# The Dental Caries Experience in Relation to Salivary Flow Rate, SIgA and Mutans Streptococci Bacteria in Smoker and Non-Smoker Patients

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## ABSRTACT

**Background:** Dental caries is a localized, progressive destructive, largely irreversible microbial based disease of multifactorial nature; these factors include (host, microbes and food) they influence differently on the initiation and progression of dental caries.

The aims of the study: was to evaluate the effect of smoking on salivary flow rate, secretory immunoglobulin (SIgA) level and viable count of mutans streptococci (M.S) bacteria in oral cavity and their relation to dental caries experience.

**Material and method:** The samples were collected from 80 male students ranging in ages from 18-22 years old. Where they divided in to two groups, 40 non-smokers (control group) and 40 smokers (study group). Unstimulated salivary samples were collected. Salivary flow rate was estimated and viable count (CFU/ml) of mutans streptococci was determined. The diagnosis and recording of dental caries were done according to WHO, 1987 criteria and the level of SIgA was determined by ELISA.

**Result:** the result revealed that the salivary flow rate and SIgA level were lower in smoker group than non-smoker, while the means value of dental caries experience Decay, Missing and Filling tooth (DMFT) and (CFU/ml)of M.S were higher in oral cavity of smoker group than non-smoker group.

**Conclusion:** the smoking has negative effect on salivary flow rate, SIgA and increase the viable bacterial count of M.S and dental caries in smoker patients.

**Key words**: dental caries, salivary flow rate, mutans streptococci, SIgA. **(Received: 22/6/2018; Accepted: 12/8/2018)**

## INTRODUCTION

Smoking cigarette is a widely recognized health hazard issue and a major cause of mortality; people continue to consume cigarettes on a regular basis, several biochemical and microbial alterations of saliva that could affect dental caries occurrence and severity among smoking individuals.

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation [1].

Mutans streptococci belong to the family collectively designated as lactic acid bacteria, lactic acid bacteria characteristically ferment sugar through the glycolytic pathway to form pyruvate, which is convert to lactate( lactic acid) ,the amount of lactic acid produce by the individual bacteria depend upon the environmental condition e.g. PH ,oxygen, amount of sugar present and competition with other microorganism, they also depend upon complement of enzymes present within the bacteria that produce alternative fermentation end products , like acetate (acetic acid) ,butyrate and ethanol [2,3] .

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Secretory IgA is the principal immunoglobulin isotype found in saliva and other body secretion, nearly 80% Secretory IgA is secreted by the three major salivary gland ,it present as a polymeric molecule composed of two IgA monomers, a J (joining) chain and a secretory component(SC) , each monomeric IgA is formed of four polypeptides, two light chains (kappa or lambda) and two α-heavy chains linked covalently by disulfide bonds [4,5]. SIgA plays an essential role in protection against infections caused by entero- pathogens and viruses in human body [4].

## MATERIAL AND METHODS

**Samples distribution**

Eighty dental students (male only) were divided in to two groups, 40 smoker and 40 non - smoker, their age ranges between 18-22 years old. The participants were healthy with no sign and symptoms of any systemic disease.

**Samples collection and bacteriological work**

Saliva Sample collection was made in early morning at time between 8A.M to 9A.M .Subject was instructed not to eat or drink in same day prior to sample collection. Around 1-3 ml of whole un-stimulated saliva was collected simply by drooling into an autoclaved sterilized glass graduated tubes, with the forward tilted head or by allowing the saliva to accumulate in the mouth

and then expectorate into a tube, while the determination of salivary flow rate done by using the graduated glass tube, the rule used was (volume of sample collected divided on time needed for collection ml /min.). Then serial dilution was prepared by using sterile saline ($10^3$,$10^5$) .The 0.1 ml of dilution was spread on mitis salivaris bacitracin selective media (HiMedia, India (, and the inoculated plates was incubated anaerobically by using the gas bag for 48 hours in $37C^0$, the identification of mutans streptococci done by detecting colony morphology using dissecting microscope, gram stain [6] and biochemical tests [6], the viable bacterial count expressed as colony forming unit per ml of saliva**.**

**Clinical part -Dental caries index (DMFT)**

DMF: Decay ,missing and filling is universally adopted index was used according to WHO[7].To detect the distribution of dental cries for each subject, DT (decay and left untreated ) MT (missing extract due to caries ) FT (filled tooth) , the total number of effected tooth by dental caries is a summation of DT+MT+FT which is known as DMFT value, the dental examination was done by using diagnostic dental instrument (probe and dental mirror) on dental chair.

**Determination of secretory IgA immunoglobulin by ELISA**

**Test principle**

All collected samples were centrifuge at 3000 rpm for 7 min. then stored in -20 $C^0$ until test done. Secretory IgA ELISA method is based on the simultaneous binding of human IgA to two antibodies, one monoclonal immobilized on micro well plates and the other, polyclonal conjugated with horseradish peroxidase (HRP). After incubation the bound/free separation was performed by a simple solid-phase washing. Then the enzyme in the bound-fraction reacts with the sbstrate tetra methyl benzidine (TMB) and ($H_2O_2$) as colored indicator, the blue color changes into yellow color when the Stop Solution sulphuric acid ($H_2SO_4$) was added. The color intensity was proportional to the IgA concentration in the sample. The IgA concentration in the sample was calculated through a standard curve. (LDN international company).


**Figure 1: Secretory IgA ELISA kit**

**Statistical analysis**

Mean value of each variable, standard error (SE), standard deviation (SD), correlation coefficient and independent t-test were done between variables in two groups , All analyses were performed by using SPSS version 24.

# RESULT

The mean value of salivary flow rate in non-smoker group (0.1925) ml/min was higher than the mean value of smoker group (0.1550) ml/min., with significant difference ($p < 0.05$) between both groups (table 1).

The mean value of viable count (CFU/ml) $\times 10^3$ of mutans streptococci in non-smoker group (164.975) CFU/ml was less than that of smoker group (171.325) CFU/ml. and the statistical analysis revealed non-significant difference between two groups ($p > 0.05$).

According to dental caries index tooth (DMFT), the mean value in non- smoker group (8.600) was less than smoker group (10.150). The statistical analysis showed non- significant difference among the two groups ($p > 0.05$).

The mean value of immunoglobulin A in saliva of non- smoker group (147.825) µg/mL was higher than smoker group (125.125) µg/mL. The statistical analysis showed no significant differences between the two groups, ($p > 0.05$).

**Table 1: the statistical analysis of salivary flow rate, viable count of mutans streptococci, Dental caries index DMFS and SIgA among non-smoker and smoker groups.**

| Variables | Non-smoker 40 | | | Smoker 40 | | | Statistical analysis | | |
|---|---|---|---|---|---|---|---|---|---|
| | mean | SE | SD | mean | SE | SD | t-test | pvalue | Sig. |
| salivary flow rate | 0.192 | 0.135 | 0.859 | 0.155 | 0.112 | 0.714 | -2.199 | 0.034 | S |
| CFU of M.S | 164.975 | 25.456 | 160.998 | 171.325 | 27.6621 | 174.950 | 0.176 | 0.861 | NS |
| DMFT | 8.600 | .850 | 5.377 | 10.150 | 0.940 | 5.950 | 1.235 | 0.224 | NS |
| SIgA | 147.825 | 10.503 | 66.428 | 125.125 | 8.514 | 53.850 | -1.640 | 0.109 | NS |

DMFT= decay, missing, filling tooth, CFU=colony forming unit, SE =standard error , SD= standard deviation ,P <0.05 Significant

**2 -Identification of Mutans Streptococci**

Identification of Mutans Streptococci was carried out by three stages which are

**a. Colony Morphology:**

The colonies of Mutans Streptococci are cultured on selective media (mitis salivaris bacitracin agar plate), incubated at 37 $C^0$ for 48 hr. and appear, either light blue in color about 1-2 mm in diameter (Smooth type) or appeared as irregular colonies with rough or frosted glass surface (Rough type) as shown in fig.(2-A). Most of mutans streptococci colonies had a depression at the middle with a drop of polysaccharide in it, or sometimes the whole colony submerged in a pool of polysaccharide fig. (2-B).

**b. Gram Stain Characteristics:**

Mutans Streptococci bacteria when subjected to Gram's stain, the result were be gram positive, appear in small ovoid or spherical shape in short or long chains as in fig.(3) .In addition mutans Streptococci was recognize as non-motile when examined under microscope by direct smear.

**c. Biochemical Tests:**

All colonies of mutans Streptococci were catalase negative, tested for catalase production by addition of 2-3 drops of 3% $H_2O_2$ to a few isolated colonies on clean slide. and had a positive reaction in fermentation of mannitol, which is indicated by the changing the indicator color from red to yellow through the formation of acid after incubation as shown in fig. (4).

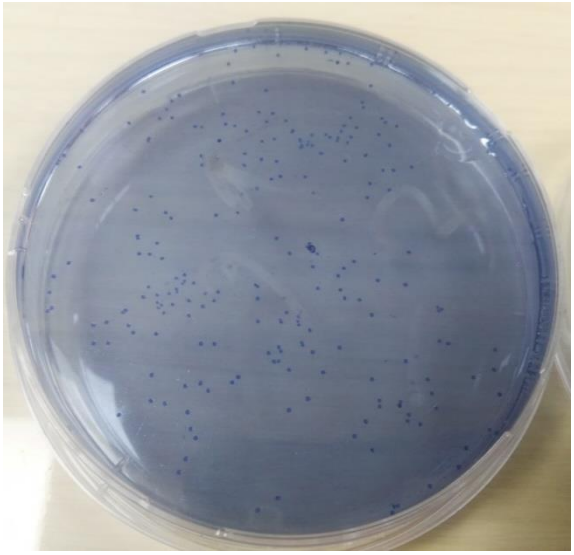

**Figure 2-A: Colonies of Mutans Streptococci on MSB agar**

**Figure 2-B: Mutans Streptococcus bacteria on mitis salivaris bacitracin agar (15× magnification)**

**Figure 3: Gram's stain of Mutans Streptococci cell from pure culture (1000× magnification)**

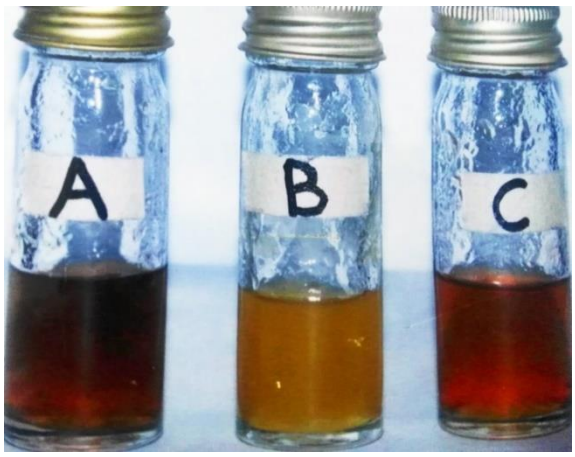


**Figure 4: Mannitol fermentation test of Mutans Streptococci**

A: Positive control tube(agar and bacteria without mannitol).
B: Study tube (agar and mannitol inoculated with MS).
C: Negative control tube (agar and mannitol).

**The correlation coefficient between the variables in both groups**

**1- The correlation coefficients between the salivary flow rate and viable count of mutans streptococci.**

The result in table (2) showed the correlation between salivary flow rate ( ml/min) and CFU/ ml $\times 10^3$ of M.S in non-smoker group which was negative, non-significant ($p > 0.05$). While the result showed negative high significant correlation was seen in smoker group ($p < 0.001$).

**Table 2: the correlation coefficient between the salivary flow rates (ml/min.) and (CFU /ml) of mutans streptococci and lactobacilli bacteria**

| Variable | Salivary flow rate (ml/min) | | | | | |
|---|---|---|---|---|---|---|
| | Non-smoker | | | smoker | | |
| | r value | P value | significance | r value | P value | Significance |
| **MS count (CFU/ml)** | -0.238 | 0.138 | NS | -0.538 | 0.000 | HS |

P < 0.05 Significant, p>0.05 non-significant

**2- The correlation coefficient between salivary flow rate and DMFT**

In table (3) the correlation coefficient between the salivary flow rate and (DMFT) was negative with highly significant correlation in both groups as ($p < 0.001$).

**Table 3: the correlation coefficients between the salivary flow rate (ml/min) and dental caries index in smoker and non-smoker groups**

| Variable | Salivary flow rate | | | | | |
|---|---|---|---|---|---|---|
| | Non-smoker group | | | Smoker group | | |
| | r value | P value | significance | r value | P value | significance |
| **DMFT** | -0.556 | 0.000 | HS | -0.629 | 0.000 | HS |

P < 0.05 Significant

**3-The correlation coefficient between the salivary flow rate and secretory immunoglobulin A**

The result in table (4) revealed that relation between the salivary flow rate and secretory IgA in non-smoker and smoker group which was positive but non-significant correlation was found (p >0.05).

**Table (4) the correlation coefficient between salivary flow (ml/min) rate and SIgA (µg/mL)**

| Variable | Salivary flow rate (ml/min) | | | | | |
|---|---|---|---|---|---|---|
| | Non-smoker | | | smoker | | |
| SIgA (µg/mL) | r value | P value | significance | r value | P value | significance |
| | 0.084 | 0.605 | NS | 0.007 | 0.967 | NS |

P < 0.05 Significant

**4-The correlation coefficients between the CFU/ml of mutans streptococci and DMFT among the non-smoker and smoker group**

Table (5) revealed a positive significant correlation between viable count of mutans streptococci and DMFT, (p<0.001) in both groups.

**Table (5) correlation coefficient between the CFU/ml of mutans streptococci and DMFT among the non-smoker and smoker group**

| Variable | Mutans streptococci (CFU/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Non-smoker | | | smoker | | |
| | r value | p value | Significance | r value | p value | significance |
| DMFT | 0.596 | 0.000 | HS | 0.694 | 0.000 | HS |

P < 0.05 Significant

**5- The correlation coefficient between the secretory immunoglobulin A and Colony Forming Units (CFU/ml) of salivary Mutans Streptococci**

In table (6) the correlation between secretory IgA and viable count of mutans streptococci show negative non-significant relation in both non-smoker and smoker groups.

**Table 6: the correlation coefficient between the secretory immunoglobulin (µg/mL) and Colony Forming Units (CFU/ml) of salivary Mutans Streptococci**

| Variable | Secretory immunoglobulin A | | | | | |
|---|---|---|---|---|---|---|
| | Non-smoker group | | | Smoker group | | |
| | r value | P value | significance | r value | P value | Significance |
| MS count | -0.303 | .015 | NS | -0.199 | 0.219 | NS |

P < 0.05 Significant

**6- Correlation coefficient between the Secretory IgA and DMFT among non-smoker and smoker groups**

The result in table (7) illustrates a negative non-significant correlation between salivary IgA and DMFT in both groups, the (p >0.05).

**Table 7: correlation Coefficient between the secretory IgA (µg/mL) and DMFT among the non-smoker and smoker group**

| Variable | Secretory IgA(µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | Non-smoker | | | Smoker | | |
| | r value | p value | Significance | r value | p value | significance |
| DMFT | -0.357 | 0.024 | NS | -0.230 | .1540 | NS |

P < 0.05 Significant

# DISCUSSION

**1-The salivary flow rate in relation to dental caries experience**

In the present study the mean value of salivary flow rate in smoker group was lower than non-smoker, the difference between two groups was statistically significant, and this result means that smoking has negative effect on salivary flow rate. The explanation of this reduction may be due to the effect of smoking on taste receptors which is consider as a primary receptor site in the oral cavity that exposed constantly to the tobacco particles, generally the use of tobacco decreases the sensitivity of taste receptors with subsequent depression in salivary reflex, presumably, this might lead to change the taste receptors response and hence alter in salivary flow rate, the importance of saliva is due to its role in maintaining a healthy oral environment , clearing of cariogenic foods from oral cavity, buffer

capacity [8] .The mean value of DMFT was higher in smoker group as compare with the non-smoker group, the result of current study agree with other previous studies [9,10]. The statistical analysis revealed no significant differences between two groups. The increased of dental caries index in smoker group may be attributed to the deficient in the salivary flow rate which is lead to deficiency in clearing capacity of the cariogenic food from the mouth and deficiency in neutralizing effect and buffer capacity of acids produced by cariogenic bacteria [11] , or due to the shifting of the bacterial population towards *lactobacillu*s and the cariogenic streptococci in smokers all might argue for increased dental caries[12] .The essential role of salivary flow rate against cariogenic bacteria and caries process confirm by the result of current study that detect the correlation between the salivary flow rate and DMFT, in both smoker and non-smoker group which was negative relation, this result agree with AL-Saadi, 2009[13], which said that salivary flow rate was negatively associated with dental caries.

**2- Mutans in relation to salivary flow rate and dental caries experience**

In present study the result showed that means value of CFU/ml of M.S bacteria was higher in smoker than non-smoker group, with no significant difference between two groups this was in agreement with other studies [14] .The explanation of this increased in count of cariogenic bacteria in oral cavity of smoker group may be due to either that tobacco smoking depress the immunoglobulin in oral cavity IgM , IgA, or could be due to reduced salivary flow rate and more carious teeth present in smokers than nonsmokers group which consider as retention site for bacteria [9] .The correlation coefficient in present study revealed positive highly significant relation between the (CFU/ml) of mutans streptococci and dental caries index(DMFT) in both groups, the current result agree with Al-mizraqchi,1998[15] .An explanation of this result due to that M.S are consider as highly cariogenic bacteria ,this is due to their several virulence factors mediated there carcinogenicity such as ,prevalent plaque adhesion-like cell surface proteins, acid production, tolerance, and production of glucosyl transferases, mutacin and intracellular polysaccharides [3].

The correlation coefficient between CFU/ml of M.S and salivary flow rate in smoker group was negative highly significant correlation and weak negative in non-smoker group, this lead to conclusion that high salivary flow rate represent as a protective factor against caries and cariogenic bacteria, since the role of saliva in maintaining a healthy oral environment which can be summarized by: diluting and eliminating sugars and other substances ,buffer capacity , balancing demineralization-reminerlization and the antimicrobial action of saliva in maintaining the equilibrium of the oral ecosystems, this is essential for dental caries control, the saliva is able to perform its function of maintaining the oral microbiota balance because it contains certain proteins, which are possess an antimicrobial effect because some of them are capable of modifying the bacteria's metabolism and inhibit their ability to adhere to the surface of the tooth, the most important proteins involved in oral ecosystem maintenance are proline-rich proteins, lysozyme, lactoferrin, peroxidases, agglutinins and histidine, as well as secretory immunoglobulin A and immunoglobulins G and M[3].

**3-Salivary IgA in relation to salivary flow rate and dental caries experience**

The secretion of salivary IgA in the oral cavity occur by the different type of salivary gland, major salivary gland secretion could be due to dripping of foreign-body inside the glands duct or sub-mucosal foreign-body insertion, while secretion from minor salivary gland due to interaction between the foreign-body (antigen)and mucosal surfaces[16] .The result of current research showed that mean value of SIgA is lower in smoker group than non-smoker group ,this agree with study of Golpasand Hagh *et al*.,2013[10]. With non-significant differences between two groups .The explanation of decreased salivary immunoglobulin A level in smoker group, may be due to either immunosuppressive effect of the tobacco products that impair T-cell immune-regulation which is responsible of B-cell differentiation and maturation this will lead to decrease in immunoglobulin A production or due to influence of smoking on the salivary gland cells itself which are responsible for the completion of secretory IgA secretion [17] .The correlation coefficient between salivary IgA and dental caries index were negative ,this is may be due to the inverse relation between SIgA and cariogenic bacteria in the oral cavity which reduced the dental caries in healthy individual in addition to others antimicrobial component of saliva, the result of current study approved with other studies [18][19] .And the correlation coefficient between SIgA and salivary flow rate was positive relation , other human studies between salivary flow rate and SIgA are conflicting , the contradictory result between different researches may be due to different sampling methods ,different criteria for

patient selection and different laboratory tests used by researchers, moreover ,the concentration of Secretory immunoglobulin A may change depending upon the salivary flow rate, hormonal factors ,emotional status and physical activity [20].

**4- The secretory IgA in relation to mutans streptococci**

The correlation coefficient in present study between the salivary immunoglobulin A and M.S was negative relation in both groups. SIgA considered to be the first line of defense of the host against pathogenic microorganism which colonize or invade surfaces bathed by external secretions , the main function of SIgA antibodies seems to be interferes with bacterial adherence to host surfaces by preventing both non-specific and stereo chemical interactions, the binding of SIgA to adhesions can reduce the negative surface charge and the hydrophobicity of bacteria, thus limiting the potential for ionic and hydrophobic interactions between bacteria and host receptors, the reduction of the hydrophobicity of bacteria is probably due to the heavy glycosylation of fragment crystalisble and secretory components, which confer hydrophilic properties to the SIgA molecule[4] .

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## الخلاصه

المقدمه :تسوس الاسنان هو مرض موضعي , مدمر , واسع الانتشار ويعتبر مرض مايكروبي ,متعدد العوامل هذه العوامل تتضمن (الكائن ,المايكروبات والطعام) والذين يؤثرون بنسب مختلفه على بدايه وتقدم تسوس الاسنان في الكائنات .

الهدف من الدراسه : الهدف من الدراسه كان دراسة تاثير التدخين على نسبة تدفق اللعاب , الجلوبيولين المناعي أ ,وعدد بكتريا المكورات المسبحيه الميوتنس وتاثيرهم على تسوس الاسنان .

المواد واساليب العمل :العينات جمعت من 80 طالب من الذكور فقط ,اعمارهم كانت تتراوح بين 18-22 سنه,مقسمين الى مجموعتين 40 منهم من المدخنين و40 من غير المدخنين. تم جمع عينات من سائل العاب الغير محفز كما تم قياس معدل تدفق اللعاب,تم تشخيص وتحديد عدد المكورات المسبيحيه الميوتنس , وفحص تسوس الاسنان وفق معايير منظمة الصحه العالميه 1987م .كما تم تحديد مستوى اللعاب المناعي الجلوبيولين أ لجميع المشتركين في البحث.

النتائج: نتائج الدراسه الحاليه اظهرت ان مستوى تدفق اللعاب والاجسام المضاده أ هي اقل في الاشخاص المدخنين من غير المدخنين , اما عدد بكتريا المكورات المسبحيه الميوتنس و تسوس الاسنان كان اكثر في مجموعة المدخنين من غير المدخنين.

الاستنتاج: التدخين يؤثر بشكل سلبي على مستوى تدفق اللعاب والاجسام المضاده أ,كما يؤدي الى زياده في عدد بكتريا المكورات المسبحيه وتسوس الاسنان في مجموعه المدخنين اكثر من غير المدخنين .


الكلمات المفتاحيه: تسوس الاسنان , تدفق سائل اللعاب , المكورات المسبحيه الميوتان, الجلوبيولين المناعي أ .