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ASSOCIATION OF DENTAL CARIES & S-IGA IN SMOKERS AND NON-SMOKERS

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Abstract

Introduction - Dental caries is a multifactorial disease that results in demineralization of dental hard tissues like enamel. The risk of dental caries is controlled by various factors like presence of carbohydrate in diet, maintenance of oral hygiene and many more. Amongst this saliva contribute an important parameter due to its complex composition, presence of Secretory Immunoglobulin A (S-IgA) which acts as an antibacterial substance. Our study focuses on the levels of salivary S-IgA in smokers and Non-smokers within the region of Nagpur and Lucknow and determines its association with dental caries. Also, a comparative study is conducted to analyze the S-IgA levels in smokers and non-smokers between the Nagpur and Lucknow population. **Methods -** A total of 240 healthy subjects were selected and divided into 2 groups (120 subjects each from Lucknow and Nagpur) and each group from these cities was divided into 4 subgroups, based on smoking and dental caries. Informed consent was taken, and 5 ml saliva was collected. Salivary S-IgA was measured using ELISA Kit. Caries status was determined according to the DMFT Index. Results were analyzed using SPSS software Version 24.0 (IBM Corporation, Chicago, USA). **Results –** Smokers in Nagpur and Lucknow showed higher number of caries with lowest concentration of S-IgA.as compared to non-smoking and caries-free subjects (123.2 ± 19.9 vs. 13.3 ± 4.1 µg/ml respectively, $p < 0.001$). The comparative study in Nagpur and Lucknow subjects was found to be insignificant. **Conclusion –** Our findings suggest that levels of salivary S-IgA are inversely proportional to prevalence of dental caries in smokers.

Keywords: S-IgA, Dental Caries, Saliva, ELISA, DMFT, Smokers, Non-Smokers

INTRODUCTION

Dental caries is a widely prevalent disease problem globally. It is one of the most common chronic infectious, transmissible diseases resulting from tooth-adherent specific bacteria, primarily Streptococcus Mutans that metabolize sugars to produce acid.[28] Thus Bacteria is said to be one of the essential factors of caries formation. The imbalance of cariogenic bacteria and commensal bacteria in dental plaque results in higher production of acid which over time resulting demineralization of the hard tooth structure i.e., enamel. dentin, cementum.[24]

Role of Secretory immunoglobulin A (S-IgA) in Saliva Immune Ecosystem

There are three major categories that regulate the oral ecosystem: **Host related, Microbe related, and External factors**. Among **host factors**, secretory immunoglobulin A (S-IgA) constitutes the main specific immune defense mechanism or predominant immunoglobulin in whole saliva and contributes an essential role in homeostasis of oral microbiota. In concoction with other factors like pH, salivary flow rate, hormonal factors, emotional stress, environmental factors and several antimicrobial substances, such as lysozyme, lactoferrin, salivary peroxidase and mucins, S-IgA helps to maintain the oral cavity in disease free state.[3, 23,21,26]

Naturally occurring S-IgA antibodies that are reactive against a variety of indigenous bacteria are detectable in saliva. These antibodies may control the oral microbiota by reducing the adherence of bacteria to the oral mucosa and teeth. Thus, the protection against bacterial etiologic agents of caries and periodontal diseases could be conferred by the induction of S-IgA antibodies via the stimulation of the mucosal immune system. However, this elucidates that the role of the S-IgA immune system in controlling the oral indigenous microbiota as a prerequisite for the development of effective vaccines against these diseases.[27]

Association of Smoking, Dental Caries and S-IgA

Numerous epidemiological studies around the world have reported a close relationship between smoking, occurrence of dental caries and level of S-IgA. Different studies have proved that smoking personnel (including 94.6% men and 5.4% women) have a higher decayed, missing, filled teeth (DMFT) score than non-smoking personnel.[5,24] The probable cause are decreased buffering effect, lower pH of smokers' saliva and higher number of lactobacillus and Streptococcus mutans increases the susceptibility to caries in smokers.[13, 11] It is contemplated from different studies that lower concentration of S-IgA is one of the risk factor for increase susceptibility for dental caries and various other periodontal diseases.[4, 9, 12]

The purpose of the present study was to explore the association between salivary S-IgA, status of dental caries and tobacco smoking habit in Lucknow and Nagpur region and aims at comparing the same association in both the cities. Our study also focuses on a comparison of S-IgA level between Lucknow and Nagpur region.

MATERIALS & METHODS

1. **Study Population and Design -**

A total of 240 individuals aged 20 – 60 Years were enrolled from Nagpur, Maharashtra and Lucknow, Uttar Pradesh in the study with 120 individuals each from Nagpur and Lucknow. Each 120 individuals from both the cities were classified into 4 subgroups described as follows:

Group I: Smokers with Dental Caries (Smoking at least 8 cigarettes/bidis a day for at least 3 years) (Minimum DMFT = 1) (n =30).

Group II: Smokers without Dental Caries ((Smoking at least 8 cigarettes/bidis a day for at least 3 years) (Caries Free DMFT = 0) (n =30).

Group III: Non - smokers individuals with Dental Caries (Minimum DMFT = 1) (n =30).

Group IV: Non -smokers individuals without Dental Caries (Caries Free DMFT = 0) (n =30)

Subjects were selected by a random sampling method from the general population of Nagpur and Lucknow. The approval of the Local Research Ethics Committee was taken for the study. After

obtaining written consent, demographic data, medical history, clinical history, and thorough intraoral examination including smoking habit (frequency and duration), DMFT was assessed.
All participated individuals were healthy, without any acute and chronic systemic diseases. Subjects with a history of focal Infection or any dental treatment in the 3 months span prior to the study or prior to the dental treatment at the time of examination, the presence of dental abscesses, tobacco chewing habit or subject undergoing any drug therapy, subjects suffering from any kind of autoimmune and allergic diseases were excluded from the study.
All Subjects were instructed not to eat or drink for 2 hours before the appointment. All Individuals were subjected to:
• Registration of the dental caries – DMFT
• Saliva Sample for S-IgA study
Each participant was examined by trained dental students. All the measurements were taken by a single examiner to ensure inter-observer variability.

Table 1 - Samples: A total of 120 individuals

Groups	Subjects	Frequency
Group I	30 Smokers with Dental Caries	>8 Cigarettes or bidis/day for at least 3 years; DMFT Score Minimum 1
Group II	30 Smokers without Dental Caries	>8 Cigarettes or bidis/day for at least 3 years; DMFT Score 0
Group III	30 Smokers with Dental Caries	DMFT score minimum 1
Group IV	30 Controls (Nonsmokers without Caries)	DMFT score 0

2. Clinical Examination and Evaluation of Dental Caries -
Thorough history and clinical examination were performed. To remove biases of false case history by the patient, routine blood investigations, random blood sugar were measured by taking their blood samples. We screened 113 subjects in which 04 subjects were diabetic and 09 subjects were anemic. Oral examination was performed for the 120 healthy subjects, their teeth examined and DMFT scores were calculated.
The teeth which were not included in this index were unerupted, congenitally missing, supernumerary and teeth which were filled for causes other than decay (like trauma, aesthetics or as abutment).

3. Saliva Collection Method -
Saliva was collected from the patients who were instructed not to eat or drink for 2 hours which was done after stimulation for 2 minutes by the means of chewing a rubber band. 5 ml of Stimulated saliva was collected in a sterile plastic container and consequently, frozen in a refrigerator at -10 Degree Centigrade.

4. ELISA Method for the study of S-IgA -
S-IgA in the saliva were determined qualitatively by ELISA method employing saliva secretory IgA kit of euro immune (Immunoglobulin A ELISA Kit, US) in both the region as per manufacturer's instructions. Salivary concentrations in S-IgA (µg/ml) in each sample were deliberated using a standard curve procured from standardizations within the kit.

5. Statistical Analysis -
All data collected were analyzed using SPSS version 24.0 (IBM Corporation, Chicago, USA). All the continuous variables were analyzed using a one-way parametric ANOVA test comparing between smoking habit, dental caries status and S-IgA level in all 04 subgroups of Nagpur and Lucknow selected populations.

The differences in the number of dental caries in all the 04 subgroups in both the selected city with caries were analyzed using the unpaired t-test. The *p*-values less than 0.05 were contemplated as statistically significant.
Also, a comparative study between Nagpur and Lucknow selected population were considered and found to be statistically insignificant.

RESULTS

Analysis:

Descriptive and analytical statistics were done. The normality of data was analyzed by the Shapiro-Wilk test. As the data follows normal distribution the parametric tests were used to analyze the data. The one-way analysis of variance (ANOVA) test was used to check mean differences among the groups. Post hoc analysis was done using Tukey's HSD test. The independent sample t-test was used to compare means wherever applicable. The level of significance was kept at $p<0.05$.
Software: SPSS (Statistical Package for Social Sciences) Version 24.0 (IBM Corporation, Chicago, USA)

Output Tables:

Table 1: Comparison of mean salivary S-IgA level among the groups (Lucknow)

Groups	N	Mean	S.D.	S.E.	Min.	Max	F-value	P-value[#]
Group I	30	17.63	3.15	0.57	13.00	24.00	3001.514	<0.001[†]
Group II	30	25.10	2.55	0.46	21.00	30.00		
Group III	30	59.16	3.96	0.72	53.00	66.00		
Group IV	30	114.73	6.78	1.23	103.00	127.00		

[#]P-value derived from one-way ANOVA test; [†]significant at $p < 0.05$

The mean salivary S-IgA level was compared among the four groups. The analysis done by one-way ANOVA showed statistically significant differences (**p<0.001**) in mean salivary S-IgA levels. The group I - Smokers with Dental Caries had the least salivary S-IgA level of 17.63 ± 3.15 followed by group II - Smokers without Dental Caries (25.10 ± 2.55) and group III - non-smokers individuals with Dental Caries (59.16 ± 3.96). The group IV - non-smokers individuals without Dental Caries had the highest salivary S-IgA level of 114.73 ± 6.78.

Table 2: Post hoc pair wise comparison of mean salivary S-IgA level among the groups (Lucknow)

Groups	M.D.	95% C.I.	P-value[*]
Group I v/s Group II	-7.46	-10.44--4.48	<0.001[†]
Group I v/s Group III	-41.53	-44.51--38.55	<0.001[†]
Group I v/s Group IV	-97.10	-100.07--94.12	<0.001[†]
Group II v/s Group III	-34.06	-37.04--31.08	<0.001[†]
Group II v/s Group IV	-89.63	-92.61--86.65	<0.001[†]
Group III v/s Group IV	-55.56	-58.54--52.65	<0.001[†]

[#]P-value derived from Tukey's HSD post hoc test; [†]significant at $p < 0.05$

The post hoc pairwise comparative analysis was done. When group I was compared with group II, a mean difference of -7.46 (95% CI: -10.44--4.48) was seen which was statistically significant (**p<0.001**). When group I was compared with group III, a mean difference of -41.53 (95% CI: -44.51--38.55) was seen which was statistically significant (**p<0.001**). When group I was compared with group IV, a mean difference of --97.10 (95% CI: -100.07--94.12) was seen which was statistically significant (**p<0.001**).

When group II was compared with group III, a mean difference of -34.06 (95% CI: -37.04--31.08) was seen which was statistically significant (**p<0.001**). When group II was compared with group IV, a mean difference of -89.63 (95% CI: -92.61--86.65) was seen which was statistically significant (**p<0.001**).

When group III was compared with group IV, a mean difference of -55.56 (95% CI: -58.54--52.65) was seen which was statistically significant (**p<0.001**).

Table 3: Comparison of mean salivary S-IgA level among the groups (Nagpur)

Groups	N	Mean	S.D.	S.E.	Min.	Max	F-value	P-value[#]
Group I	30	16.63	3.15	0.57	12.00	23.00	2999.125	<0.001[†]
Group II	30	24.10	2.55	0.46	20.00	29.00		
Group III	30	58.16	3.96	0.72	52.00	65.00		
Group IV	30	113.80	6.79	1.24	102.00	126.00		

[#]P-value derived from one-way ANOVA test; [†]significant at p < 0.05

The mean salivary S-IgA level was compared among the four groups. The analysis done by one-way ANOVA showed statistically significant differences (**p<0.001**) in mean salivary S-IgA levels. The group I - Smokers with Dental Caries had the least salivary S-IgA level of 16.63 ± 3.15 followed by group II - Smokers without Dental Caries (24.10 ± 2.55) and group III - non-smokers individuals with Dental Caries (58.16 ± 3.96). The group IV - non-smokers individuals without Dental Caries had the highest salivary S-IgA level of 113.80 ± 6.79.

Table 4: Post hoc pair wise comparison of mean salivary S-IgA level among the groups (Nagpur)

Groups	M.D.	95% C.I.	P-value[*]
Group I v/s Group II	-7.46	-10.44--4.48	<0.001[†]
Group I v/s Group III	-41.53	-44.51--38.55	<0.001[†]
Group I v/s Group IV	-97.16	-100.14--94.18	<0.001[†]
Group II v/s Group III	-34.06	-37.04--31.08	<0.001[†]
Group II v/s Group IV	-89.70	-92.68--86.71	<0.001[†]
Group III v/s Group IV	-55.63	-58.62--52.65	<0.001[†]

P-value derived from Tukey's HSD post hoc test; [†]significant at p < 0.05

The post hoc pairwise comparative analysis was done. When group I was compared with group II, a mean difference of -7.46 (95% CI: -10.44--4.48) was seen which was statistically significant (**p<0.001**). When group I was compared with group III, a mean difference of -41.53 (95% CI: -44.51--38.55) was seen which was statistically significant (**p<0.001**). When group I was compared with

group IV, a mean difference of --97.16 (95% CI: -100.14--94.18) was seen which was statistically significant (**p<0.001**).

When group II was compared with group III, a mean difference of -34.06 (95% CI: -37.04--31.08) was seen which was statistically significant (**p<0.001**). When group II was compared with group IV, a mean difference of -89.70 (95% CI: -92.68--86.71) was seen which was statistically significant (**p<0.001**).

When group III was compared with group IV, a mean difference of -55.63 (95% CI: -58.62--52.65) was seen which was statistically significant (**p<0.001**).

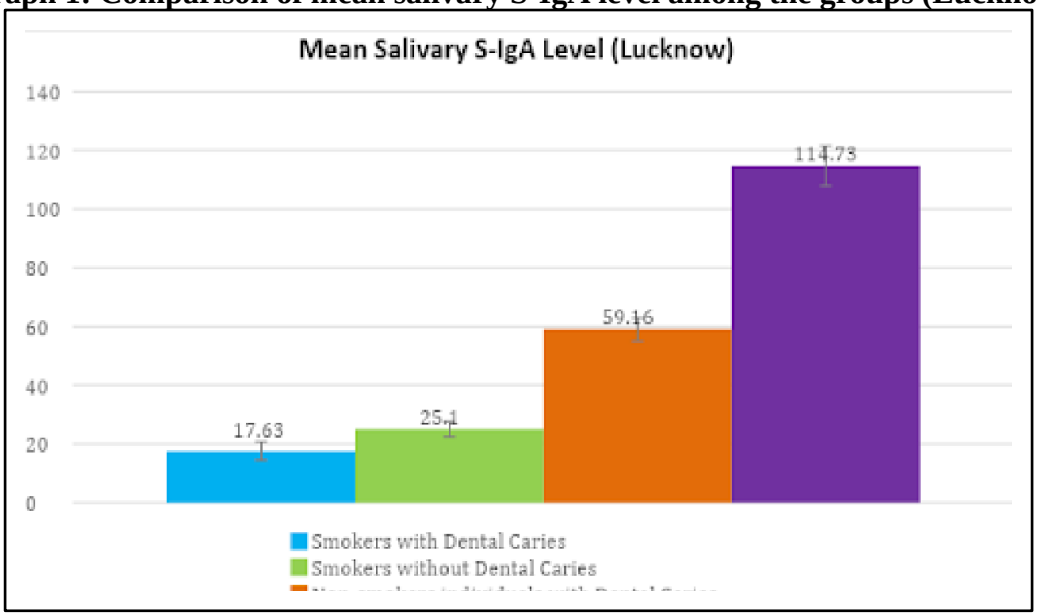
Table 5: Comparison of mean salivary S-IgA level between study participants of Lucknow and Nagpur

Group Type	Lucknow (n=30)	Nagpur (n=30)	P-Value*
	Mean ± S.D.	Mean ± S.D.	
Smokers with Dental Caries	17.63 ± 3.15	16.63 ± 3.15	0.225
Smokers without Dental Caries	25.10 ± 2.55	24.10 ± 2.55	0.134
Non-smokers individuals with Dental Caries	59.16 ± 3.96	58.16 ± 3.96	0.333
Non-smokers individuals without Dental Caries	114.73 ± 6.78	113.80 ± 6.79	0.597

[#]P-value derived from one-way ANOVA test; [†]significant at p < 0.05
The mean salivary S-IgA level was compared between study participants of Lucknow and Nagpur. The analysis done by independent sample t-test showed **NO** significant statistical differences in mean differences between study participants of Lucknow and Nagpur region.

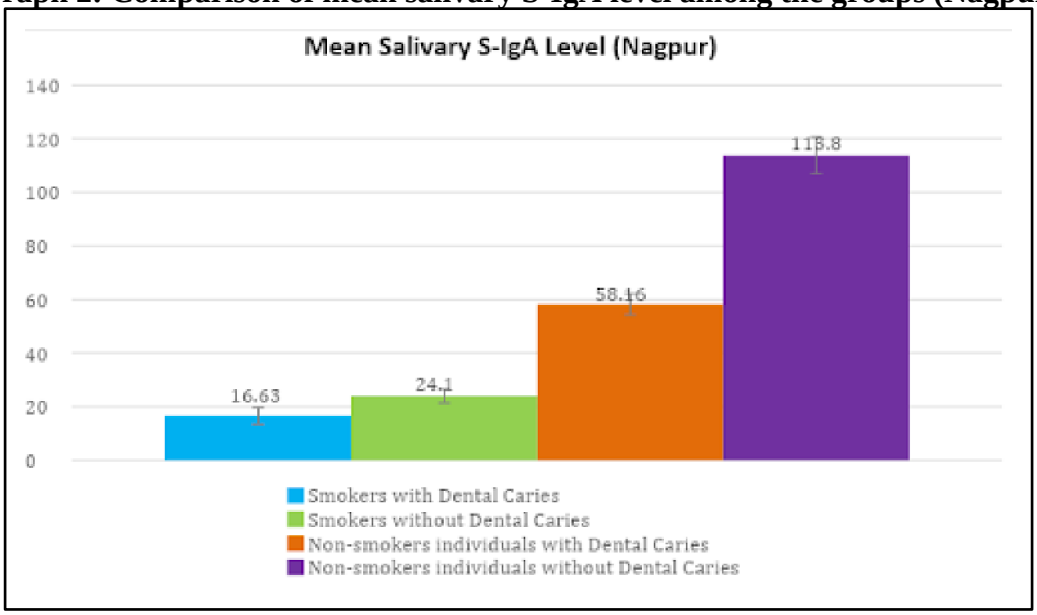
Output Graphs:

Graph 1: Comparison of mean salivary S-IgA level among the groups (Lucknow)



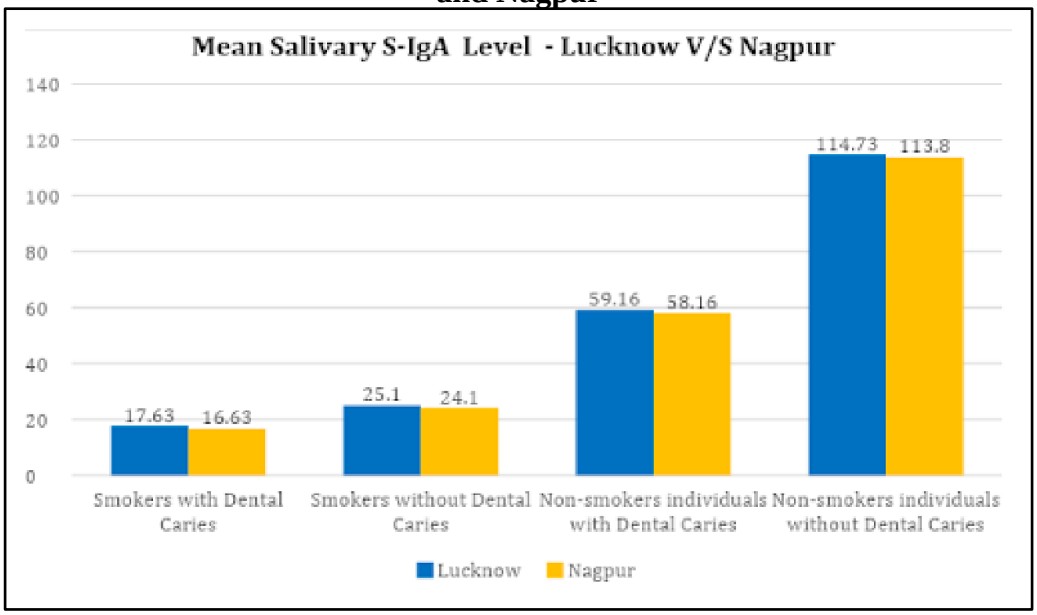
Note: The error bar represents standard deviation

Graph 2: Comparison of mean salivary S-IgA level among the groups (Nagpur)



Note: The error bar represents standard deviation

Graph 3: Comparison of mean salivary S-IgA level between study participants of Lucknow and Nagpur



DISCUSSION

Dental caries is a Chemico- Parasitic process consisting of decalcification of dental hard tissues like enamel and dentin followed by its dissolution. The etiology of dental caries is elucidated by numerous parameters like our oral hygiene practice, imbalance of various cariogenic bacteria like *Streptococcus mutans, Streptococcus salivaris, Streptococcus oralis Lactobacilli, Streptococcus gordonii, Actinomyces, Candida albicans* etc. and commensal bacteria such as *Streptococcus sanguinis*.[30]

Also, various studies like Jiayi Wu et. al. 2019 showed that there is a close relationship between smoking and dental caries. A component of cigarettes i.e., nicotine enhances the carious activity of these cariogenic microorganisms thus, promoting the formation of a caries-susceptible environment. Smoking also affects saliva by lowering the buffer capability and altering its chemical agent thus again enhances the caries-susceptible environment.[24.]

Struzycka I, 2014 also proves that when there is a compromised balance between these commensal bacteria, they start acting as pathogens causing dental caries.[34]

Other important effects of smoking in modulating oral environment as analyzed by Nesrin Tarbiah et. al.2019, F Pan et. al. 2010, I Cloez-Tayarani et. al. 2007, P Moszczyński et. al 2001, Q Yang et. al. 1999, YM Geng et. al. 1995, RL Gregory et. al. 1992, Chan MA et. al. 1990 is that it causes dysregulation and B lymphocytes impairment.[7,10,15,20,22] nicotinic receptors are expressed by B cells,[8, 17] and long-term exposure to nicotine can suppress B-cell development, proliferation and immune functions.[20]

In 1975, Ørstavik and Brandtzaeg reported that low titers of parotid S-IgA appeared to correspond with higher rates of dental caries. However, since the levels of IgA antibody rather than IgA immunoglobulin may be important, these studies were not definitive, in co relating the dental caries and secretory IgA.[19]

Thereafter, Challacombe S.J, in 1980 studied serum and salivary antibodies to Streptococcus mutans in relation to the development and treatment of dental caries and stated that salivary IgA is not directly related to protection against dental caries but reflects a past exposure of the host to cariogenic microorganisms.[6]

In 1981, McGhee and Michalek found that subjects with IgA deficiency fell into two groups in terms of oral antibody i.e., those with compensatory IgM antibodies against S. mutans in saliva and those without. Only in the group without compensatory IgM was the caries activity significantly higher than age sex matched controls. This study was indicating a co relation of secretory IgA and dental caries and smoking.[14]

In 1982, Bolton, R.W. and G.L. Hlava examined children for caries activity and their salivary IgA were evaluated for reactivity to antigens of cariogenic bacteria by an enzyme-linked immunosorbent assay (ELISA). IgA levels to Streptococcus mutans were higher in children with no detectable caries. Analysis of IgA specific for Lactobacillus casei, teichoic acid, and glucan revealed no protective role for these specificities in children.[2]

G Norhagen Engstrom, 1998 analyzed the level of salivary immunoglobulins like S-IgA in smoking and non-smoking individuals and showed decreased level of S-IgA in both stimulated and unstimulated saliva in non-smokers individuals as compared to smokers which is similar to our study results.[18]

J R Barton et. al., 1990 investigated the influence of cigarette smoking on salivary immunoglobulins i.e. S-IgA, S-IgM in pure parotid saliva and showed result similar to our study i.e. smokers had significantly lower S- IgA and higher IgM concentrations than did non-smokers.[25]

Skok MV et. al. 2007 also investigated the effects of tobacco smoking on saliva immunoglobulin (S-IgA) levels and conclude similar results as in our study in smokers and nonsmoker's individuals.[31,32,33]

In 2013, L Golpasand et al studied the association of dental caries and salivary S-IgA with tobacco smoking. The findings indicate that Smokers showed a higher number of caries and the lowest concentration of S-IgA. Thus, it reveals that low concentrations of salivary S-IgA are correlated with a higher prevalence of dental caries in smokers as implicated in our study.[26]

In 2014, N Upadhyaya reviewed that the tobacco use is directly associated with various medical problems like cancer, low birth weight, pulmonary cardiovascular diseases including oral cavity which negatively affects first.[16]

Both the studies were conducted before COVID 19 period. The study in Nagpur was conducted in 2013-14 and in Lucknow in 2016-17.

CONCLUSION

Numerous studies showed that smoking is considered as one of the risk factors in promoting dental caries amongst others like poor oral hygiene, different eating habits esp. diet including higher sugar amount, poor brushing habits for increased caries activity. In Smokers saliva has a decreased buffering effect and a higher number of lactobacilli and Streptococci mutans, consequently increasing susceptibility to caries. Smoking is also seen to be associated with lower salivary cystatin activity which inhibits certain proteolytic enzymes.

Our studies indicate that higher incidence of dental caries is associated with the lowest concentration of salivary S-IgA in smokers which is also shown in other studies. The decreased S-IgA content in

smoking individuals is due to the presence of some components in cigarettes, mainly nicotine, which promotes the growth of cariogenic bacteria (e.g., the Streptococci mutans) in smokers than nonsmokers. Several studies also prove the strong association between dental caries and smoking. But the exact association of dental caries with tobacco smoking remains unknown although smoking seems to be a risk factor for increased dental caries. Further studies, clinical trials and experiments are required to prove the effect of smoking as one of the main causes among others of dental caries.

Abbreviations:
DMFT = decayed, missing, or filled teeth; DMFS = decayed, missing, or filled surfaces;
ELISA = Enzyme Linked Immunosorbent Assay; S-IgA = Salivary Secretory IgA

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