

Association of Dental Caries, *Streptococcus Mutans* Counts and Secretory IgA with Tobacco Smoking

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Abstract: This article reviewed smoking oral diseases Cigarette smoke affects the oral cavity first, so it is evident that smoking has many negative influences on oral cavity, for example, dental caries. The article also discusses the relationship between smoking, *Streptococcus mutans* counts, secretory IgA (S-IgA) and dental caries in detail. Dental caries were significantly related to the presence of *Streptococcus mutans* as well as tobacco smoking. Furthermore, higher levels of microbial antigenic loads present in the oral cavity of the individuals under investigation probably increase the production of S-IgA. On the other hand the levels of S-IgA in saliva were significantly decreased tobacco smokers either with dental caries or without dental caries in comparison with non-smokers ones.

Key words: Tobacco smoking, Dental caries, *Streptococcus mutans*, IgA.

INTRODUCTION

Martin *et al.* (2006) stated that mutans streptococci participate in the formation of biofilms on tooth surfaces. These biofilms are known as dental plaque(s). Sucrose is required for the accumulation of mutans streptococci. Initial attachment of mutans streptococci to tooth surfaces, this attachment is thought to be the first event in the formation of dental plaque. The mutans streptococcal adhesin (known as antigen I/II) interacts with α -galactosides in the saliva-derived glycoprotein constituents of the tooth pellicle. Accumulation of mutans streptococci on tooth surfaces in the presence of sucrose, glucosyltransferases enzymes (GTFs) synthesize extracellular glucans from glucose (after the breakdown of sucrose into glucose and fructose), and this is thought to be the second event in the formation of dental plaque. The mutans streptococcal Glucan-binding protein (GBP) is a receptor-like protein that is distinct from GTFs, and it specifically binds glucans. GTFs themselves also have a glucan-binding domain and can therefore also function as receptors for glucans. So, mutans streptococci bind pre-formed glucans through GBP and GTFs, and this gives rise to aggregates of mutans streptococci. Acid production by mutans streptococci. The metabolism of various saccharides (including glucose and fructose) by the accumulated bacterial biofilm results in the production and secretion of considerable amounts of the metabolic end-product lactic acid, which can cause demineralization of the tooth structure when present in sufficient amounts in close proximity to the tooth surface. This is thought to be the third event in the formation of dental plaque, and it eventually results in a carious lesion.

Jha *et al.* (2006) reported that cigarette smoking and other tobacco use imposes a huge and rowing burden for public health globally. Approximately 5 million people are killed annually by tobacco use. By the year 2030, according to current trends, it is assumed that this number will increase to 10 million with 70% of deaths occurring in low and middle income countries. Numerous studies from high-income countries, and a growing number from low and middle income countries, provide strong evidence that tobacco taxes increase, dissemination of information about health risks from smoking, restrictions on smoking in public places and in work-places, comprehensive bans on advertising and promotion and increased access to cessation therapies are all effective in reducing tobacco use and its consequences. Despite this evidence, tobacco control policies have been unevenly applied, partially due to political constraints.

Smoking and its relation to dental caries is a subject of controversy, however several studies indicate an association with smokeless tobacco and caries, particularly in terms of root surface caries. This may be due to high proportion of sugar in some type of smokeless tobacco. It is interesting to note that maternal smoking and environmental tobacco smoke is also considered as a risk factor for dental caries in children. In 1951, the increase in tobacco smoking was followed by a decrease in caries rate. Smoking increases thiocyanate level in saliva. Thiocyanate, a normal constituent of saliva, was found to have a possible caries inhibiting effect.

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On the other hand, studies showed that smoking is associated with lower salivary cystatin activity and output of cystatin C during gingival inflammation. Cystatins are thought to contribute to maintaining oral health by inhibiting certain proteolytic enzymes. In addition, studies have confirmed from earlier results that there were no significant differences in salivary flow rates between smokers and non-smokers. The decreased buffering effect of smoker's saliva and the higher number of lactobacilli and *S. mutans* group may indicate an increased susceptibility to caries (Sajith *et al.*, 2007).

Saliva is a complex oral fluid consisting of a mixture of secretions from the major salivary glands and the minor glands of the oral mucosa. The normal stimulated secretion rate in adults is 1-2 ml per minute. However, it may be reduced to less than 0.1 ml per minute in individuals with severe salivary gland malfunction. Salivary sampling protocols are advantageous in that they make for frequent and easy collection of samples by non-invasive, stress-free techniques. Patients find little difficulty in salivating into disposable tubes and can provide an adequate volume in ~10 min. Saliva represents the first line of defense against foreign pathogens as well as commensal residents when high population densities can also be pathogenic (Balwant *et al.*, 2008).

Secretory IgA (S-IgA) is the prominent immunoglobulin in whole saliva and is considered to be the main specific defense mechanism in the oral cavity. In conjunction with several antimicrobial substances, including lysozyme, lactoferrin, salivary peroxidase, and mucins, S-IgA may help maintain the oral cavity disease free by limiting microbial adherence to epithelial and tooth surfaces by neutralizing virulence factors. S-IgA may also prevent the penetration of antigens into the oral mucosa (Sroisiri *et al.*, 2008). Passive smoking was associated with a decrease in secretory IgA concentration in young children (Aysun *et al.*, 2009).

MATERIAL AND METHODS

Samples:

A total of 60 individuals aged 20 – 50 years were selected and classified into 4 groups of 15 each as follows:

Group I: Smokers individuals with dental caries (smoking at least 8 cigarettes a day for at least 3 years).

Group II: Smokers individuals without dental caries (caries free).

Group III: Non-smokers individuals with dental caries (smoking at least 8 cigarettes a day for at least 3 years).

Group V: Non-smokers individuals without dental caries (caries free).

All selected individuals were required have no history of focal infection in the three months prior to the study or prior to the dental treatment at the time of examination, the absence of dental abscesses, the absence of any medication therapy. Each person was instructed not to eat or drink any thing for two hours before the appointment. The dental examination was performed in a dental chair, using a dental mirror and an explorer. The investigation was conducted at Dental Clinic at Faculty of Dentistry, Al-Azhar University, Cairo, Egypt.

Saliva Collection and Preparation:

Sugarless gum was given to the patients aiming at the stimulation of salivary flow. Whole saliva was collected in sterile cups (Biomedica Lab., Egypt), discharging the first portion. In a maximum 2 hours after sampling, saliva samples were diluted to 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} and *Streptococcus mutans* counts were performed. The levels of microorganisms were expressed as CFU/ml.

Streptococcus mutans Isolation:

Streptococcus mutans were cultivated on a medium composed of mitis salivarius agar (Difco Lab., USA) supplemented with 0.0001% potassium tellurite, 2.8 (ug/ml) of bacitracin (Sigma chemical Co., USA) and 20% (w/v) sucrose. The bacitracin was freshly prepared immediately before use. *Streptococcus mutans* was grown in 37 °C incubator under anaerobic conditions. The biochemical tests were carried out to identify the mutans Streptococci (Shklair *et al.*, 1974 and 1976). A phenol red broth base was used as the basal medium used for fermenting mannitol, sorbitol, raffinose, and melibiose. The carbohydrates are added aseptically to the warm basal media, where the final concentration of carbohydrates was 1.0 %. The media were dispensed into sterile screw cap tubes that had been inoculated with the organism to be tested and read after 48 hours of anaerobic incubation. After 48 hours incubation, 0.1 ml of the Nessler's reagent was added directly to the medium and the production of ammonia was indicated by the development of an orange-yellow colour.

Determination of secretory IgA by using Radial Immuno-Diffusion (RID) Method:

In radial immunodiffusion method (RID, ASTRA s.r.l via Ciro Menotti 1/A 20129 Milano, Italy), patients specimen in a small well is let to react with an antiserum suspended agarose gel to form Ab-Ag complexes. After the incubation, the protein to be examined and the corresponding antibody in the agarose results in a precipitation ring which diameter is proportional to the level of antigen. The value of the measured diameter in millimeters can be compare to a conversion table and the analyte concentration is determined.

Carefully, pipette 5 ul of saliva sample into the center of each well of RID plate (Fig. 1). Cover RID plate and incubate for 48 hours at 30°C, then measure the diameter of precipitation ring and compare to the corresponding value on the conversion table and record the concentration. Two RID controls (positive and negative) were included in each test series. Normal values ranging from 90 to 450 mg/dl.

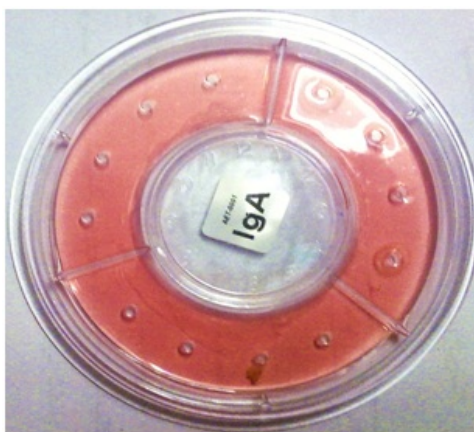


Fig. 1: Radial Immuno-Diffusion (RID) plate (RID, ASTRA s.r.l via Ciro Menotti 1/A 20129 Milano, Italy).

RESULTS AND DISCUSSION

Correlation among Dental Caries, Streptococcus mutans Counts and Tobacco Smoking:

As shown in Table (1), a total of 60 individuals aged 20 to 50 years were included in the analysis. Association between smoking, dental caries and numbers of *Streptococcus mutans* is well documented in group (I). Increased numbers of *Streptococcus mutans* have been associated with tobacco smoking in groups (I and II) Non-smokers reported more frequent healthy oral health behavior than did daily smokers even in case of group (III).

Correlation Between Dental Caries, Secretory IgA (S-IgA) and Tobacco Smoking:

Data recorded in Table (2) showed that the total salivary concentration of S-IgA (using Radial immunodiffusion method) was significantly decreased in some individuals of groups I and II While significantly higher levels of S-IgA were observed in Group III in comparison with group V (control). Non-smokers caries-active patients have significantly higher levels of naturally induced S-IgA compared with non-smokers caries-free patients.

Discussion:

The present results are consistent with the view that, significant association between *Streptococcus mutans* counts, tobacco smoking, secretory IgA (S-IgA) and risk of dental caries. In this study, dental caries were significantly related to the presence of *Streptococcus mutans* as well as tobacco smoking. Furthermore, higher levels of microbial antigenic loads present in the oral cavity of the individuals under investigation probably increase the production of S-IgA. On the other hand the levels of S-IgA in saliva were significantly decreased in tobacco smokers either with dental caries or without dental caries in comparison with non-smokers ones. It has been suggested that salivary S-IgA antibodies generated by the mucosal immune system play an important role in the immune response against dental caries (Benderli *et al.*, 2000). These antibodies may not only reduce the adherence of bacteria to saliva-coated tooth surfaces but may also neutralize extracellular enzymes (Hajishengallis *et al.*, 1992). It has been reported that caries-free patients have significantly higher levels of naturally induced S-IgA compared with caries-active subjects (Benderli *et al.*, 2000). Evidence from

previous studies has resulted in the perception that there should be a correlation between S-IgA and dental caries. Nevertheless, a discrepancy between S-IgA levels and caries prevalence still remains. It is due to this that studies have found either a positive (Bolton and Hlava, 1982) or a negative correlation (Benderli *et al.*, 2000). Results of this study indicate a significant positive association between S-IgA and the presence of dental caries. Similar findings were observed in other studies involving children and young adults (Farias and Bezerra, 2003 and Koga-Ito *et al.*, 2004). In these studies, dental caries were significantly related to high titers of S-IgA and the presence of *Streptococcus mutans* as well as large numbers of microorganisms.

Table 1: Levels of *Streptococcus mutans* detected for each studied group.

Individuals	<i>Streptococcus mutans</i> counts (CFU/ml)			
	Group (I)	Group (II)	Group (III)	Group (V)
1	4.2 x 10 ⁴ ±0.41	2.1 x 10 ⁴ ±0.26	5.7 x 10 ⁴ ±0.53	2.0 x 10 ³ ±0.13
2	6.4 x 10 ⁵ ±0.22	7.2 x 10 ³ ±0.29	3.2 x 10 ⁴ ±0.44	4.6 x 10 ⁴ ±0.27
3	5.2 x 10 ⁵ ±0.31	1.4 x 10 ⁵ ±0.18	3.7 x 10 ³ ±0.22	3.2 x 10 ² ±0.30
4	4.6 x 10 ⁴ ±0.44	6.5 x 10 ⁵ ±0.37	2.6 x 10 ³ ±0.17	1.7 x 10 ³ ±0.15
5	4.8 x 10 ⁶ ±0.52	6.2 x 10 ⁶ ±0.43	2.2 x 10 ⁴ ±0.29	2.8 x 10 ³ ±0.45
6	7.6 x 10 ⁵ ±0.11	3.8 x 10 ³ ±0.53	1.4 x 10 ⁴ ±0.30	3.4 x 10 ³ ±0.54
7	3.1 x 10 ⁴ ±0.14	4.9 x 10 ⁴ ±0.21	5.3 x 10 ³ ±0.28	2.5 x 10 ² ±0.10
8	2.4 x 10 ⁴ ±0.33	7.7 x 10 ⁴ ±0.23	4.3 x 10 ³ ±0.20	5.7 x 10 ⁴ ±0.27
9	4.5 x 10 ⁴ ±0.24	5.2 x 10 ³ ±0.13	6.5 x 10 ⁵ ±0.47	6.1 x 10 ⁴ ±0.48
10	2.2 x 10 ⁵ ±0.27	1.8 x 10 ⁵ ±0.63	2.8 x 10 ⁵ ±0.21	3.2 x 10 ⁴ ±0.62
11	1.3 x 10 ⁴ ±0.38	4.1 x 10 ⁶ ±0.65	5.1 x 10 ⁴ ±0.11	3.5 x 10 ² ±0.16
12	5.3 x 10 ⁶ ±0.18	1.9 x 10 ⁵ ±0.17	1.7 x 10 ³ ±0.64	4.3 x 10 ² ±0.39
13	5.8 x 10 ⁶ ±0.12	3.3 x 10 ⁵ ±0.27	4.4 x 10 ⁴ ±0.19	2.6 x 10 ⁴ ±0.50
14	1.7 x 10 ⁵ ±0.37	1.2 x 10 ³ ±0.34	3.6 x 10 ⁵ ±0.28	3.7 x 10 ⁴ ±0.71
15	3.2 x 10 ⁶ ±0.62	4.3 x 10 ⁴ ±0.54	5.1 x 10 ⁶ ±0.40	1.9 x 10 ² ±0.22

Group (I): Smokers individuals with dental caries; Group (II): Smokers individuals without dental caries.

Group (III): Non-smokers individuals with dental caries; Group (V): Non-smokers individuals without dental caries.

Table 2: Secretory IgA concentration in each studied group.

Individuals	Secretory IgA (S-IgA) concentrations (mg/dl)			
	Group (I)	Group (II)	Group (III)	Group (V)
1	91.41±0.06	83.35±0.06	523.52±0.05	222.10±0.07
2	201.39±0.03	91.41±0.03	598.62±0.07	201.39±0.04
3	191.29±0.02	75.46±0.02	509.01±0.03	211.66±0.05
4	134.27±0.03	99.64±0.05	553.05±0.06	232.70±0.02
5	181.36±0.01	67.74±0.03	614.15±0.04	299.91±0.04
6	222.510±0.04	108.04±0.07	494.67±0.07	323.67±0.03
7	60.18±0.06	99.64±0.05	466.49±0.05	276.83±0.08
8	83.35±0.03	116.62±0.03	710.89±0.09	254.43±0.02
9	75.46±0.07	143.35±0.07	727.61±0.02	348.11±0.09
10	99.64±0.04	99.64±0.05	677.97±0.04	335.81±0.01
11	211.66±0.02	125.36±0.05	645.72±0.06	425.51±0.07
12	108.04±0.04	162.02±0.04	629.85±0.08	360.59±0.06
13	232.70±0.03	152.60±0.08	568.07±0.02	373.23±0.05
14	83.635±0.03	91.41±0.03	480.50±0.04	412.18±0.08
15	75.46±0.07	171.60±0.1	645.72±0.01	439.00±0.04

Group (I): Smokers individuals with dental caries; Group (II): Smokers individuals without dental caries.

Group (III): Non-smokers individuals with dental caries; Group (V): Non-smokers individuals without dental caries(control).

Challacombe (1980) stated that salivary IgA is not directly related to protection against dental caries, but reflects a past exposure of the host to cariogenic microorganisms. High levels of salivary antibodies have been found to be related to dental caries (Rose *et al.*, 1994 and Benderli *et al.*, 2000). Patients with dental caries show high amounts of acidogenic microorganisms, such as *Streptococcus mutans* (Grindejford *et al.*, 1991) in their oral cavities. The presence of caries lesions can lead to more retentive areas for dental plaque accumulation and more difficulty in carrying out good oral hygiene. This may be the reason for the high levels of *Streptococcus mutans* detected in their saliva. Furthermore, higher levels of microbial antigenic loads present in the oral cavity of these individuals probably increases the immune reaction which leads to high levels of antibody production. Sroisiri *et al.* (2008) demonstrated that, The presence of dental caries was associated with increased S-IgA, *Streptococcus mutans* levels in the oral cavity. Sajith *et al.* (2007) mentioned that smoking is hazardous especially to women and children. Cigarette smoking negatively influences oral cavity. It has been established that it also causes diseases such as oral cancer, periodontitis, leukoplakia and several other oral lesions, but the direct influence of smoking on dental caries is still not verified. It has been proved that smoking along with bad oral hygiene, food habits, preventive dental visits and over all health standards, is associated with high caries incidence.

A Swedish study carried out in 1991 shows that smoking, as a habit and an increased number of cigarettes smoked per day, are positively correlated with increased number of decayed, missing and filled teeth (Hirsch *et al.*, 1991). Cigarette smoking was shown to be associated with the prevalence of caries (Heng *et al.*, 2006). Association between smoking and dental caries is well documented in older age groups (Locker, 1992 and Jette *et al.*, 1993). Among middle-age (Axelsson *et al.*, 1998) or young adults (Sgan-Cohen *et al.*, 2000) results are inconsistent. Non-smokers reported more frequent healthy oral health behavior than did daily smokers (Telivuo *et al.*, 1995). Aysun *et al.* (2009) discussed that, Passive smoking was associated with a decrease in secretory IgA concentration in young children.

REFERENCES

- Axelsson, P., J. Paulander and J. Lindhe, 1998. Relationship between smoking and dental status in 35–, 50–, 65– and 75-year-old individuals. *J. Clin. Periodontol.*, 25(4): 297-305.
- Aysun, A., D. Ozge, H.B. Ebru and B. Yuksel, 2009. Evaluation of the relation between passive smoking and salivary electrolytes, protein, S-IgA, Sialic acid and amylase in young children. Ondokuz Mayıs Univ., Fac. of Dentistry, Pediatric Dentist. Dept., 55139, Samsun, Turkey. Doi: 10.1016/ J. Archoralbio., 1-17.
- Balwant Rai, B.D.S. Intern, Simmi Kharb, (M.D.) Bio-chemistry and S.C. Anand, (M.D.S.) Oral & Maxillofacial Surgery and Orthodontics, 2008. Government Dental College, Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Science, Rohtak, Haryana (INDIA) Pt. Bhagwat Dayal Sharma, Post Graduate Institute of Medical Science, Rohtak, Haryana (INDIA) Balwant Rai, B.D.S. Intern, Simmi Kharb, (M.D.) Bio-chemistry and S.C. Anand, (M.D.S.) Oral & Maxillofacial Surgery and Orthodontics: Saliva as a Diagnostic Tool in Medical Science: a Review Study, *Adv. in Med. Dent. Sci.*, 2(1): 9-12.
- Benderli, Y., D. Erdilek, F. Koray, A. Telci and N. Turan, 2000. The relation between salivary IgA and caries in renal transplant patients. *Oral surg. Oral Med. Oral Pathol. Oral Radiol. Endod.*, 89: 588- 593.
- Bolton, R.W., G.L. Hlava, 1982. Evaluation of salivary IgA antibodies to cariogenic microorganisms in children: correlation with dental caries activity. *J. Dent. Res.*, 61: 1225-1228.
- Challacombe, S.J., 1980. Serum and salivary antibodies to *Streptococcus mutans* in relation to the development and treatment of dental caries. *Arch Oral Biol.*, 25: 495-502.
- Farias, D.G., A.C. Bezerra, 2003. Salivary antibodies, amylase and protein from children with early childhood caries. *Clin Oral Investing*, 7: 154-157.
- Grindeford, M., G. Dahllof, S. Wikner, 1991. Prevalence of mutans streptococci in one-year-old children. *Oral Microbiol Immunol.*, 6: 280- 283.
- Hajishengallis, G., E. Nikolova, M.W. Russell, 1992. Inhibition of *Streptococcus mutans* adherence to salivacoated hydroxyapatite by human secretory immunoglobulin A (S-IgA) antibodies to cell surface protein antigen I/II: reversal by IgA protease cleavage. *Infect Immun.*, 60: 5057-5064.
- Heng, C.K., V.M. Badner, K.D. Freeman, 2006. Relationship of cigarette smoking to dental caries in a population of female inmates. *Journal of correctional health care.*, 12(3): 164-174.
- Hirsch, J.M., G. Livian, S. Edward, J.G. Noren, 1991. Tobacco habits among teenagers in the city of Goteborg, Sweden, and possible association with dental caries. *Swed. Dent. J.*, 15(3): 117-123.
- Jette, A.M., H.A. Feldman, S.L. Tennstedt, 1993. Tobacco use: a modifiable risk factor for dental disease among the elderly. *Am. J. Public Health.* 1993 Sep., 83(9): 1271-1276.
- Jha, P., F.J. Chaloupka, M. Corrao, B. Jacob, 2006. Reducing the burden of smoking world-wide: effectiveness of interventions and their coverage. *Drug Alcohol. Rev.*, 25(6): 597-609.
- Koga-Ito, C.Y., C.A. Martins, I. Balducci, A.O. Jorge, 2004. Correlation among mutans streptococci counts, dental caries, and IgA to *Streptococcus mutans* in saliva. *Braz Oral Res.*, 18: 350-355.
- Locker, D., 1992. Smoking and oral health in older adults. *Can. J. Public Health*, 83(6): 429-432.
- Martin, A.T. and A.N. David, 2006. The molecular pathogenesis of dental caries associated with mutans streptococci. *Nature Reviews Immunology* doi:10.1038/nri1857, 6: 555-563.
- Rose, P.T., R.L. Gregory, L.E. Gfell, C.V. Hughes, 1994. IgA antibodies to *Streptococcus mutans* in caries resistant and susceptible children. *Pediatr. Dent.*, 16: 272-275.
- Sajith, V., F. Zdeněk, Š. Jindra J. Vimal and S. Rakesh, 2007. Smoking related systemic and oral diseases. Charles University in Prague, Faculty of Medicine in Hradec Kralove and University Hospital Hradec Kralove, Czech Republic: Department of Hygiene and Preventive Medicine1, Department of Dentistry. *ACTA MEDICA (Hradec Kralove)*; 50(3): 161-166.
- Sgan-Cohen, H.D., J. Katz, T. Horev, A. Dinte, A. Eldad, 2000. Trends in caries and associated variables among young Israeli adults over 5 decades. *Community Dent Oral Epidemiol.*, 28(3): 234-240.
- Shklair, I.L. and H.J. Keene, 1974. A biochemical scheme for the separation of the five varieties of *Streptococcus mutans*. *Arch. Oral Biol.*, 19: 1079-1081.
- Shklair, I.L. and H.J. Keene, 1976. Biochemical characterization and distribution of *Streptococcus mutans* in three diverse populations. p. 201-210. In *Microbial aspects of dental caries*, Stiles, H.M. W.J. Loesche, and T.C. O'Brien (ed), Information Retrieval Inc., Washington, D.C., USA.

Sroisiri, T., T. Boonyanit, N. Siriruk and J. Sukritta, 2008. Salivary secretory IgA, pH, flow rates, *mutans* streptococci and *candida* in children with rampant caries. Department of Microbiology, Department of Pediatric Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand, 39: 5.

Telivuo, M., P. Kallio, M.A. Berg, H.J. Korhonen, H. Murtomaa, 1995. Smoking and oral health: a population survey in Finland. *J. Public Health Dent.*, 55(3): 133-138.