow can cause oral alterations such as an increase in the concentration of mucin and glucose.[5]

In this study the HbA1c levels were recorded to assess the glycaemic levels of the subjects. Only those individuals with the history of diabetes, at least for 3 years with HbA1c levels between 7-8% and who were under medication were included in this study. Increased HbA1c levels in diseased group indicated higher glycaemic levels in diabetics which was statistically significant. These results are in accordance to the study done by Carda et al, where he found that 100% of diabetic patients presented periodontal diseases compared to the control group.[11] Nelson and Taylor also stated higher incidence of periodontitis in type 2 diabetes mellitus compared to healthy subjects.[14,15]

The mean plaque levels were higher in diseased groups compared to periodontally healthy groups and was statistically significant. This may be due to the accumulation of plaque and calculus leading to poor oral hygiene. This explains the higher PI scores in group II and group III as compared to group I and group IV. These results are in accordance with the studies conducted by Lertpimonchai et al.[16] The mean gingival index levels were higher in diseased groups as compared to periodontally healthy groups and was statistically significant. This is because the subjects in group II and group III had more plaque and calculus leading to gingival inflammation. These results are similar to the studies conducted by Cakmak et al, who reported increase in the incidence of periodontitis with increase in gingival index scores.[17]

The mean probing pocket depth scores were higher in diseased groups compared to periodontally healthy groups and was statistically significant. Therefore, PPD $\geq$ 5 mm were higher in both group II and group III attributing to the presence of periodontal disease. These results are in accordance with the studies conducted by Gigante et al.[18] The mean CAL levels were higher in diseased groups as compared to periodontally healthy groups and was statistically significant. These results are in accordance with the studies conducted by Nanbara et al and Borges et al.[19]

MUC4 was detected in all groups. The MUC4 levels were lower in group I and was statistically significant as compared to group II. Group I compared to group III showed higher MUC4 levels and was statistically significant. Comparing group I to group IV showed no statistical significant difference in MUC4 levels. The mean MUC4 levels in group II was lower as compared to group III but it was not statistically significant. The MUC4 levels in group II was higher as compared to group IV and was statistically significant. The MUC4 levels in group III was higher compared to group IV and was statistically significant. Therefore, in this study the levels of MUC4 were higher in group II and group III as compared to group I and group IV and was statistically significant. The results of this study is in accordance with the study done by Lundmark & Bage et al, 2015 where higher expression of MUC4 was found in patients with periodontitis as compared to healthy controls.[8] Contrary to these findings, a study done by Lundmark et al, in the year 2017 stated that MUC4 levels were significantly lower in saliva and GCF from periodontitis patients relative to healthy controls.[6]

Multiple linear regression analysis was carried out to know the association between MUC4 levels for all variables considered. The MUC4 levels were higher in periodontitis subjects with or without diabetes compared to diabetics without periodontitis. This could be because of the interaction of salivary proteins to change their localization thereby increasing the retention time and altering their biological activity, providing increased protection for oral cavity. The formation of biofilm in periodontitis subjects may increase the levels of MUC4 to protect the gingival tissue.[20] Previous studies have also reported higher levels of mucins in saliva samples from periodontitis patients as compared to healthy subjects. This may be because of the response of salivary glands to periodontitis as mucins are mainly involved in non-immunological defense mechanism of the oral cavity.[8,9]

The results in this study also showed that the mean MUC4 levels were higher in group III (periodontitis subjects with diabetes) as compared to group II (periodontitis subjects

without diabetes). The reason for this could be, in response to infection, there are alterations in mucins, that are driven directly by epithelial cells and in response to signals from underlying innate and adaptive immunity. These alterations include increased mucin secretion and altered mucin glycosylation affecting microbial adhesion.[20] Therefore, diabetes may act as a significant risk factor for periodontitis altering the levels of MUC4 and because of the increase in membrane bound MUC4 as a response to inflammation, as well as a systemic inflammatory state owing to increased glycemic levels.

However further longitudinal studies with an increased sample size have to be conducted to provide a strong evidence to justify the role of MUC4 in association with type 2 diabetes mellitus.

CONCLUSION :

MUC4 levels were increased in diseased subjects compared to healthy subjects. MUC4 levels were increased in diabetic subjects with periodontitis suggesting that diabetes may act as a risk factor in altering the levels of MUC4.

Tables

Table 1 : Comparison of Age among Study groups

Demographic parameters	Group I	Group II	Group III	Group IV	p value
Age(yrs)	27±2.9	41±7.4	45.9±6.3	49.2±4.7	<0.001*

* significant at 5% level of significance (p<0.05)

Table 2 : Comparison of study parameters among groups

Study variable	Group I	Group II	Group III	Group IV	p value
Plaque index	0.3±0.3	1.5±0.4	1.3±0.3	0.4±0.3	<0.001*
Gingival index	0±0	2.1±0.3	2.1±0.3	0±0	<0.001*
Pocket depth	1.2±0.1	4.1±0.7	4.5±0.7	1.4±0.2	<0.001*
CAL	1.2±0.1	4.6±0.7	4.9±0.8	1.4±0.2	<0.001*
Hb A1c levels	5±1	5.9±0.6	7.8±0.3	7.4±0.3	<0.001*
Mucin 4 values	8.5±5.5	25.9±1.9	26.8±2.5	8.8±5.5	<0.001*

Note: * significant at 5% level of significance (p<0.05)

Table 3 : Intergroup comparison of study parameters

Study variable	Kolmogorov-Smirnov Z p value					
	Group I vs Group II	Group I vs Group III	Group I vs Group IV	Group II vs Group III	Group II vs Group IV	Group III vs Group IV
Plaque index	<0.001*	<0.001*	0.994	0.475	<0.001*	<0.001*
Gingival index	<0.001*	<0.001*	<0.001*	0.879	<0.001*	<0.001*
Pocket depth	<0.001*	<0.001*	0.557	0.169	<0.001*	<0.001*
CAL	<0.001*	<0.001*	0.547	0.369	<0.001*	<0.001*
Hb A1c levels	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.259
Mucin 4 values	<0.001*	<0.001*	0.995	0.911	<0.001*	<0.001*

Note: * significant at 5% level of significance (p<0.05)

Table 4 : Multiple linear regression analysis of the association between Mucin 4 values adjusted for selected variables

Predictor variable	Regression coeff	Std. Error	95% Confidence Interval		p value
Group II (ref Group I)	21.09	6.24	8.64	33.54	0.001*
Group III (ref Group I)	24.66	6.98	10.73	38.59	0.001*
Group IV (ref Group I)	4.01	3.04	-2.05	10.08	0.191
Age(yrs)	-0.02	0.09	-0.20	0.17	0.849
Sex (ref female)	0.50	1.05	-1.59	2.60	0.633
Plaque index	-0.45	1.69	-3.81	2.91	0.790
Gingival index	-1.68	2.40	-6.47	3.12	0.487
Pocket depth	-0.18	1.91	-3.98	3.63	0.927
CAL	0.65	1.93	-3.21	4.51	0.738
Hb A1c levels	-1.47	0.82	-3.10	0.17	0.079
Constant	15.76	4.92	5.95	25.56	0.002*

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