ORIGINAL RESEARCH PAPER
Volume-6 | Issue-10 | October-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ELECTROPHORETIC ANALYSIS OF WHOLE SALIVA AND PREVALENCE OF DENTAL CARIES – A STUDY IN INDIAN POPULATION

Dental Science

Rajiv Mengi B-grade Specialist, Department of Health, J&K Government, Jammu, Jammu and Kashmir, India

Rudra Kaul Lecturer, Department of Conservative Dentistry and Endodontics, Indira Gandhi Government Dental College and Hospital, Jammu, Jammu and Kashmir, India

Vibhuti Kaul * Registrar, Department of Oral Medicine and Radiology, Indira Gandhi Government Dental College and Hospital, Jammu, Jammu and Kashmir, India *Corresponding Author

Himja Mengi Private Practitioner, General Dentistry, Private Clinic, Jammu, Jammu and Kashmir, India

ABSTRACT

Background: Genetically determined variation in salivary protein composition may play an important role in dental caries etiology and other oral diseases.
Aim: To evaluate the correlation between various salivary proteins and DMFT with the help of SDS-PAGE.
Materials and methods: Using standardized parameters, salivary samples were collected from 50 individuals, which were subjected to SDS-PAGE. Subsequently, the DMFT was correlated with various salivary proteins amongst other parameters like age, sex, diet etc.
Statistical Analysis: Chi square test was used to test the association of the two independent variables with $p < 0.05$ considered as significant.
Results: DMFT significantly increased (p value for, MG1<0.005, MG2<0.001 and PRP-1<0.048) with increasing age and reduction in the expression of salivary proteins.
Conclusion: DMFT was increased due to decrease in proteins (except myosin) and poor oral hygiene while DMFT increased due to increase in age.

KEYWORDS

Caries diagnosis, electrophoresis, dental caries, saliva, salivary diagnostics, SDS-PAGE

INTRODUCTION

Dental caries is all pervading in modern man living in highly industrialized societies. It is a major health problem affecting mankind, in that its manifestations persist throughout life despite treatment. There are practically no geographic areas in the world whose inhabitants do not exhibit some evidence of dental caries. It affects more than 90% of population of both genders in all races, all socio-economic strata and every age group.[1]

It is generally accepted, however, that saliva secretion and salivary components secreted in saliva are important for dental health. The final result, "caries to be or not to be", is a complex phenomenon involving a number of external factors- for example diet, microbial flora-colonizing the teeth, oral hygiene, and age and internal defense factors, such as general health, nutritional and hormonal status, tooth surface morphology and saliva which is regarded as to play a potent role in dental caries.[2]

Saliva is critical to the preservation and maintenance of oral health, and any changes in its amount or quality may alter the oral health status.[3] Oral fluids often called 'the mirror of the body' or 'window on health status' is the perfect medium to be explored for health and disease surveillance.[4] Saliva has protective effects against dental caries, which has proved its way that it is not a single factor but has a major role in dental decay.[4,5] Saliva is needed in the mineralization of the teeth; it has antibacterial, antifungal, and antiviral capacity; and it has additional properties beneficial to the oral environment.[6] Genetically determined variation in salivary protein composition may play an important role in dental caries etiology and other oral diseases.[7] Human whole saliva contains, among other proteins, oligomeric mucin (MG1) with molecular mass of 200 kDa, monomeric mucin (MG2) with molecular mass of 110 kDa and proline rich protein 1 (PRP). Upto 26% of salivary proteins are mucins.[8] These proteins play an important role in protecting oral surfaces and as precursors of acquired enamel pellicle; therefore, they can modulate and influence the enamel demineralization – remineralization process dental caries formation.[7] Therefore, in the present study, we have tried to demonstrate the proteins particularly; MG1, MG2, PRP and its correlation with dental caries with the help of gel electrophoresis.

MATERIALS AND METHODOLOGY

A total of 50 samples between age group 15-60 were randomly selected from out-patient department of Dr. D.Y PATIL dental college and hospital. The age of the patients included in study were categorized into 3 groups, of which 0-20 years group has 4 (8%)patients, 21-45 years group has 39 (78%) patients and 45 years group and above has 7(14%). Out of 50 patients, there were 23 (46%) females and 27 (54%) males.

All subjects were selected randomly. Written informed consent was taken. Patients were asked to abstain from eating and drinking (except water) on the day of saliva collection. The patients were all healthy and free from any medication for at least 1 month preceding the saliva collection. Resting (unstimulated) human whole saliva (HWS) samples were collected. To reduce possible circadian fluctuations, sample collection was done under the same conditions and by the same examiner.

Caries status was determined with decayed, missing, or filled teeth (DMFT) index by a single examiner and calibrated according to World Health Organization (WHO) diagnostic criteria.[86] Based on epidemiologic data of our population, two scores were utilized to correlate presence or absence of proteins with DMFT: subjects with low DMFT index (>7.0)

Resting (unstimulated) human whole saliva (HWS) samples were collected between 8 am and 11 a.m. prior to clinical examination to reduce possible circadian contributions under the same conditions and by the same examiner. For resting secretion collection, the patient was instructed to sit in a relaxed position, with the elbows resting on the knees and the head lowered between the arms, the so-called coachman's position.[87] After rinsing their mouths with water and 1 min. of becoming comfortable, subjects were asked to swallow all remaining saliva. With the lips only slightly apart, the patient allowed the saliva to drool passively over the lower lip into the measuring cylinder, avoiding actively spitting.

Saliva from each individual was collected over a single 5-min. period by spitting into chilled, pre weighted disposable polypropylene sterile "Eppendorf tubes" with adapted conical disposable cup. Saliva samples were then stored at -80^{0}C.

The sample was subjected to sodium do-decyl sulphate-poly acryl amide gel electrophoresis (SDS-PAGE). Proteins were identified according to relative mobility in gel and stain patterns. Salivary molecules MG1, MG2, and PRP-1 were scored according to band size and stain intensity as:

absent (-),
low intensity (+),
moderate intensity (++),
high intensity (+++).
However, due to subjectivity inherent in these parameters, salivary molecules were additionally scored only as present and absent.

STASISTICAL ANALYSIS AND RESULTS

The results of the present study were inferred using Chi square test to test the association of the two independent variables with $p < 0.05$ *considered as significant as* the study aims to demonstrate the proteins particularly; MG1, MG2, PRP and its correlation with dental caries with the help of gel electrophoresis. [FIGURE 1 and 2]

The data for their dietary habits, sex, oral hygiene and age were considered in this study. Of the 50 patients selected, Table 1 shows that, since p-value is less than 0.05 we reject null hypothesis; for all proteins except protein with molecular weight of 205,000 Da i.e. Myosin. So, we conclude that there is association between presence or absence of bands and DMFT, and it can be observed that the relationship of DMFT and presence of proteins is negative, and maximum value is - 0.514 and the correlation of cystatins, histatins, and statherins and DMFT has the maximum value. We have also tried to correlate the relationship for age, fruit intake, oral hygiene against DMFT and it is found that there is significant association of Age and DMFT; and also of oral hygiene and DMFT. All the results are tabulated for better understanding.

DISCUSSION

The etiology of dental caries is a generally agreed to be a complex problem complicated by many indirect factors which obscure the direct cause/causes. There is universally no accepted opinion of the etiology of the dental caries. The contributing factors in dental caries are tooth composition, morphological characteristics, position, saliva composition, inorganic, organic, antibacterial factors; Diet-physical factors, quality of diet, local factors, carbohydrate content.[1]
DMFT is known to be affected by various factors like sex, age, oral hygiene, dietary intake and saliva proteins.

In the current study, comparing saliva samples by sex, gels stained with coomassie brilliant blue R250 showed that females (mean=7.0) have less no. of bands than males (mean=8.0), p>0.05 which is not statistically significant. In a study conducted by Banderas-Tarabay et al., (2002)[7] which is in contrast to our study showed that females have more bands (mean=25.0) than males (p>0.05) which is also not statistically significant. In another study conducted by Dodds et al., (1997)[9] of parotid saliva protein profiles in caries free and caries active adults noted that woman had significantly higher concentration of proteins than males while there was no difference attributable to caries activity which is in contrast to our study.

When diet is taken into consideration, it was noticed that diet deficiency results in absence of proteins and increase in DMFT. In our study it was observed that people vegetarian or non-vegetarian does not seem to have any effect on DMFT. Whereas Johnson et al., (1995)[10] concluded that the basic PRP were increased several-folds in saliva of protein deficient rats.

In our study, there appears to be a correlation among MG1, MG2 and DMFT, indicating importance of saliva quality per-se, reflected in oral health status of these subjects.

In terms of age current study found significant effect of age on MG1 and MG2, the concentration of MG1 and MG2 significantly increased in younger age groups as compared to older patients. Study done by Navazesh et al., (1992)[11] also found similar correlation.
Our study showed DMFT significantly increased (p value for, MG1<0.005, MG2<0.001 and PRP-1<0.048) with increasing age and reduction in the expression of salivary proteins. Studies by Pedersen et al.,(2005)[12] was in accordance with our study

In our study of association between PRP-1 and DMFT, there is a significant association between PRP-1 and DMFT (p<0.048) while studies by Anderson et al.,(1982)[13] and Mandel and Bennick, in their study of caries free and caries resistant individuals and salivary protein polymorphism found no association between salivary phenotype (parotid saliva) and dental caries. The difference of results in our study may be probably due to whole saliva samples as compared to their parotid saliva samples.

In a study of association between the peptide composition of human parotid saliva and dental decay (caries), Ayad et al., (2000)[14] found that proteolytic processing of parotid salivary proteins differs among individuals who have remained caries free and those who have experienced dental decay, which is in similar to our study which shows that the DMFT increases with decrease in protein content.

CONCLUSION

When other factors like age, dietary habits, sex and oral hygiene were taken into consideration, it was observed that age and oral hygiene has a significant effect on DMFT in the presence or absence of proteins which implies that as age increases, DMFT increases and protein decreases. There is significant increase in DMFT with poor oral hygiene. There was no significant relation of DMFT with respect to sex and dietary habits.

In conclusion, we observed that MG1, MG2 and PRP-1 and all other proteins studied showed that DMFT was increased due to decrease in proteins (except myosin) and poor oral hygiene while DMFT increased due to increase in age. These proteins are also considered as biomarkers for caries now a days[15]. But sex and dietary habits does not seem to play any role in increase or decrease of dental caries.

Table 1: Table showing protein band probability (Cross-tabulation)

Protein MW	Band		Total
	Absent	Present	
Myosin (205KDa)	12 (24%)	38 (76%)	50
Phosphorylase b (97KDa)	8 (16%)	42 (84%)	50
Bovine Serum Albumin (66KDa)	11 (22%)	39 (78%)	50
Ovalbumin (43KDa)	9 (18%)	41 (82%)	50
Carbonic Anhydrase (29KDa)	14 (28%)	36 (72%)	50
MG1(>210KDa)	15 (30%)	35 (70%)	50
MG2(~110KDa)	15 (30%)	35 (70%)	50
PRP 1(<30,000Da)	27 (54%)	23 (46%)	50
Cystatins, Histatins, Statherins(21.5KDa)	16 (32%)	34 (68%)	50
Total	127	323	450

Table 2: Showing intensity of bands

Protein MW)	Intensity				Total
	Lowest/ Absent	Moderate	High	Very high	
Myosin (205KDa)	12	30	8	0	50
Phosphorylase b (97KDa)	9	19	19	3	50
Bovine Serum Albumin (66KDa)	11	22	16	1	50
Ovalbumin (43KDa)	10	33	7	0	50
Carbonic Anhydrase (29KDa)	14	14	16	6	50
MG1(>200kDa)	14	26	9	1	50
MG2(>110kDa)	15	30	3	2	50
PRP1(30,000Da)	26	16	8	0	50
Cystatins, Histatins, Statherins(21.5KDa)	17	27	6	0	50
Total	128	217	92	13	450

Table 3: Shows Chi - Square value, P – Value and Spearman Correlation value for Presence or Absence of Protein band and DMFT

Chi-Square results DMFT and Presence or absence of Protein				Correlation of DMFT
Protein MW (Da)	Value	Degrees of Freedom	P value	Correlation Value
Cystatins, Histatins, Statherins (<21,000Da)	13.235a	1	.000	-.514
PRP1(30,000Da)	3.926a	1	.048	-.280
MG 2(>210)	12.054a	1	.001	-.491
MG 1(>110)	8.003a	1	.005	-.400
Carbonic Anhydrase (29 KDa)	10.938a	1	.001	-.468
Ovalbumin (43 KDa)	6.174a	1	.013	-.351

Bovin Serum Albumin (66 KDa)	4.432a	1	.035	-.298
Phosphorylase b (97,4KDa)	5.357a	1	.021	-.327
Myosin (205 KDa)	2.562a	1	.109	-.226

Table 4: Shows Chi - Square value, p – Value and Spearman Correlation value for Protein band intensity and DMFT

Chi-Square test Results DMFT X Band Intensity				**Correlation of DMFT**
Protein MW	**Value**	**Degrees of Freedom**	**P value**	**Correlation Value**
Cystatins, Histatins, Statherins(21.5KDa)	10.871	2	.004	-.461
PRP1(30 KDa)	10.880	2	.004	-.433
MG 2(>110kDa)	14.554	3	.002	-.480
MG 1(>210kDa)	8.280a	3	.041	-.328
Carbonic Anhydrase (29 KDa)	11.519	3	.009	-.334
Ovalbumin (43 KDa)	9.603	2	.008	-.438
Bovin Serum Albumin (66 KDa)	9.951	3	.019	-.017
Phosphorylase b (97,4KDa)	9.338	3	.025	-.224
Myosin (205 KDa)	2.836	2	.242	-.231

Table 5: showing different factors; age, fruit intake, diet and oral hygiene and their correlation value with DMFT

Factors	**Value**	**P value**	**Correlation Value**
Age X DMFT	6.992	.030	-.062
Fruit intake X DMFT	.069	.793	-.037
Diet X DMFT	.006	.941	.011
Oral Hygeine X DMFT	10.557	.005	-.459
SEX X DMFT	0.573	0.449	-0.107

Table 6: shows correlation between DMFT and Protein

Control variable	DMFT	Myosin (205 KDa)	Phosphorylase b (974 KDa)	BSA (66 KDa)	Ovalbumin (43 KDa)	carbonic anhydrase (29 KDa)	MG1 (210 KDa)	MG2 (110 KDa)	PRP1 (<30000 KDa)	cystatins, histatins, statherins (≤21.5 KDa)
Age		-.244	-.341	-.319	-.388	-.474	-.417	-.491	-.287	-.522
Oral Hygine		-.199	-.280	-.255	-.290	-.414	-.404	-.441	-.209	-.456
Food Habit		-.226	-.329	-.299	-.351	-.470	-.404	-.491	-.276	-.515
Fruit Intake		-.226	-.331	-.300	-.359	-.468	-.421	-.491	-.280	-.516
Sex		-.233	-.326	-.291	-.339	-.460	-.401	-.493	-.281	-.512

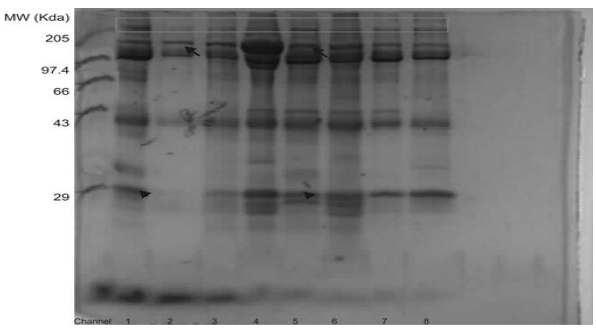

FIG: 1 Genetic polymorphism determination of salivary phenotypes in a representative SDS-PAGE stained with Coomassie brilliant blue R250. Differences and variability in molecular pattern among different subjects (channel 1-8) can be observed. Inside lined rectangle, phenotypic molecular patterns of MG1 with extensive heterogeneity are present, while MG2 is shown by an arrow and in comparison, proline-rich proteins are shown by head of an arrow. MW = molecular weight standards.

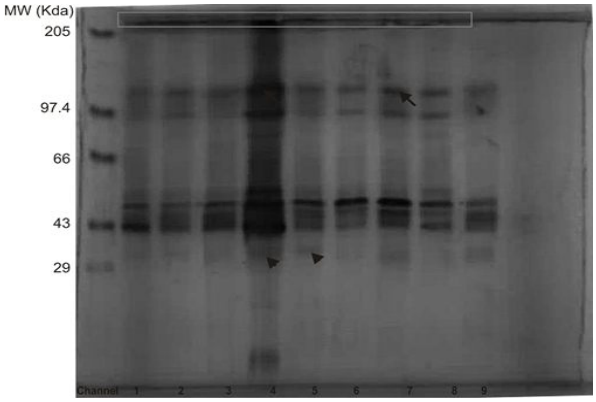

FIG 2: Representative SDS polyacrylamide slab gel analysis of HWS stained with Coomassie blue. Specific biochemical characterization of glycoproteins in HWS; MG1 (shown in rectangle), MG2 (shown in arrow), proline rich protein (head of arrow).

REFERENCES

1) Shafer, Hine, Levy. Text of Oral Pathology [6th Edn.]. Elsevier. 2009; 409-473.
2) M. Lenander-Lumikari and V. Loimaranta. Saliva and dental caries. Adv Dent Res; 2000:14:40-47.
3) Sue P. Humphrey, Russell T. Williamson. A review of saliva: normal composition, flow, and function. The Journal of Prosthetic Dentistry;2001:85:162-169 .
4) Alik Segal and David T. Wong. Salivary diagnostics: enhancing disease detection and making medicine better. Eur J Dent Educ .; 2008:12(1)22-292
5) Bernhard G. Zimmermann and David T. Wong. Salivary Mrna targets for cancer diagnostics. Oral Oncol. ;2008:44(5):425-429.
6) Eliaz Kaufman and Ira B. Lamster. The diagnostic applications of saliva-A Review. Crit Rev Oral Biol Med; 2002:13(2):197-212.
7) Jose Antonio Banderas-Tarabay, Ivonne Gardenia Zacarias-D'Oleire, Richardo Garduno-Estrada, Enrique Aceves-Luna and Mireya Gonzalez-Begne. Electrophoretic analysis of whole saliva and prevalence of dental caries. A study in Mexican dental students. Archives of medical research;2002:33:499-505.
8) Anna Zalewska, Krzysztof Zwierz, Krzysztof Zolkowski and Andrej Gindzienski. Structure and biosynthesis of human salivary mucins. Acta Biochemica Polonica;2000:47(4):1067-1079.
9) Dodds M W J, Dorthea A. Johnson, Connie C. Mobley, Kathryn M. Hattay. Parotid saliva protein profiles in caries-free and caries-active adults. Oral Surg Oral Med Oral Path Oral Radiol Endo: 1997:83:244-251
10) D.A. Johnson, H. Lopez, and J.M. Navia. Effects of protein deficiency and diet consistency on the parotid gland and parotid saliva of rats. J Dent Res; 1995: 74(8): 1444-1452.
11) M. Navazesh, R. A. Mulligan, V. Kipnis, P. A. Denny, P. C. Denny. Comparison of whole saliva flow rates and mucin concentrations in healthy Caucasian young and aged adults. J Dent Res; 1992: 71(6):1275-1278.
12) Anne Marie Lynge Pedersen, Allan Bardow and Birgitte Nauntofte. Salivary changes and dental caries as potential oral markers of autoimmune salivary gland dysfunction in primary sjogrens syndrome. BMC Clinical Pathology; 2005:5(4):1-13
13) L.C. Anderson, B. L. Lamberts, and W. F. Bruton. Salivary protein polymorphisms in caries-free and caries-active adults. J Dent Res 1982; 61:2: 393-396.
14) M. Ayad, B.C. Van Wuychuyse, K. Minaguchi, R. F. Raubertas, G.S. Bedi, R. J. Billings, W. H. Bowen, and L. A. Tabak. The association of basic Proline-rich peptides from human parotid gland secretions with caries experience. J Dent Res ;2000: 79(4):976-982.
15) Martins C, Buczynski AK, Maia LC, Siqueira WL, Castro GF. Salivary proteins as a biomarker for dental caries-a systematic review. J Dent. 2013 Jan;41(1):2-8.