Chinese Journal of Laboratory Medicine

Role of Dietary habits, antioxidants and Lipid profile in Dental caries of smokers

Naresh Yajamanam [1], Haridas Somayaji [2], Nireeksha Shetty [3]*, Preethi Hegde [4]

1 Associate professor, Department of Biochemistry, Santhiram Medical College, Nandyal
2 Associate Professor, Department of Orthopaedics, K.S. Hegde Medical Academy, Nitte (Deemed to be University)
3* Assistant Professor, Conservative, and Endodontics, ABSMIDS, Nitte (Deemed to be University)
4 Assistant Professor, Department of Physiology, K.S Hegde Medical Academy, Nitte (Deemed to be University)

DOI: https://doie.org/10.0714/Cjlm.2024727271

Abstract:

Background: Dental caries is a complicated multifactorial disease characterized by demineralization of dental hard tissue (enamel, dentine, and cementum) in both deciduous and permanent teeth (1-4). Dental caries can be prevented and reversed with correct management. The present study aimed to study the role of dietary habits, antioxidants and lipid profile in Dental caries of smokers. **Methodology:** 5ml of the blood sample was collected from these subjects. A portion of the blood was mixed with EDTA (2mg/ml.) The other portion was used for the preparation of serum. Erythrocytes were isolated from blood samples by adding blood in to tubes containing EDTA Venous blood samples were centrifuged at 1000 x g for 15 min at 4 °C and the plasma was removed The packed erythrocyte pellet was washed three times with an equal volume of sterile 0.9% NaCl. pH = 74 Stock hemolysate was obtained by dilution of packed erythrocytes with distilled water and kept at 4°C. Isolation of the main etiological agent in dental caries *Streptococcus mutans* was carried out and biochemical characterization was conducted. Its susceptibility to cause dental caries was also checked. Status of Vitamin C. Total antioxidant, iron, MDA and Lipid profile in serum and Erythrocyte SOD activity in case were estimated and compared with the control.

Results: In the present study we found small, round, cream-coloured colonies with smooth margins and glistering surface was obtained on blood agar plate. The microorganism isolated was found to be non motile, non spore and gave purple coloured colonies on gram staining.The microorganisms isolated gave positive results for indole, Methyl red, citrate utilization, nitrate reduction and sugar fermentation test and negative for voges Proskauer and catalase test. This confirms that the isolate is *S. mutans,* Vitamin C, iron, Total antioxidant, SOD, HDL, level was found to be low in case group compared to that of the control, where as MDA, Total Cholesterol, LDL and Triacyglycerides were high in the case group compared to that of the control. ANOVA was performed for multiple comparison which showed statistical significance between the groups.

Conclusion: Dental caries infection causes an increase of free radicals in body moreover in smokers the ROS produced is very high. Low levels of antioxidants and micronutrients in all the case groups indicates the inefficient neutralization of free radicals. This inefficient neutralization results in other harmful effects like increased lipid peroxidation rate and lipid profile level alteration. Antioxidant depletion can be treated and its level in the body can be normalized by using adjuvant antioxidant supplementation. Studies can be carried out on *S. mutans* at genomic level and drugs or gene therapy which diminishes the adverse effects of the microbe in dental caries can be reduced. Dietary habits, smoking habits should be properly maintained to reduce the incidence of dental caries.

Key words: Dietary habits, dental caries, smoking

Introduction:

Dental caries is a complicated multifactorial disease characterized by demineralization of dental hard tissue (enamel, dentine, and cementum) in both deciduous and permanent teeth (1-4). Dental caries can be prevented and reversed with correct management (5). Four factors are required for the formation of dental caries. These factors include bacterial biofilm (plaque), fermentable carbohydrates, dental hard tissue, and time (6). Furthermore, personal and oral environmental factors have a significant impact on the onset and progression of the disease (2,7).

Oxidative stress is generated by an imbalance in the generation of free radicals and non-radical species, such as reactive oxygen species (ROS), as well as the activity of enzymatic and non-enzymatic antioxidant systems, both of which are effective defense mechanisms against oxidative damage (8-10). As a result, pro-oxidant species can damage cells and tissues by destroying lipids, proteins, enzymes, and DNA (11-13). Oxidative stress is primarily assessed by the relationship between antioxidants (total antioxidant capacity, reduced glutathione, oxidized glutathione, glutathione peroxidase activity, superoxide dismutase, and catalase) and pro-oxidants (reactive species and levels of nitrates and nitrites), as well as biomarkers of oxidative damage (lipid peroxidation and protein oxidation).

Tobacco use has placed a significant and growing cost on the public, resulting in higher death and morbidity rates. Tobacco kills 5 million people each year. According to current trends, the mortality rate is predicted to reach 10 million by 2030, with 70 percent of deaths occurring in low- and middle-income nations. Oral health is also negatively impacted. Oral difficulties include tooth and dental restoration discoloration, loss of smell and taste, disease development such as smoker's palate and melanosis, coated tongue, precancerous lesions and cancer, oral candidiasis, periodontitis, implant failure, and dental caries (14). Recent studies have shown that smokers had a higher prevalence of caries, contrary to earlier research that suggested smoking helped to reduce caries. Since caries is a multifactorial disease of lifestyle, socioeconomic, and sociodemographic gradients, tobacco usage acts as a confounding variable rather than a direct etiological factor (15,16).

Long-term smoking has been shown to reduce the activity of endogenous salivary enzymatic antioxidants such as SOD, CAT, and Px, as well as the efficiency of non-enzymatic endo- and exo-antioxidant systems: GSH, UA, and vitamin C (17). Long-term use of traditional cigarettes and e-cigarettes decreases the concentration of salivary components of specific and non-specific immunity, such as IgA, peroxidase, lactoferrin, and lysozyme (18).

Smoking has been proven to affect lipid and lipoprotein levels.A putative mechanism for how cigarette smoking affects lipid levels in serum has been proposed.6 Nicotine absorption triggers the release of catecholamines, cortisol, and growth hormones, which activate adenyl cyclase in fat tissue. This causes lipolysis of stored TG and the release of free fatty acids. This leads to increased hepatic TG and VLDL production (19)..

Early research suggests that smokers are extremely sensitive to periodontal disease and caries. Identifying sociodemographic characteristics, behaviours, and disease prevalence is also critical for implementing innovative techniques for managing periodontal diseases and dental caries in smoking patients. The present study aimed to study the **role of dietary habits, antioxidants and lipid profile in Dental caries of smokers.**

Methodology:

1.Ethical Clearance:

This study was approved by the ethical committee of A B.Shetty Memorial Institute of Dental College, Mangalore.

2. Study Population:

Dental caries patients with and without smoking habits were selected from the outpatients from the Department of Conservative Dentistry and Endodontics A B.Shetty Memorial Institute of Dental College, Mangalore. The control group consisted of volunteers with no caries and smoking habits.

The subjects were divided in to following four groups

Control: Nonsmokers without Caries-25

Case:

Smokers without Caries - 25

Smokers with Caries-25

Non smoker with Caries-25

5ml of the blood sample was collected from these subjects. A portion of the blood was mixed with EDTA (2mg/ml.) The other portion was used for the preparation of serum.

Erythrocytes were isolated from blood samples by adding blood in to tubes containing EDTA Venous blood samples were centrifuged at 1000 x g for 15 min at 4 °C and the plasma was

removed The packed erythrocyte pellet was washed three times with an equal volume of sterile 0.9% NaCl. pH = 74 Stock hemolysate was obtained by dilution of packed erythrocytes with distilled water and kept at 4-C

3.(a) Isolation of Streptococcus mutans:

The cariogenic bacterium *Streptococcus mutans* was isolated from the dental swab using blood agar medium Organisms were streaked on blood agar plates and were incubated at 37°C for 24 hours. A single colony was taken from the plate and was inoculated in to nutrient broth and kept in shaking condition overnight. The microorganism was sub-cultured on Nutrient agar slants for further studies. The colony isolated was subjected to various morphological and biochemical tests for characterization.

(b) Morphological Tests

Colony morphology: The colony developed on the blood agar plate was observed and the colony characteristics was noted down.

Gram Staining

Gram staining was carried out to differentiate whether the microorganism isolated was Gram-positive or Gram-negative**.** Smear was prepared on a clean glass slide, air dried and heat fixed The smear was covered with crystal violet and left for 1 minute The slide was then washed with water and Gram's iodine was added and allowed to stand for one minute decolourizer(alcohol) was added, counterstained with saffranin for 1-2 minutes and observed under oil immersion lens.

(c) Susceptibility test

The susceptibility of *Streptococcus mutans* to cause Dental caries was checked by inoculating Synders test agar media with the isolate. After inoculation, the media was incubated at 37°C for a week, and the result was observed.

(d) Biochemical Tests

i.Indole Test: Tryptophan or peptone broth is inoculated with the isolate culture and incubated for 24-48 hours. Following incubation, 5 drops of Kovac's reagent was added to the culture broth.

ii Methyl-Red Test : MR-VP broth was inoculated with test culture and incubated for 24 hours After incubation, 5 drops of methyl red reagent solution was added to the cultured MR- VP broth and mixed well. Colour was observed. Red colour signifies positive result and yellow colour signifies negative result.

iii. Voges-Proskauer Test: MR-VP broth was inoculated with test culture and incubated for 24 hours After incubation, 5 drops of Barrit's reagent was added to the cultured MR-VP broth and mixed well Colour was observed.

iv. Citrate Utilization Test: Simmons citrate agar slant was prepared The SC agar is inoculated with the test culture and incubated at 37°C for 24 hours.

v. Catalase Test: Hydrogen peroxide was placed at the centre of a clean glass slide and loopful of the test culture was mixed. Observed the culture for the formation of effervescence.

vi. Nitrate Reduction Test: Nitrate broth prepared was inoculated with the test organism The tubes inoculated were incubated at 37°C for 24 hours Following incubation, the ability to reduce nitrate to nitrite was determined by the addition of Nessler's reagent.

vii. Sugar Fermentation: Phenol red broth with sugars like glucose, and maltose was prepared and Durham's tubes were inserted, the broth was inoculated with the test culture and incubated at 37°C for 48 hours and observed for acid production

4.Estimation of Vitamin C:

Standardisation: The standardization protocol should be carried out as given below Plot the optical densities of the standard solutions on a graph against their respective concentrations.

Sl. No.	Vol. of std. Vit C (mL)	Conc. of std. Vit C (mL)	Vol. of 5% TCA (mL)	Vol. of DTC reg (mL)		Vol. of 4.5M H_2SO_4 (mL)	O.D at 512 nm
B	0.0	0.0	1.0	0.4 mL	**INCUBATE AT 60°C IN WATER BATH FOR 60 MINUTES**	2 mL	
1	0.2	0.4	0.8	0.4 mL		2 mL	
2	0.4	0.8	0.6	0.4 mL		2 mL	
3	0.6	1.2	0.4	0.4 mL		2 mL	
4	0.8	1.6	0.2	0.4 mL		2 mL	
5	1.0	2.0	0.0	0.4 mL		2 mL	

100 µL of the sample (serum/Saliva) was taken in a clean test tube. 900 µL of 5% TCA was added to it and allowed to precipitate proteins for about ten minutes and centrifuged. 500 µL of the supernatant was taken and transferred into another test tube. To this 200 µL of DTC reagent added, plug the tube and incubated the mixture at 60 o C for 60 min in a water bath. Simultaneously a blank with 1 mL of TCA and 200 µL of DTC reagent was also maintained under similar conditions. The reaction mixture was cooled following 60 minutes of incubation, in an ice bath. 1mL of 4.5M sulphuric acid added to it and cooled to room temperature. Optical density was measured at 540 nm against blank.

5.Estimation of Total Antioxidants:-

The assay is based on the principle of conversion of Molybdenum (Mo VI) by reducing agents like antioxidants to molybdenum (Mo V), which further reacts with phosphate under acidic pH resulting in the formation of a green coloured complex, the intensity of which can be read spectrophotometrically at 695nm. 100µL of the sample was pipetted into a clean test tube. 5% TCA was added to precipitate out the proteins in the sample. The mixture is allowed to stand for five minutes and centrifuged. Transfer 100µL of the clear supernatant into a clean test tube with 1mL of TAC reagent added to it and incubate the mixture in a water bath at 90 o C for 90 minutes. Simultaneously a blank is also maintained by substituting 100µL of water instead of sample in the reaction mixture. Cool and read the optical density of the greenish to bluish colour formed at 695nm against blank. The concentration of the total antioxidants in the serum obtained by plotting the absorbance of the test against the standard graph, and the concentration is expressed as µg/mL.

6.Estimation of MDA:

MDA has frequently been measured in serum thiobarbituric acid – reactive substances (TBAARS) assay ccording to Kei (1978). Here TBA reacts with MDA to form pink 2:1, maximally at 532 nm. This colored complex was measured by a spectrophotometer. The optical densities of the test samples is directly proportional to the concentration of MDA in the sample and calculated by the plotting against the standard graph and multiplied by the respective dilution factors the final concentration is expressed as µM/L.

7.Spectrophotometric analysis for superoxide dismutase:

The substrate used for the assay consists of nitro blue tetrazolium chloride (NBT) which reacts with superoxide anions produced upon illumination of riboflavin in the presence of methionine as an electron donor, to produce formazan which is a blue coloured complex. The SOD present in the sample will act on the superoxide anions produced by riboflavin and thereby reduce the net superoxide anions in the substrate leading to decreased production of formazan manifested by decreased intensity of the blue color formed. The decrease in the formation of formazan is directly proportional to the amount of SOD in the sample, 50% decrease in the formation of formazan is taken as one unit of SOD. Centrifuge 500µL of heparinised blood at 1800 rpm for 10 min. Separate the upper plasma layer, add 500 µL of normal saline to the erythrocyte layer, mix well and centrifuge, Discard the upper layer and add fresh normal saline to the erythrocytes, repeat this step two more times to wash the erythrocytes. Control for each sample analyzed has to be maintained. Common standard & blanks for each set of illumination is maintained. 100 µL of RBC lysate is diluted further by the addition of 400 µL of 0.05 M phosphate buffer to get a final erythrocyte dilution of 1:20.

i. Test: 0.3 mL Riboflavin, 2.5 mL Methionine, 0.1 mL NBT, 0.1 mL RBC lysate.

ii. Control: 2.5mL Methionine, 0.3 mL Riboflavin, 0.1mL 0.05 M phosphate buffer,

0.1mL RBC lysate.

iii. Standard: 2.5 mL Methionine, 0.3 mL Riboflavin, 0.1 mL NBT,

0.1 mL 0.05 M phosphate buffer

iv. Blank: 2.5 mL Methionine, 0.3 mL Riboflavin, 0.2 mL 0.05 M phosphate buffer

Following illumination, immediately read the optical density of all the reaction mixtures at 560nm. Calculate the units of enzyme present in the sample using the formula and expressed as U/mg Hb

SOD present/mg Hb = SOD activity/Hb/20 Dilution factor = 20

SOD activity*20 SOD activity/mg Hb = ——————Hb

= —————— U/mg Hb.

8.Estimation of Iron:

Procedure Standardization:

Working standard (Ammonium ferrous sulfate) prepared in aliquots of 0.1, 0.2, 0.3, 0.4, 0.5mL respectively in different test tubes. The volume of solution was made up to 1mL by adding deionized water.2 ml of the chromogen solution was added to all the tubes. Placed at room temperature for 5 minutes. The optical density of all the reaction mixtures was measured Spectro photometrically at 535 nm against the blank replacing the working standard with deionised water. The obtained optical densities was then plotted on a graph with the concentrations on the X-axis and the respective optical densities along the y-axis.

Estimation of iron in sample

100 µL of the sample (serum/saliva)was taken in clean microcentrifuge tube and makeup to 250µL with deionized water. Added 500 µL of protein precipitating solution. Centrifuged the mixture at 2000 rpm for 10 minutes. 500 µL of the supernatant was taken and added to 500 µL of the chromogen solution. The optical density immediately (within 10 minutes) measured at 535nm against a blank treated in a similar way as the test wherein the sample is replaced with the deionized water

Calculation

The concentration of iron in the sample is obtained by plotting the optical densities of the test against the standard graph. The obtained concentration is then multiplied by 2.5 (dilution factor).

9.Estimation of Triglycerides:

Lipid profiles were measured by the commercial kit method. Triglycerides were estimated by the GPO-PAP method. 3 test tubes were taken as Blank (B), Standard(S), and Test(T) and added reagent, standard and sample to the respective tubes. Mixed well and incubated for 10 minutes at 37℃ room temperature for 15 minutes. Absorbance read at 505-540nm by spectrophotometer.

10.Estimation of Cholesterol and HDL:

Cholesterol and HDL-C were estimated by CHOD-PAP method.

For HDL-C

The sample and reagent were added to a centrifuge tube, kept for 5 minutes at room temperature and then centrifuged for 10 min at 3000rpm. 3 test tubes were taken as Blank(B), Standard(S), Test(T) and added reagent standard and sample to the respective tubes. Mixed well and incubated for 10 minutes at 37℃ room temperature for 15 minutes. Absorbance can read at 505-540nm by spectrophotometer.

For Total Cholesterol:

3 test tubes were taken as Blank(B) Standard(S), and Test(T) and added reagent, standard and sample to the respective tubes. Mixed well and incubated for 10 minutes at 37℃ room temperature for 15 minutes Absorbance can read at 505-540nm by spectrophotometer.

11.Estimation of VLDL and LDL:

VLDL & LDL-C can calculated by following the formula.

VLDL TG / 5

LDL TC-[HDL+VLDL]

12.Statistical Analysis:

Statistical analyses were performed using the following software tools: Statistical Package for the Social Sciences (version 16.2 for Windows; SPSS, Inc., Chicago, Illinois, USA). Data expressed as mean± standard deviation. Multiple comparison were done by ANNOVA. For all statistical analyses, $p<0.05$ was considered statistically significant.

Results:

The present study aimed to study the role of dietary habits, antioxidants and lipid profile in Dental caries of smokers. The ethical committee of A B. Shetty Memorial Institute of Dental College, Mangalore approved this study. Dental caries patients with and without smoking habits were selected from the outpatients from the Department of Conservative Dentistry and Endodontics A B.Shetty Memorial Institute of Dental College, Mangalore. The control group consisted of volunteers with no caries and smoking habits.

The subjects were divided in to following four groups,

Control: Nonsmokers without Caries-25

Case: Smokers without Caries-25, Smokers with Caries-25, Non-smokers with Caries-25

5ml of the blood sample was collected from these subjects. A portion of the blood was mixed with EDTA (2mg/ml.) The other portion was used for the preparation of serum. Erythrocytes were isolated from blood samples by adding blood in to tubes containing EDTA Venous blood samples were centrifuged at 1000 x g for 15 min at 4 °C and the plasma was removed The packed erythrocyte pellet was washed three times with an equal volume of sterile 0.9% NaCl. pH = 74 Stock hemolysate was obtained by dilution of packed erythrocytes with distilled water and kept at 4°C. Isolation of the main etiological agent in dental caries *Streptococcus mutans* was carried out and biochemical characterization was conducted. Its susceptibility to cause dental caries was also checked. Status of Vitamin C. Total antioxidant, iron, MDA and Lipid profile in serum and Erythrocyte SOD activity in case were estimated and compared with the control.

In the present study we found small, round, cream coloured colonies with smooth marginsand glistering surface was obtained on blood agar plate. The microorganism isolated was found to be non motile, non spore and gave purple coloured colonies on gram staining.The microorganisms isolated gave positive results for indole, Methyl red, citrate utilization, nitrate reduction and sugar fermentation test and negative for voges Proskauer and catalase test. This confirms that the isolate is *S. mutans* (Table 1 and 2).

Table 1: Colony morphology of the isolates

Characteristics	**N-Agar**	**Blood Sugar**
Size	Small	Small
Shape	Round	Round
Colour	Cream	Cream
Margin	Smooth	Smooth
Elevation	Low convex surface	Low convex surface
Appearance	Normal	Glistering

Table 2: Biochemical Tests for the Isolates

SI No	**Biochemical Tests**	**Results**
1	Indole Test	Positive
2	Methyl red Tests	Positive
3	Voges-Proskauer Test	Negative
4	Citrate Utilization Test	Positive
5	Sugar Fermentation Test	Positive
6	Catalase Test	Negative
7	Nitrate reduction Test	Positive

Vitamin C, iron, Total antioxidant, SOD, HDL, level was found to be low in case group compared to that of the control, where as MDA, Total Cholesterol, LDL and Triacyglycerides were high in the case group compared to that of the control (Table 3).

Table 3: Biochemical Analysis of the blood

Parameters	Group I Control (Mean±SD)	Group II Smokers without Caries (Mean±SD)	Group III Smokers with Caries (Mean±SD)	Group IV Non-smokers with Caries (Mean±SD)
Vitamin-C (mg/dl)	1.21±0.23	0.61±0.12	0.23±0.05	0.36±0.18
Iron (ug/dl)	140.12±12.37	55.15±5.02	26.28±5.21	69.36±10.68
MDA (µM/L)	2.6±0.65	5.71±0.58	6.96±0.83	3.88±0.92
Total Antioxidant (ug/dl)	157.77±47.32	77.19 ±13.61	90.04±11.18	86.00 ±21.60
SOD (µg/Hb)	1249.2±292.62	824.85±84.184	544.35±66.18	948.44±192.72
Total Cholesterol (mg/dl)	167.46±7.26	181.36±6.74	187.58±4.07	170.24 ± 9.39
HDL (mg/dl)	58.92±2.59	50.20±4.62	50.20±4.62	47.44±4.62
LDL(mg/dl)	90.86±1.70	105.39±17.79	110.98±8.84	103.25±8.63
Triacylglycerides (mg/dl)	102.35±8.53	135.38 ±40.13	160.72±36.81	126.04±43.76

ANOVA was performed for multiple comparison which showed statistical significance between the groups (Table 4).

Table 4: ANOVA for multiple comparison between the groups

Parameters	F	P value
MDA (µM/L)	**159.656**	**0.000*****
Iron (ug/dl)	**749.799**	**0.000*****
Vitamin-C (mg/dl)	**183.542**	**0.000*****
SOD (µg/Hb)	**65.951**	**0.000*****
Triacylglycerides (mg/dl)	**46.595**	**0.000*****
Total Cholesterol (mg/dl)	**43.392**	**0.000*****
HDL	**36.469**	**0.000*****

LDL	**15.554**	**0.000*****

In the present study correlation between the dental caries, dietary habits and smoking habits was made. The results showed a higher incidence of dental caries among vegetarian smokers than nonvegetarian smokers. The heme iron from the meat is better absorbed by the body than non heme iron which is found in dairy products vegetables and fruits (Figure 1 and Figure 2).

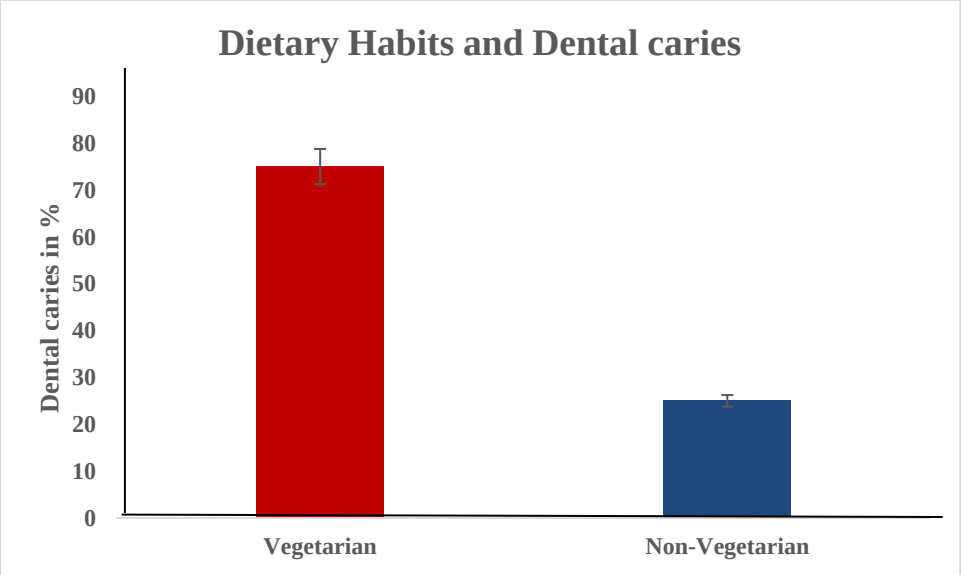

Figure 1: Showing the dietary habits and dental caries

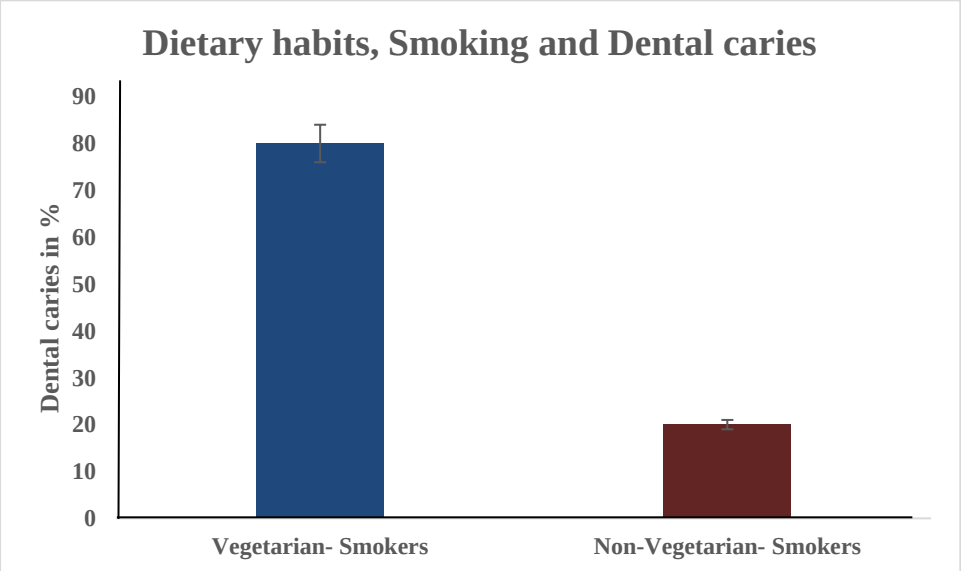

Figure 1: Showing the dietary habits, smoking and dental caries

Discussion:

Dental caries is a complicated multifactorial disease characterized by demineralization of dental hard tissue (enamel, dentine, and cementum) in both deciduous and permanent teeth. Dental caries can be prevented and reversed with correct management. Identifying sociodemographic characteristics, behaviours, and disease prevalence is also critical for implementing innovative techniques for managing periodontal diseases and dental caries in smoking patients. The present study aimed to study the role of dietary habits, antioxidants and lipid profile in Dental caries of smokers.Dental caries patients with and without smoking habits were selected for the study. The control group consisted of volunteers with no caries and smoking habits.The subjects were divided in to following four groups,

Control: Nonsmokers without Caries-25

Case: Smokers without Caries-25, Smokers with Caries-25, Non-smokers with Caries-25

5ml of the blood sample was collected from these subjects. A portion of the blood was mixed with EDTA (2mg/ml.) The other portion was used for the preparation of serum. Erythrocytes were isolated from blood samples by adding blood in to tubes containing EDTA Venous blood samples were centrifuged at 1000 x g for 15 min at 4 °C and the plasma was removed The packed erythrocyte pellet was washed three times with an equal volume of sterile 0.9% NaCl. pH = 74 Stock hemolysate was obtained by dilution of packed erythrocytes with distilled water and kept at 4°C. Isolation of the main etiological agent in dental caries *Streptococcus mutans* was carried out and biochemical characterization was conducted. Its susceptibility to cause dental caries was also checked. Status of Vitamin C. Total antioxidant, iron, MDA and Lipid profile in serum and Erythrocyte SOD activity in case were estimated and compared with the control.

In the present study, we found small, round, cream-coloured colonies with smooth margins and a glistering surface was obtained on a blood agar plate. The microorganism isolated was found to be nonmotile, non spore and gave purple-coloured colonies on gram staining.The microorganisms isolated gave positive results for indole, Methyl red, citrate utilization, nitrate reduction and sugar fermentation test and negative for Voges Proskauer and catalase test. This confirms that the isolate is *S. mutans.*

Vitamin C, iron, Total antioxidant, SOD, and HDL, level was found to be low in case group compared to that of the control, whereas MDA, Total Cholesterol, LDL and Triacyglycerides were high in the case group compared to that of the control. Tobacco chewers reported reduced salivary total antioxidant capacity and a higher caries risk than controls with and without caries. This evidence emphasizes the importance of tobacco chewing in the development of dental caries (20). Current smokers have more periodontal damage than former and nonsmokers with chronic periodontitis. MDA can be used as a valid biomarker for oxidative stress because it corresponds directly with oral polymorphonuclear leukocyte levels (21). Cigarette smoking can alter the critical enzymes of lipid transport, lowering lecithin: cholesterol acyltransferase

(LCAT) activity and altering cholesterol ester transfer protein (CETP) and hepatic lipase activity, which attributes to its impact on HDL metabolism and HDL subfractions distribution. In addition, HDL is susceptible to oxidative modifications by cigarette smoking, which makes HDL become dysfunctional and lose its atheroprotective properties in smokers (22,23). study on the relationship between the lipid profile and chronic smoking, Neki NS [24] discovered that smokers had very low levels of HDL and increased levels of TC, LDL, VLDC, and TG. Dim John Kennedy [25] found elevated levels of total cholesterol, triglycerides, and LDL-C. ANOVA was performed for multiple comparisons which showed statistical significance between the groups.

In the present study correlation between the dental caries, dietary habits and smoking habits was made. The results showed a higher incidence of dental caries among vegetarian smokers than nonvegetarian smokers. The heme iron from the meat is better absorbed by the body than non heme iron which is found in dairy products vegetables and fruits. There is evidence that those who eat little or non-meat or poultry and drink large amounts of mile juice and milk are risk of iron depletion (26, 27).

Conclusion

S. mutans converts carbohydrates into lactic acid, this acid causes demineralization of tooth enamel. The positive result obtained in synders agar test carried out in the present study confirms this. This study concludes that lactic acid formation by the microorganism based on the diet we consume cause dental caries. In the present study the levels of vitamin C, Iron, TA, SOD, HDL were found o be low in the case group when compared to the control. Within the case group when comparison was done the lowest level of the above-mentioned parameters was found in SC group Levels of TC, TG, LDL, and MDA was high in the case group when compared to control group. Within case group when comparison was done the highest level of the above mentioned parameters was found in SC group. The major difference found in SC group concludes the increase in severity of the disease with smoking

Dental caries infection causes an increase of free radicals in body moreover in smokers the ROS produced is very high. Low levels of antioxidants and micronutrients in all the case groups indicates the inefficient neutralization of free radicals. This inefficient neutralization results in other harmful effects like increased lipid peroxidation rate and lipid profile level alteration. Antioxidant depletion can be treated and its level in the body can be normalized by using adjuvant antioxidant supplementation. Studies can be carried out on *S. mutans* at genomic level and drugs or gene therapy which diminishes the adverse effects of the microbe in dental caries can be reduced. Dietary habits, smoking habits should be properly maintained to reduce the incidence of dental caries.

Acknowledgements:

Gratefully acknowledging the support from NITTE – Deemed to be University.

Conflict of Interest:

No potential conflicts of interests relevant to this work were reported

References:

1.Duncan HF, Galler KM, Tomson PL, Simon S, El-Karim I, Kundzina R, Krastl G, Dammaschke T, Fransson H, Markvart M. European Society of Endodontology position statement: Management of deep caries and the exposed pulp. International Endodontic Journal. 2019 Jul;52(7):923-34.

2. Barrett B, O'Sullivan M. Management of the deep carious lesion: a literature review. Journal of the Irish Dental Association. 2021 Feb 1;67(1, February-March).

3. Deery C. Caries detection and diagnosis, sealants and management of the possibly carious fissure. *British Dental Journal* . 2013;214(11):551–557.

4. Schwendicke F, Walsh T, Lamont T, Al-Yaseen W, Bjørndal L, Clarkson JE, Fontana M, Rossi JG, Göstemeyer G, Levey C, Müller A. Interventions for treating cavitated or dentine carious lesions. Cochrane Database of Systematic Reviews. 2021(7).

5. Urquhart O, Tampi MP, Pilcher L, Slayton RL, Araujo MW, Fontana M, Guzmán-Armstrong S, Nascimento MM, Nový BB, Tinanoff NJ, Weyant RJ. Nonrestorative treatments for caries: systematic review and network meta-analysis. Journal of dental research. 2019 Jan;98(1):14-26.

6. Frencken JE, Peters MC, Manton DJ, Leal SC, Gordan VV, Eden E. Minimal intervention dentistry for managing dental caries–a review: report of a FDI task group. International dental journal. 2012 Oct 1;62(5):223-43.

7. Urquhart O, Tampi MP, Pilcher L, Slayton RL, Araujo MW, Fontana M, Guzmán-Armstrong S, Nascimento MM, Nový BB, Tinanoff NJ, Weyant RJ. Nonrestorative treatments for caries: systematic review and network meta-analysis. Journal of dental research. 2019 Jan;98(1):14-26.

8. Pyati SA, Naveen Kumar R, Kumar V, Praveen Kumar NH, Parveen Reddy KM. Salivary flow rate, pH, buffering capacity, total protein, oxidative stress and antioxidant capacity in children with and without dental caries. Journal of Clinical Pediatric Dentistry. 2018 Jan 1;42(6):445-9.

9. Kirschvink N, de Moffarts B, Lekeux P. The oxidant/antioxidant equilibrium in horses. The Veterinary Journal. 2008 Aug 1;177(2):178-91.

10. Túnez I, Feijóo M, Huerta G, Montilla P, Munoz E, Ruiz A, Collantes E. The effect of infliximab on oxidative stress in chronic inflammatory joint disease. Current medical research and opinion. 2007 Jun 1;23(6):1259-67.

11. Sheikhi M, Bouhafs RK, Hammarström KJ, Jarstrand C. Lipid peroxidation caused by oxygen radicals from Fusobacterium-stimulated neutrophils as a possible model for the emergence of periodontitis. Oral diseases. 2001 Jan;7(1):41-6.

12. Baltacıoğlu E, Akalın FA, Alver A, Balaban F, Ünsal M, Karabulut E. Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in post-menopausal women with chronic periodontitis. Journal of clinical periodontology. 2006 Jun;33(6):385-92.

13. Canakci V, Yildirim A, Canakci CF, Eltas A, Cicek Y, Canakci H. Total antioxidant capacity and antioxidant enzymes in serum, saliva, and gingival crevicular fluid of preeclamptic women with and without periodontal disease. Journal of periodontology. 2007 Aug;78(8):1602-11.

14. Since caries is a multifactorial disease of lifestyle, socioeconomic, and sociodemographic gradients, tobacco usage acts as a confounding variable rather than a direct etiological factor

15. Since caries is a multifactorial disease of lifestyle, socioeconomic, and sociodemographic gradients, tobacco usage acts as a confounding variable rather than a direct etiological factor

16. Mittal N, Singh N, Kumar PN. Prevalence of dental caries among smoking and smokeless tobacco users attending dental hospital in Eastern region of Uttar Pradesh. Indian Journal of Community Medicine. 2020 Apr 1;45(2):209-14.

17. Waszkiewicz N, Zalewska A, Szajda SD, et al. The effect of chronic alcohol intoxication and smoking on the activity of oral peroxidase. Folia Histochem Cytobiol. 2012;50(3):450–55.

18. Cichońska D, Kusiak A, Kochańska B, et al. Influence of electronic cigarettes on selected antibacterial properties of saliva. Int J Environ Res Public Health. 2019;16(22):4433.

19. Jain RB, Ducatman A. Associations between smoking and lipid/lipoprotein concentrations among US adults aged≥ 20 years. Journal of circulating biomarkers. 2018 May 30;7:1849454418779310.

20. Halkai KR, Halkai R. Prevalence of dental caries among coronavirus disease 2019-recovered patients and correlation with salivary total antioxidant capacity in Kalaburagi region of Indian subpopulation. Journal of Conservative Dentistry and Endodontics. 2024 Apr 1;27(4):414-8.

21. Padhye NM, Padhye AM, Gupta HS. Quantification and comparison of the impact of the smoking status on oral polymorphonuclear leukocyte and malondialdehyde levels in individuals with chronic periodontitis: A double-blinded longitudinal interventional study. Contemporary Clinical Dentistry. 2019 Jul 1;10(3):517-24.

22. He BM, Zhao SP, Peng ZY. Effects of cigarette smoking on HDL quantity and function: implications for atherosclerosis. Journal of cellular biochemistry. 2013 Nov;114(11):2431-6.

23. Batic-mujanovic O, Pranjic N. influence of smoking on serum lipid and lipoprotein levels among familial medicine patients. M ed Arch. 2008;62:264–67

24. Neki NS. lipid profile in chronic smokers. JIACM. 2002;3:51–54.

25. Nnno dimjohnkennedy. effect of smoking on lipid profile among adult smoklers in owerri,Nigeria. j med ab sci. 2010;2(1):18.

26. Moustarah F, Daley SF. Dietary iron. InStatPearls [Internet] 2022 Oct 22. StatPearls Publishing.

27. Lynch SR, Hurrell RF, Dassenko SA, Cook JD. The effect of dietary proteins on iron bioavailability in man. Mineral absorption in the monogastric GI tract. 1989 Jan 1:117-32.