CARIES RISK ASSESSMENT AND DETECTION OF STREPTOCOCCUS MUTANS COUNT IN PLAQUE AND SALIVA USING MUTANS-SANGUIS AGAR

Abstract:

Caries diagnosis is considered as a three-step process including identification of the lesion–caries detection, assessment of lesion severity and assessment of lesion activity. Many factors such as bacteria, carbohydrate diet, and host response cause initiation of dental caries and its progression.

S.mutans are potential human odontopathogens and colonize the tooth after the eruption. However, if the colonization is delayed by other bacterias, there is the possibility that the decay will not occur or its occurrence will be greatly reduced. Assessment of the caries risk of individual patients is a critical component in determining an appropriate management strategy.

A total of 160 samples were taken from the outpatient department of Surendera Dental College and Research Institute. We have used the ADA caries risk assessment form among our study samples to ascertain their caries risk and compared it with their Streptococcus mutans levels in saliva and plaque using mutans-sanguis agar.

The findings of the present study indicated the Streptococcus mutans counts among high risk and moderate risk group were statistically insignificant when compared to low risk and control group even though the mean value showed an increase. However, there was no statistical significance when the low-risk group were compared to the control group.

We observed that the CFU yield was higher in unstimulated saliva than the plaque samples in contrast to reported literature.

Moreover, Dental caries risk assessment should become a routine component in dental practice. Estimation of the caries risk will help to establish the periodicity and intensity of caries management protocol.

Our data suggest that the MS count in oral microflora are influenced by age and various other factors such as diet, time and host response. As dental caries is multifactorial disease further clinical studies are needed to identify the actual pathogenesis.

Keywords – Caries risk status, S Mutans Count, Plaque, Saliva, Low risk, Moderate risk, High risk

INTRODUCTION:

Dental caries is the most prevalent chronic disease throughout the world. Worldwide, approximately 3.6 billion people (48% of the population) have dental caries in their permanent teeth as of 2016. The World Health Organization estimates that nearly all adults have dental caries at some point in time.[1]Dental caries are caused by decalcification of the inorganic portion and destruction of the organic matrix of the teeth in the presence of three major factors, i.e. host, fermentable carbohydrates, and

acid-producing bacteria.[2]Therefore, efforts to prevent dental caries have often focused on methodsto control the activity of oral bacteria.[3]

Bacteria in dental plaque produce acidsthat degrade the tooth tissues and the local reductionof pH leads to the selection of an aciduric microbiota,which contributes further to lesion development. The most common bacteria associated with dental cavities are the mutans streptococci, most prominently Streptococcus mutans and Streptococcus sobrinus, and lactobacilli. However, cariogenic bacteria (the ones that can cause the disease) are present in dental plaque, but they are usually in too low concentrations to cause problems unless there is a shift in the balance.[4]

The study of microorganisms of the genusstreptococci is of great clinical interest due to their pathogenic potential. They cause a wide variety of diseaseswhich include dental caries and also serious systemic diseases like bacterial endocarditis, rheumatic fever,puerperal fever and various pyogenic infections.[5] The warm and moist condition in the oral cavity,combined with its variety of sites suited for prospectivebacterial colonization offers oral streptococci, an optimalenvironment for their growth.[6]The composition of oralmicroflora at different surfaces within the mouth is basedon physical and biological properties like the presence ofreceptors for microbial adhesion, the redox potential of the site and provision of essential nutrients.[7]

Microbes that were formerly associated only with oraldiseases are increasingly pathogenic ingeneral. Almost 50% of the oral microflora is constitutedby oral streptococci. Bacteremia may occur after dentaltreatment, but also after vigorous tooth brushing especiallyin patients with periodontitis. Thus, for many microorganisms,oral cavity acts as an important pathway into the human body.[8]

Taking into account, the important role of mutansstreptococci in the etiopathogenesis of dental caries, theirquantification and identification is relevant forepidemiological and early intervention studies.[9,10,11]

Detectionand identification of mutans streptococci have beenperformed by different methods, namely microbial culturetechniques, biochemical identification, bacitracin typing and molecular techniques. The media that can be used to grow Streptococcus mutans bacteria are mitis-salivarius (MS) agar, MC agar, mitis-sucrose-bacitracin (MSB), BCY agar, and MM10 sucrose agar, mutanssanguis agar. However, Mutans-sanguis agar showed the maximum results for streptococcus mutans.[12,13,14]

Cariogenic microorganisms are defined by their ability to colonize teeth causing a marked reduction in pH in the presence of sugar substrate and consequently induce caries. Rogers in a south Australian study isolated 82 streptococcal strains from the mouth of individuals aged 13-25 years with active caries and classified them into five biotypes using twenty biochemical tests. Two of these biotypes were related to Streptococcus sanguis and Streptococcus mutans.[5]

The fluctuation in the frequency of MS (Mutans streptococci) may occur due to the technical variations. Amoroso et al reported that the bacterial Counts of MS as CFU/ml increased in number from 3-8 years of age whereas, in the 9-14 years of age, it remained constant. Salivary analysis of MS could be performed by standard technique and tongue depressor technique.[5]

Currently, management of caries and its prevention is based on altering the complex dental biofilm, modify the oral factors and diet to favour oral health.

Burt (2005) said that Risk is a probability that an event will occur. Young (2010) had described that Caries risk assessment (CRA) is a prediction of future caries based on

the diagnosis of current disease by evaluation of risk and protective factors for making evidence-based clinical decisions.

There are many CRA tools but the same is not validated in the Indian population. Hence, we planned to perform CRA among different age groups and compare it with the MS count in saliva and plaque by culture on Mutans-Sanguis agar.

MATERIALS AND METHODS:

Source of data:

Samples were collected from outpatients of dental clinics in Sriganganagar, Rajasthan. A total of 80 subjects were used to collect 80 samples of saliva and 80 samples of plaque. Hence, the study was performed on 160 samples.

Inclusion Criteria:

1. ADA caries risk assessment form [Annexure 1] was followed.
2. Subjects who gave the signed consent to carry out the study.
3. Patients of age >6years were included.
4. Male to female ratio was random.

Exclusion criteria:

1. Physically or mentally handicapped children.
2. History of antibiotic therapy or fluoride treatment in the past 2- 4 weeks.
3. Children undergoing any kind of interceptive orthodontic treatment.
4. Patients with dentures.
5. Patients who give a history of chronic diseases.

6. Immunocompromised patients.

7. Current or former smokers (> 10 pack).

8. Patients with prosthodontic crowns

Armamentarium:

Stainless steel Mouth mirror; Probe; Explorer; Tweezer (DPI, India)

HiMediaMutans-Sanguis Agar

HiMedia Sterile loops for culture

HiMedia Sterile Petri plates – 90mm

Top-loading Autoclave (Stericlave, India)

Incubator (JSGW, India)

Stickers Label,

Pre-autoclaved Saliva collection bottles (RomsonsSpecican, India)

24 gauge sterile Needles (Dispovan, India)

Digital colony counter (Electronics India)

Method:

Using ADA caries risk assessment form [Annexure 1] as a standard. The patients will be grouped into four groups:

GROUP A – CONTROL/CARIES-FREE [n=20]

GROUP B - LOW CARIES RISK [n=20]

GROUP C - MODERATE CARIES RISK[n=20]

GROUP D- HIGH CARIES RISK [n=20]

AMERICAN DENTAL ASSOCIATION- CARIES RISK ASSESSMENT FORM

ADA American Dental Association®
America's leading advocate for oral health

Caries Risk Assessment Form (Age >6)

Patient Name:				
Birth Date:			**Date:**	
Age:			**Initials:**	

		Low Risk	Moderate Risk	High Risk
Contributing Conditions		**Check or Circle the conditions that apply**		
I.	**Fluoride Exposure** (through drinking water, supplements, professional applications, toothpaste)	☐ Yes	☐ No	
II.	**Sugary Foods or Drinks** (including juice, carbonated or non-carbonated soft drinks, energy drinks, medicinal syrups)	**Primarily at mealtimes** ☐		**Frequent or prolonged between meal exposures/day** ☐
III.	**Caries Experience of Mother, Caregiver and/or other Siblings** (for patients ages 6–14)	**No carious lesions in last 24 months** ☐	**Carious lesions in last 7–23 months** ☐	**Carious lesions in last 6 months** ☐
IV.	**Dental Home:** established patient of record, receiving regular dental care in a dental office	☐ Yes	☐ No	
General Health Conditions		**Check or Circle the conditions that apply**		
I.	**Special Health Care Needs** (developmental, physical, medical or mental disabilities that prevent or limit performance of adequate oral health care by themselves or caregivers)	☐ No	**Yes (over age 14)** ☐	**Yes (ages 6–14)** ☐
II.	**Chemo/Radiation Therapy**	☐ No		☐ Yes
III.	**Eating Disorders**	☐ No	☐ Yes	
IV.	**Medications that Reduce Salivary Flow**	☐ No	☐ Yes	
V.	**Drug/Alcohol Abuse**	☐ No	☐ Yes	
Clinical Conditions		**Check or Circle the conditions that apply**		
I.	**Cavitated or Non-Cavitated** (incipient) **Carious Lesions or Restorations** (visually or radiographically evident)	**No new carious lesions or restorations in last 36 months** ☐	**1 or 2 new carious lesions or restorations in last 36 months** ☐	**3 or more carious lesions or restorations in last 36 months** ☐
II.	**Teeth Missing Due to Caries in past 36 months**	☐ No		☐ Yes
III.	**Visible Plaque**	☐ No	☐ Yes	
IV.	**Unusual Tooth Morphology** that compromises oral hygiene	☐ No	☐ Yes	
V.	**Interproximal Restorations – 1 or more**	☐ No	☐ Yes	
VI.	**Exposed Root Surfaces Present**	☐ No	☐ Yes	
VII.	**Restorations with Overhangs and/or Open Margins: Open Contacts with Food Impaction**	☐ No	☐ Yes	
VIII.	**Dental/Orthodontic Appliances** (fixed or removable)	☐ No	☐ Yes	
IX.	**Severe Dry Mouth** (Xerostomia)	☐ No		☐ Yes

Overall assessment of dental caries risk: ☐ Low ☐ Moderate ☐ High

Patient Instructions:

Laboratory Procedure

PLAQUE SAMPLING

The plaque was collected using needles from an occlusal/interproximal site of premolars and molars. Each sample was labelled. Contamination was avoided.

SALIVA SAMPLING

1-2ml of Unstimulated Saliva was collected from patients. The bottles were labelled and stored to avoid contamination.The culture of S. *mutans*in saliva and plaque samples using Mutans-Sanguis (M-S) agar was done.

Preparation of M-S agar was done as follows:

- 98.1 grams of M-S agar powder was suspended in 1000ml of distilled water.
- It was mixed well and sterilized by autoclaving at 15 lbs. pressure at 121^{o}C for 15 minutes.
- It was cooled at room temperature to form a gel and poured into sterilized Petri dishes.
- Each petri dish was divided into 2 halves. A loop full of saliva sample was streaked on one half of the Petri dish. The needle with the plaque sample was streaked on the other half of the petri dish.
- The Petri dishes were incubated at 35-37^{o}C for 18-24 hours.
- *Streptococcus mutans*formed greyish-yellow colonies.
- The colonies were counted using the Digital colony counter.

The values were tabulated in Microsoft Excel sheet and submitted for statistical analysis using SPSS V 22.0. ANOVA and Independent Samples T-test were performed for statistical significance.

FIG 1: DIAGNOSTIC INSTRUMENTS [MOUTH MIRROR, PROBE AND TWEEZER]*

FIG 2: STERILE NEEDLES AND STERILE BOTTLE USED FOR COLLECTION OF PLAQUE AND SALIVA

FIG: 3 STERILE HI MEDIA PETRI PLATES USED FOR CULTURING STREPTOCOCCUS MUTANS

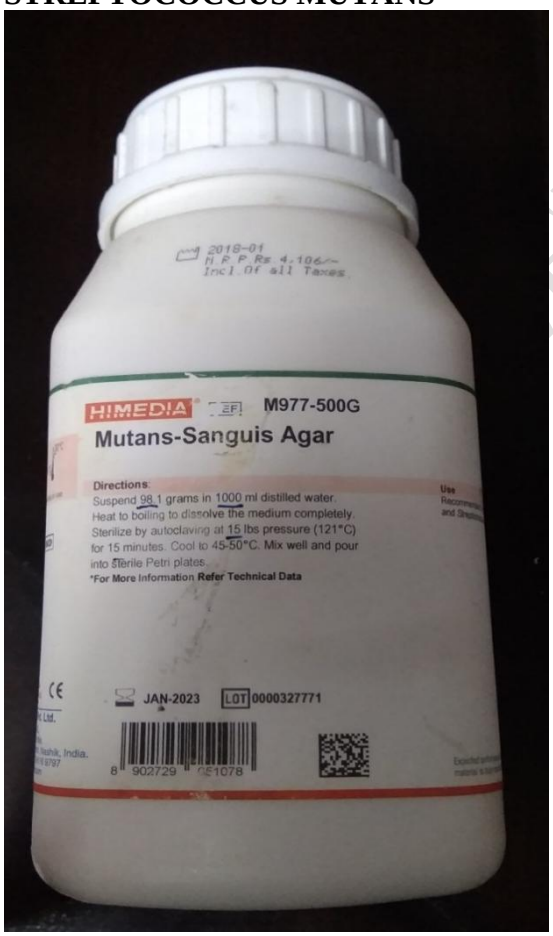


FIGURE 4: MUTANS SANGUIS AGAR FROM HI MEDIA LABORATORIES

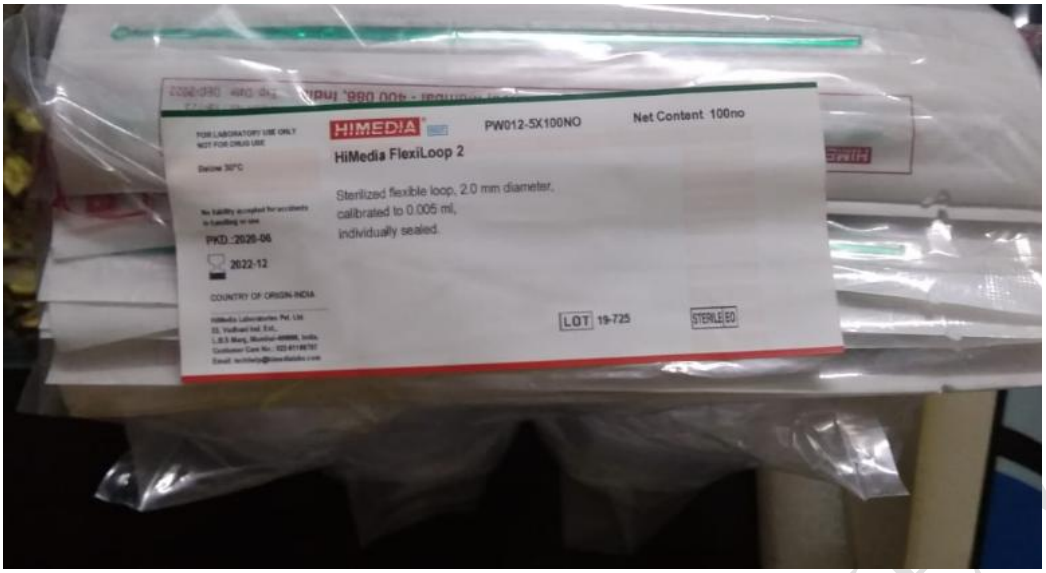


FIGURE 5: STERILE FLEXIBLE LOOPS FROM HI MEDIA LABORATORIES

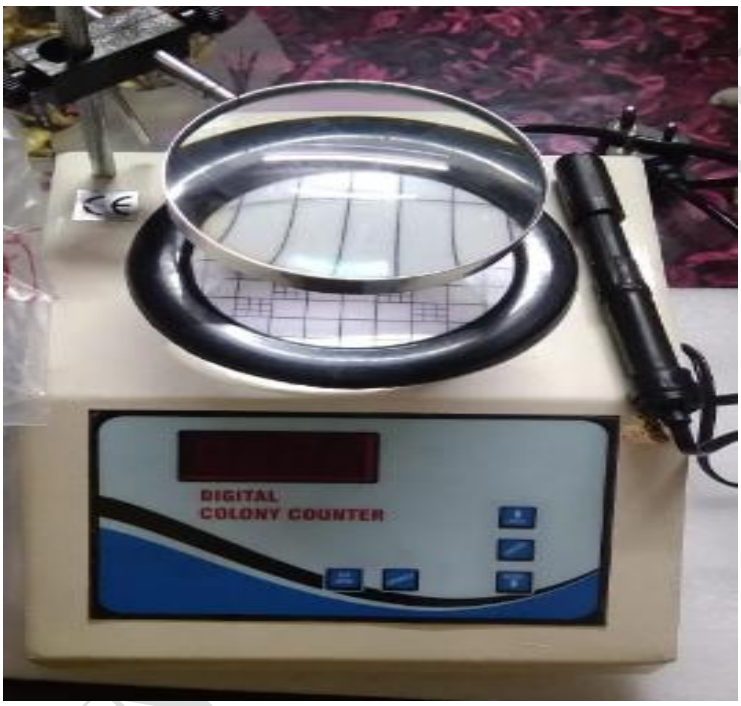


FIGURE 6: DIGITAL COLONY COUNTER

FIGURE 7: SHOWING CLINICAL INTRAORAL PHOTOGRAPHS OF CONTROL GROUP IN OUR STUDY

FIGURE 8: SHOWING PHOTOGRAPHS OF INTRAORAL PICTURES OF HIGH CARIES RISK INDIVIDUALS

FIGURE 9: SHOWING A CLINICAL PHOTOGRAPH OF MODERATE CARIES RISK INDIVIDUAL

FIGURE 10: SHOWING A CLINICAL LOW PHOTOGRAPH OF LOW CARIES RISK INDIVIDUAL

FIGURE 11: COLLECTION OF SALIVA FROM PATIENT IN STERILE CONTAINER

FIGURE 12: SHOWING COLLECTION OF PLAQUE FROM PATIENTS MOUTH WITH STERILE NEEDLE

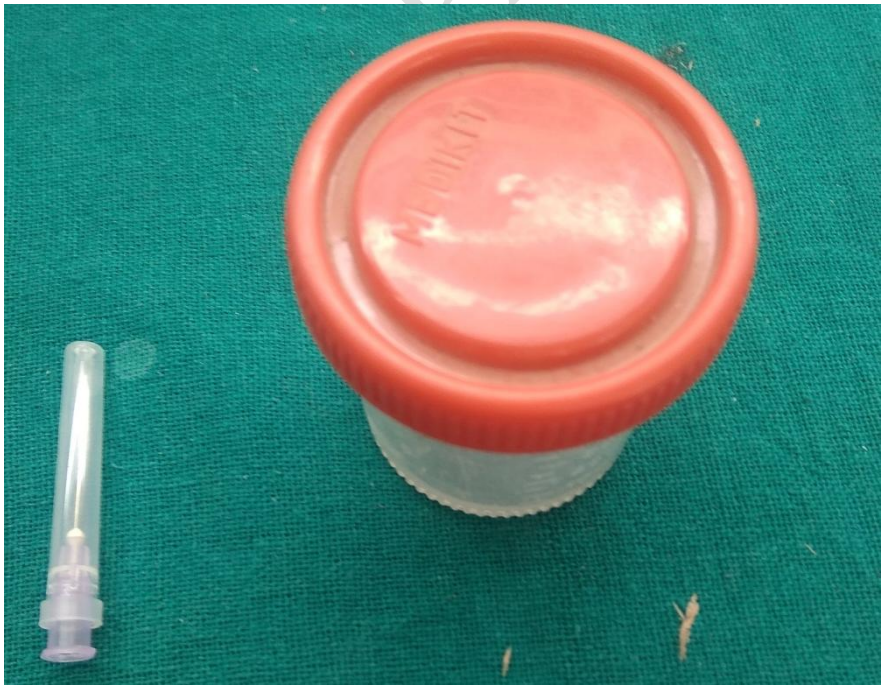


FIGURE 13: SHOWING COLLECTED PLAQUE AND SALIVA SAMPLES AND STORED PROPERLY

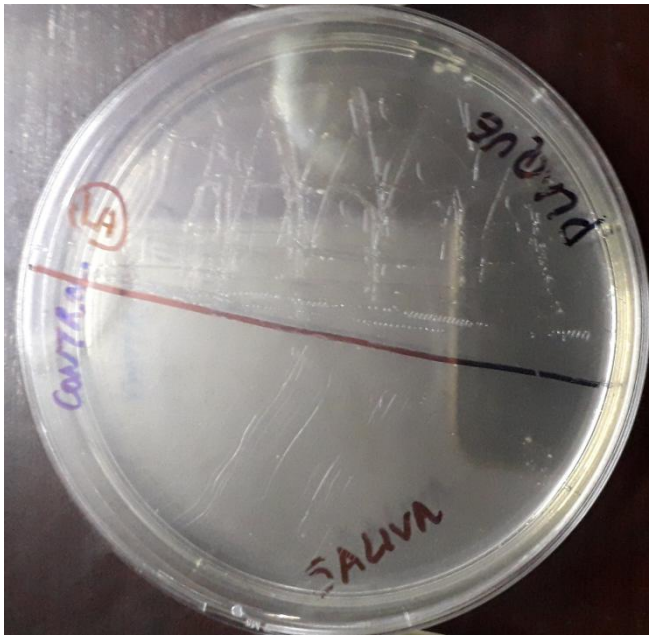


FIGURE 14: HI MEDIA PETRI PLATES SHOWING THE CFU IN CONTROL GROUP

FIGURE 15: HI MEDIA PETRI PLATES SHOWING CFU IN LOW CARIES RISK GROUP

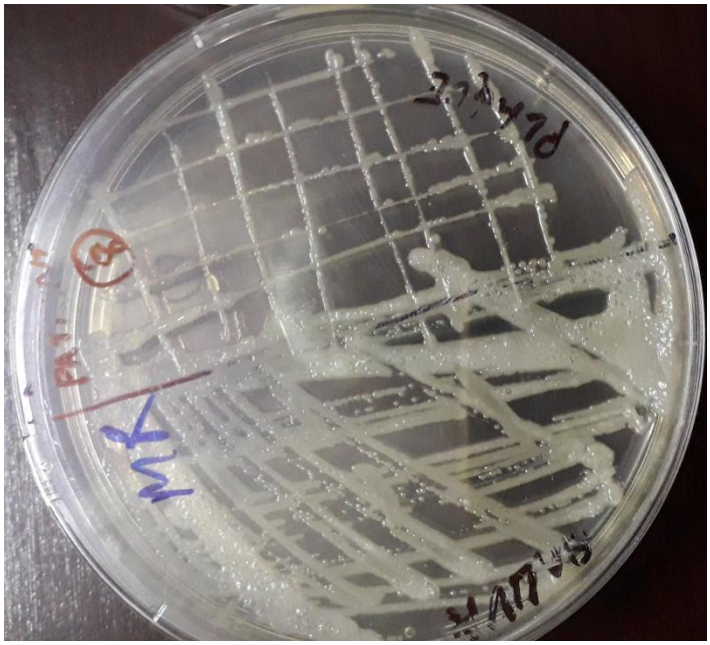


FIGURE 16: HI MEDIA PETRI PLATES SHOWING CFU IN MODERATE CARIES RISK INDIVIDUALS

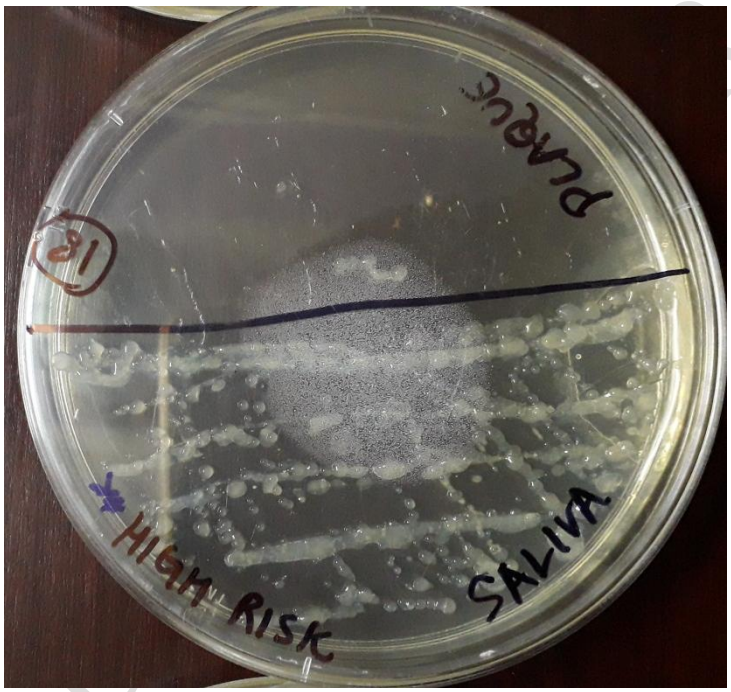


FIGURE 17: HI MEDIA PETRI PLATES SHOWING CFU IN HIGH CARIES RISK INDIVIDUALS

RESULTS:

The present study was conducted in the outpatients of dental clinics of Sriganganagar, Rajasthan.Written informed consent was obtained from the selected participants.

ADA caries risk assessment form was used to ascertain the caries risk of the individual participant. Subsequently, the plaque and saliva samples were collected from each patient. The bacterial culture was performed on Mutans-Sanguis agar. The colonies were counted after 18 hours of incubation at 37^{o}C. The S.mutans colonies were greyish-yellow in colour and those of S.sanguis were colourless.

The tabulated data were subjected to statistical analysis using ANOVA and t-test.

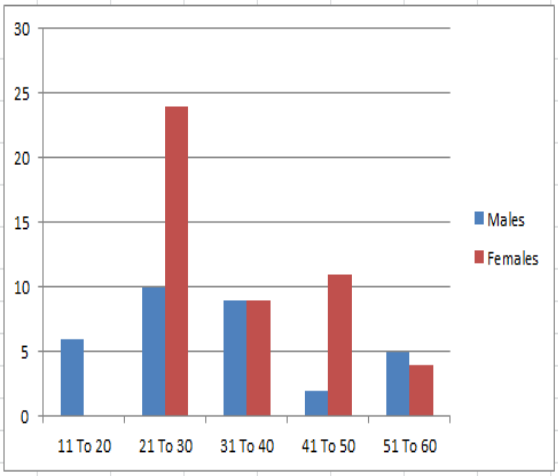
TABLE: 1 depicting the age and sex distribution

No. of cases	Males	Females
16-20 yrs	6	0
21-30 yrs	10	24
31-40 yrs	9	9
41-50 yrs	2	11
51-60 yrs	5	4
	32	48

Table 1 depicting the age and sex distribution of our 80 study samples represents the age distribution. All six samples in the age group of 16-20 years were males. we had 34 samples in 21-30 years' age group with 24 females and 10 males.18 samples in 31-40 years group with 9 females and 9 males.13 samples in 41-50 years group with 11 females and 2 males. We have 9 samples in 51-60 years group with 4 females and 5 males.11 to 60 years, with a mean age of 33.2 years. The maximum number of patients was in the age group of 21-30 years. It is represented in Graph 1.

Graph **1**

No. of cases	Males	Females
11 To 20	6	0
21 To 30	10	24
31 To 40	9	9
41 To 50	2	11
51 To 60	5	4



Graph-1 shows the sex distribution and age distribution among the study samples.

The bar diagram of males and females are shown in blue and red. the maximum no of cases [34] are in 21-30 years of age group, and the least are in 11-20 years of age group.

TABLE:2

Sex	**No. of cases**	**%**
Male	32	40
Female	48	60
Total	80	100

TABLE 2 shows the gender distribution among our study samples

Table 2 represents gender distribution among our study samples. We had 32 males and 48 females in our study. Amongst patients of both sexes, female preponderance was observed with the female to male ratio being 1.5:1. It is represented in Graph 2.

Graph 2

Graph 2 represents sex distribution among the study samples.

This graph represents the number of cases related to sex among various groups of our study. 60% of cases were females in our study and 40% of cases were males in our study. The number of females was predominantly found to be more than males in our study.

TABLE:3

Contributing Conditions	No of cases
Fluoride Exposure	20 Controls 20 Low risk
Sugary Foods or Drinks	20 Low risk 20 High risk
Caries experience of mother, caregiver, siblings	0
Patient dental records for receiving regular dental care	20 Controls 20 Low risk

Table 3 represents the contributing conditions of caries risk assessment form

Table 3 shows the contributing conditions and the number of cases among our study samples. We had 40 subjects [control group and low caries risk group each] related to fluoride exposure. 40 samples [20 low caries risk group and 20high caries risk group]

contributing to sugary foods and drinks. We had 40 samples [20 control group and 20 low caries risk] that was related to regular dental visits.

TABLE:4

General Health conditions		**Number of cases**
Special health care needs		0
Chemo/Radiation therapy		0
Eating disorders		0
Medications that reduce salivary flow		0
Drug/Alcohol Abuse		0
Clinical Conditions		**No of cases**
Cavitated or Non-Cavitated, carious lesions or restorations	10(1 finding) 10(2 findings) 05(3 findings) 08(4 findings) 07(5 & more findings)	20 – Moderate risk 20 – High risk
Teeth Missing due to caries in the past 36 months		20(High risk)
Visible Plaque		18(Moderate risk)
Unusual Tooth morphology		19(Moderate risk) 20 (High risk)
Interproximal Restorations – 1 or more		18(Moderate risk)
Exposed Root surfaces		19(Moderate risk)
Restorations with overhangs/open margins/ open contacts with food impaction		19(Moderate risk)

Dental/Orthodontic Appliances		15(Moderate risk)
Severe Dry Mouth (Xerostomia)		17(High risk)

Table no 4 showing the distribution of general health conditions and clinical conditions of caries risk assessment form

Table 4 represents the number of cases related to the different clinical conditions in our study groups. We had 20 moderate cases related to drug /alcohol abuse.in clinical condition related to cavitated or non cavitated category we had around 10 cases had 1 carious tooth, 10 cases had 2 carious teeth, 5 cases had 3 carious teeth, 8 cases had 4 carious teeth, 7 cases had 5 or more carious teeth. 20 cases in the high caries risk category had missing teeth. the visible plaque was observed clinically in 18 moderate caries risk group individuals. 19 moderate risk and 20 high-risk individuals had unusual tooth morphology that was related to improper hygiene. Interproximal restorations were present in 18 moderate caries risk group of our study. 19 moderate caries risk patients had exposed root and restorations .15 moderate caries risk patients had dental/orthodontic appliances. We had 17 high caries risk group individuals that were related to xerostomia.

TABLE: 5

	No. of cases
Contributing conditions	20 – Controls 20 – Low risk 20 – High risk
General Health Conditions	0
Clinical conditions	20 – Moderate risk 20 – High risk

Table: 5 represent the summary of findings in caries risk assessment form

Table 5 shows the number of cases in each group of conditions in the caries risk assessment form. Contributing conditions were noted in 20 controls, 20 low risk and 20 high-risk patients. No general health conditions were observed in our study. Clinical conditions were identified in 20 moderate risk and 20 high-risk patients.

Table 6 A

SALIVA [CFU]	**PLAQUE [CFU]**
146	44
118	22
142	54
96	24
2	30
136	48
23	6
98	36
30	28
56	41
49	14
89	15
52	2
71	59
84	25
162	62
92	36
118	15
78	8
116	18
87.9	29.35

TABLE: 6A showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the study group.

Table 6B

S.No.	Age	Gender	Group	SALIVA [CFU]	PLAQUE [CFU]
1	20	MALE	CONTROL	146	44
2	21	MALE	CONTROL	118	22
3	25	FEMALE	CONTROL	142	54
4	32	FEMALE	CONTROL	96	24
5	45	FEMALE	CONTROL	2	30
6	42	FEMALE	CONTROL	136	48
7	32	FEMALE	CONTROL	23	6
8	22	FEMALE	CONTROL	98	36
9	45	FEMALE	CONTROL	30	28
10	46	FEMALE	CONTROL	56	41
11	45	FEMALE	CONTROL	49	14
12	42	FEMALE	CONTROL	89	15
13	45	FEMALE	CONTROL	52	2
14	32	FEMALE	CONTROL	71	59
15	34	FEMALE	CONTROL	84	25
16	36	MALE	CONTROL	162	62
17	39	MALE	CONTROL	92	36
18	36	MALE	CONTROL	118	15
19	36	MALE	CONTROL	78	8
20	35	MALE	CONTROL	116	18
				87.9	29.35

TABLE: 6B showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the study group.

Table 7A

SALIVA [CFU]	**PLAQUE [CFU]**
162	26
66	15
96	36
64	63
96	46
46	35
48	25
45	42
126	65
36	46
90	41
112	36
125	28
86	25
210	42
114	12
169	65
46	36
114	18
102	16
97.65	35.9

Table 7A showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the low-risk group.

Table 7B

S.No.	Age	Gender			Group		SALIVA [CFU]	PLAQUE [CFU]
21	46	MALE			Low		162	26
22	52	MALE			Low		66	15
23	52	FEMALE			Low		96	36
24	54	MALE			Low		64	63
25	16	MALE			Low		96	46
26	18	MALE			Low		46	35
27	19	MALE			Low		48	25
28	21	MALE			Low		45	42
29	25	FEMALE			Low		126	65
30	21	FEMALE			Low		36	46
31	26	FEMALE			Low		90	41
32	27	FEMALE			Low		112	36
33	29	FEMALE			Low		125	28
34	26	FEMALE			Low		86	25
35	24	FEMALE			Low		210	42
36	34	FEMALE			Low		114	12
37	35	FEMALE			Low		169	65
38	25	FEMALE			Low		46	36
39	25	FEMALE			Low		114	18
40	24	FEMALE			Low		102	16
							97.65	35.9

Table 7B showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the low-risk group.

Table 8A

SALIVA [CFU]	PLAQUE [CFU]
210	12
122	16
96	69
86	26
96	46
76	46
114	36
125	43
165	14
125	26
115	72
65	36
122	86
210	45
56	12
86	8
89	45
45	26
112	46
125	36
112	37.3

Table 8A showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the moderate-risk group.

Table 8B

S.No.	Age	Gender			Group		SALIVA [CFU]	PLAQUE [CFU]
41	26	FEMALE			MODERATE		210	12
42	28	FEMALE			MODERATE		122	16
43	29	FEMALE			MODERATE		96	69
44	26	FEMALE			MODERATE		86	26
45	45	FEMALE			MODERATE		96	46
46	46	FEMALE			MODERATE		76	46
47	24	MALE			MODERATE		114	36
48	25	MALE			MODERATE		125	43
49	28	FEMALE			MODERATE		165	14
50	26	MALE			MODERATE		125	26
51	34	FEMALE			MODERATE		115	72
52	35	MALE			MODERATE		65	36
53	38	FEMALE			MODERATE		122	86
54	39	MALE			MODERATE		210	45
55	45	MALE			MODERATE		56	12
56	34	FEMALE			MODERATE		86	8
57	26	MALE			MODERATE		89	45
58	25	FEMALE			MODERATE		45	26
59	25	MALE			MODERATE		112	46
60	29	FEMALE			MODERATE		125	36
							112	37.3

Table 8B showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the moderate-risk group.

Table 9A

SALIVA [CFU]	PLAQUE [CFU]
96	21
176	80
86	2
192	96
206	18
62	58
186	46
112	12
42	31
89	46
78	40
65	12
35	28
136	22
112	36
110	41
114	76
81	15
34	56
164	21
108.8	37.85

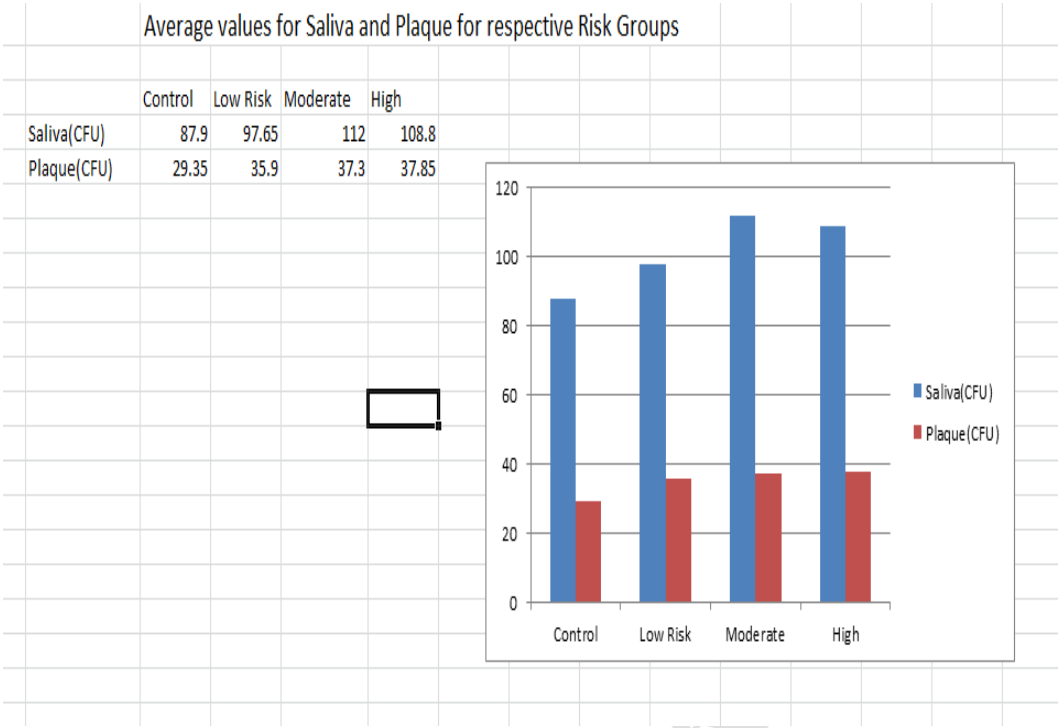
Table 9A showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the high-risk group.

S.No.	Age	Gender			Group		SALIVA [CFU]	PLAQUE [CFU]
61	27	MALE			HIGH		96	21
62	30	FEMALE			HIGH		176	80
63	15	MALE			HIGH		86	2
64	30	FEMALE			HIGH		192	96
65	20	MALE			HIGH		206	18
66	21	FEMALE			HIGH		62	58
67	25	MALE			HIGH		186	46
68	25	FEMALE			HIGH		112	12
69	36	MALE			HIGH		42	31
70	48	FEMALE			HIGH		89	46
71	54	MALE			HIGH		78	40
72	54	FEMALE			HIGH		65	12
73	52	MALE			HIGH		35	28
74	53	FEMALE			HIGH		136	22
75	26	MALE			HIGH		112	36
76	21	FEMALE			HIGH		110	41
77	52	MALE			HIGH		114	76
78	52	FEMALE			HIGH		81	15
79	32	MALE			HIGH		34	56
80	42	FEMALE			HIGH		164	21
							108.8	37.85

Table 9B showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the high-risk group.

Tables 6A and 6B reveal that we had 7 male and 13 female patients in the control group. The average mean value of CFU in saliva and plaque is 87.9 and 29.35 respectively. Tables 7A & 7B reveal that 7 males and 13 females were in the low caries risk group of our study. The average mean values of CFU in saliva and plaque are 97.65 and 35.9 respectively. Table 8A & 8B reveals the CFU in 20 moderate caries risk group individuals. We have 8 males and 12 female patients in the low caries risk group of our study. The average mean value of CFU saliva and plaque is 112 and 37.3 respectively. Table 9A & 9B reveals the CFU in 20 high caries risk group individuals. we have 10 males and 10 female patients in the high caries risk group of our study. The average mean value of CFU saliva and plaque is 108.8 and 37.85 respectively. The comparison is depicted in Graph 3.

Graph 3



Graph 3 representing the average CFU values in saliva and plaque among our study samples

Table 10

ANOVA

V2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7265.837	3	2421.946	1.107	.352
Within Groups	166279.550	76	2187.889		
Total	173545.387	79			

Table 10 shows the ANOVA comparison of salivary CFU between the study groups

In Table 10, V2 represents Saliva(CFU). F test on 4 groups namely Control, Low Risk, Moderate Risk and High Risk gives the p-value of 0.352 which is greater than 0.05. Hence, all four groups do not vary significantly in Saliva (CFU).

Table 11

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	922.100	3	307.367	.746	.528
Within Groups	31307.100	76	411.936		
Total	32229.200	79			

Table 11 represents the ANOVA comparison of plaque CFU among the study groups. In table 11, VAR00002 means Plaque(CFU), F test on 4 groups namely Control, Low Risk, Moderate Risk and High Risk gives the p-value of 0.528 which is greater than 0.05

Hence, all four groups do not vary significantly in the formation of Plaque (CFU).

t- TEST

COMPARISON BETWEEN ALL FOUR GROUPS

A] PLAQUE

Table 12 - COMPARISON BETWEEN CONTROL AND HIGH-RISK GROUPS

Group Statistics

	VAR00003	N	Mean	Std. Deviation	Std. Error Mean
VAR00002	1.00	20	29.3500	17.72383	3.96317
	4.00	20	37.8500	25.13181	5.61964

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
VAR00002	Equal variances assumed	1.792	.189	-1.236	38
	Equal variances not assumed			-1.236	34.152

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
VAR00002	Equal variances assumed	.224	-8.50000	6.87656	-22.42087
	Equal variances not assumed	.225	-8.50000	6.87656	-22.47257

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
VAR00002	Equal variances assumed	5.42087	
	Equal variances not assumed	5.47257	

The p-value is 0.189 which is greater than 0.05 which means that the null hypothesis must be rejected and Results are not significantly different for Control vs High-Risk Groups in the formation of Plaque(CFU).

Table 13 - COMPARISON BETWEEN CONTROL AND MODERATE RISK GROUPS.

Group Statistics

	VAR00003	N	Mean	Std. Deviation	Std. Error Mean
VAR00002	1.00	20	29.3500	17.72383	3.96317
	3.00	20	37.3000	21.12893	4.72457

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
VAR00002	Equal variances assumed	.209	.650	-1.289	38
	Equal variances not assumed			-1.289	36.884

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
VAR00002	Equal variances assumed	.205	-7.95000	6.16671	-20.43385
	Equal variances not assumed	.205	-7.95000	6.16671	-20.44626

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
VAR00002	Equal variances assumed	4.53385	
	Equal variances not assumed	4.54626	

The p-value is 0.650 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Control and Moderate Risk Groups in the formation of Plaque(CFU).

Table 14 - COMPARISON BETWEEN CONTROL AND LOW-RISK GROUPS.

Group Statistics

	VAR00003	N	Mean	Std. Deviation	Std. Error Mean
VAR00002	1.00	20	29.3500	17.72383	3.96317
	2.00	20	35.9000	15.98651	3.57469

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
VAR00002	Equal variances assumed	.556	.461	-1.227	38
	Equal variances not assumed			-1.227	37.603

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
VAR00002	Equal variances assumed	.227	-6.55000	5.33715	-17.35449
	Equal variances not assumed	.227	-6.55000	5.33715	-17.35824

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
VAR00002	Equal variances assumed	4.25449	
	Equal variances not assumed	4.25824	

The p-value is 0.461 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Control and Low-Risk Groups in the formation of Plaque(CFU).

Table 15 - COMPARISON BETWEEN LOW AND HIGH-RISK GROUPS.

Group Statistics

	VAR00003	N	Mean	Std. Deviation	Std. Error Mean
VAR00002	2.00	20	35.9000	15.98651	3.57469
	4.00	20	37.8500	25.13181	5.61964

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
VAR00002	Equal variances assumed	3.612	.065	-.293	38
	Equal variances not assumed			-.293	32.213

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
VAR00002	Equal variances assumed	.771	-1.95000	6.66024	-15.43295
	Equal variances not assumed	.772	-1.95000	6.66024	-15.51296

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
VAR00002	Equal variances assumed	11.53295	
	Equal variances not assumed	11.61296	

The p-value is 0.065 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Low Risk and High-Risk Groups in the formation of Plaque(CFU).

Table 16 - COMPARISON BETWEEN LOW AND MODERATE RISK GROUPS.

Group Statistics

	VAR00003	N	Mean	Std. Deviation	Std. Error Mean
VAR00002	2.00	20	35.9000	15.98651	3.57469
	3.00	20	37.3000	21.12893	4.72457

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
VAR00002	Equal variances assumed	1.159	.289	-.236	38
	Equal variances not assumed			-.236	35.384

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
VAR00002	Equal variances assumed	.814	-1.40000	5.92453	-13.39357
	Equal variances not assumed	.815	-1.40000	5.92453	-13.42276

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
VAR00002	Equal variances assumed	10.59357	
	Equal variances not assumed	10.62276	

The p-value is 0.289 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Low Risk and Moderate Risk Groups in the formation of Plaque(CFU).

Table 17 - COMPARISON BETWEEN MODERATE AND HIGH-RISK GROUPS.

Group Statistics

	VAR00003	N	Mean	Std. Deviation	Std. Error Mean
VAR00002	3.00	20	37.3000	21.12893	4.72457
	4.00	20	37.8500	25.13181	5.61964

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
VAR00002	Equal variances assumed	.672	.417	-.075	38
	Equal variances not assumed			-.075	36.911

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
VAR00002	Equal variances assumed	.941	-.55000	7.34180	-15.41269
	Equal variances not assumed	.941	-.55000	7.34180	-15.42710

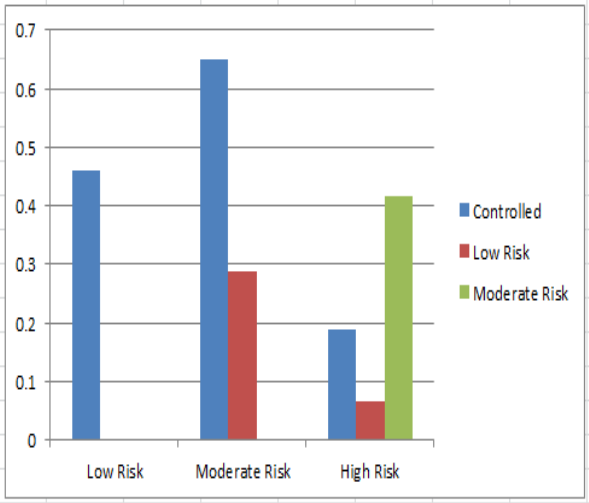
Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
VAR00002	Equal variances assumed	14.31269	
	Equal variances not assumed	14.32710	

The p-value is 0.417 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Moderate Risk and High-Risk Groups in the formation of Plaque(CFU).

Graph 4

	P-value		
Groups	Low Risk	Moderate Risk	High Risk
Controlled	0.461	0.65	0.189
Low Risk		0.289	0.065
Moderate Risk			0.417



Graph 4 reveals the comparison of p values of CFU in plaque in between different caries risk groups in our study using T-test.

B] SALIVA

Table 18 - COMPARISON BETWEEN CONTROL AND HIGH-RISK GROUPS.

Group Statistics

	V3	N	Mean	Std. Deviation	Std. Error Mean
V2	1	20	87.9000	43.56230	9.74083
	4	20	108.8000	52.86089	11.82005

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
V2	Equal variances assumed	.694	.410	-1.365	38
	Equal variances not assumed			-1.365	36.661

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
V2	Equal variances assumed	.180	-20.90000	15.31657	-51.90678
	Equal variances not assumed	.181	-20.90000	15.31657	-51.94400

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
V2	Equal variances assumed	10.10678	
	Equal variances not assumed	10.14400	

The p-value is 0.410 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Control and High-Risk Groups in the formation of Saliva (CFU).

Table 19 - COMPARISON BETWEEN CONTROL AND LOW-RISK GROUPS.

Group Statistics

	V3	N	Mean	Std. Deviation	Std. Error Mean
V2	1	20	87.9000	43.56230	9.74083
	2	20	97.6500	46.38089	10.37108

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
V2	Equal variances assumed	.011	.916	-.685	38
	Equal variances not assumed			-.685	37.852

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
V2	Equal variances assumed	.497	-9.75000	14.22825	-38.55358
	Equal variances not assumed	.497	-9.75000	14.22825	-38.55729

Independent Samples Test

		t-test for Equality of Means 95% Confidence Interval of the ... Upper
V2	Equal variances assumed	19.05358
	Equal variances not assumed	19.05729

The p-value is 0.916 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Controlled and Low-Risk Groups in the formation of Saliva (CFU).

Table 20 - COMPARISON BETWEEN CONTROL AND MODERATE RISK GROUPS.

Group Statistics

	V3	N	Mean	Std. Deviation	Std. Error Mean
V2	1	20	87.9000	43.56230	9.74083
	3	20	112.0000	43.68548	9.76837

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
V2	Equal variances assumed	.145	.706	-1.747	38
	Equal variances not assumed			-1.747	38.000

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
V2	Equal variances assumed	.089	-24.10000	13.79510	-52.02672
	Equal variances not assumed	.089	-24.10000	13.79510	-52.02672

Independent Samples Test

		t-test for Equality of Means 95% Confidence Interval of the ... Upper
V2	Equal variances assumed	3.82672
	Equal variances not assumed	3.82672

The p-value is 0.706 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Control and Moderate Risk Groups in the formation of Saliva (CFU).

Table 21 - COMPARISON BETWEEN LOW AND HIGH-RISK GROUPS.

Group Statistics

	V3	N	Mean	Std. Deviation	Std. Error Mean
V2	2	20	97.6500	46.38089	10.37108
	4	20	108.8000	52.86089	11.82005

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
V2	Equal variances assumed	.478	.494	-.709	38
	Equal variances not assumed			-.709	37.368

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
V2	Equal variances assumed	.483	-11.15000	15.72492	-42.98343
	Equal variances not assumed	.483	-11.15000	15.72492	-43.00112

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
V2	Equal variances assumed	20.68343	
	Equal variances not assumed	20.70112	

The p-value is 0.494 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Low Risk and High-Risk Groups in the formation of Saliva (CFU).

Table 22 - COMPARISON BETWEEN LOW AND MODERATE RISK GROUPS.

Group Statistics

	V3	N	Mean	Std. Deviation	Std. Error Mean
V2	2	20	97.6500	46.38089	10.37108
	3	20	112.0000	43.68548	9.76837

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
V2	Equal variances assumed	.209	.650	-1.007	38
	Equal variances not assumed			-1.007	37.865

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
V2	Equal variances assumed	.320	-14.35000	14.24712	-43.19178
	Equal variances not assumed	.320	-14.35000	14.24712	-43.19517

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
V2	Equal variances assumed	14.49178	
	Equal variances not assumed	14.49517	

The p-value is 0.650 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Low Risk and Moderate Risk Groups in the formation of Saliva (CFU).

Table 23 - COMPARISON BETWEEN MODERATE AND HIGH-RISK GROUPS.

Group Statistics

	V3	N	Mean	Std. Deviation	Std. Error Mean
V2	3	20	112.0000	43.68548	9.76837
	4	20	108.8000	52.86089	11.82005

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
V2	Equal variances assumed	1.262	.268	.209	38
	Equal variances not assumed			.209	36.698

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
V2	Equal variances assumed	.836	3.20000	15.33410	-27.84227
	Equal variances not assumed	.836	3.20000	15.33410	-27.87848

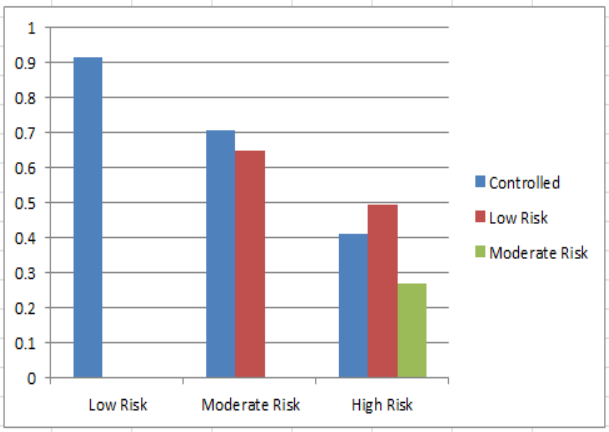
Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the ...	
		Upper	
V2	Equal variances assumed	34.24227	
	Equal variances not assumed	34.27848	

The p-value is 0.268 which is greater than 0.05 which means that the null hypothesis must be rejected. Results are not significantly different for Moderate Risk and High-Risk Groups in the formation of Saliva (CFU).

Graph 5

P-value			
Groups	Low Risk	Moderate Risk	High Risk
Controlled	0.916	0.706	0.41
Low Risk		0.65	0.494
Moderate Risk			0.268



Graph 5 reveals the comparison of p values of CFU in saliva among different caries risk groups in our study using T-test.

Table: 24

CONTROL GROUP	LOW CARIES RISK	MODERATE CARIES RISK	HIGH CARIES RISK
3.5×10^5 CFU/ml	3.9×10^5 CFU/ml	4.5×10^5 CFU/ml	4.4×10^5 CFU/ml

Table 24 shows the Average Mean value of CFU/ml.

DISCUSSION:

Featherstone JD et al has reported that Keyes triad of the primary factors responsible for dental caries (fermentable substrate, cariogenic bacteria, and a susceptible host) still holds, however, it is now well established that dental caries is a multifactorial, chronic infectious disease, with fluctuating cycles of demineralization and remineralization.[52] The carious process is driven by a diet high in fermentable carbohydrates, suboptimal oral hygiene and elevated numbers of virulent, cariogenic bacteria.[6-56]

Dental caries was identified as a silent epidemic two decades ago. Analysis of dental insurance claims in the US found a lifetime cost of single carious molar can reach upto 6105USD. All dental restorations have finite longevity and require repair or replacement over time. Hence, dental care providers should focus on disease prevention and strategize to address the aetiology of dental caries.[53]

A healthy mouth has a symbiotic relationship between cariogenic and non-cariogenic bacteria that make up the dental plaque. Disruption of this balance into an acidic environment produces an ecological shift forming a pathobiome – MS/Lactobacillus acidophilus. Continuous production of acids and frequent consumption of fermentable carbohydrates results in the dissolution of calcium/phosphate from enamel that can progress into dentine and cause physical breakdown/cavitation of teeth. [57] Hausen has defined caries risk as to the probability that an individual will develop a certain number of carious lesions (cavitated or non-cavitated) or reach a given level of disease progression, over a specific period, provided his or her exposure status remains the same during this period.[52]

AAPD had developed a clinical protocol for caries management based on peer-reviewed literature, expert panel opinion and clinical experience. A standard diagnostic, preventive and restorative recommendation could be given based on the risk status and patient compliance.[43]

The dynamic balance between pathological factors that favour demineralization like High levels of Mutans streptococci (MS), frequent sugar exposure and protective factors that favour remineralization like fluorides in the oral environment, adequate plaque control determines the development or progress or halt of the carious process. Other contributing factors include deep pits and fissures, salivary factors and socio-economic status.[52,54] Suneja et al[53] have listed various caries risk indicators. Pathological factors and protective factors include dietary factors, socio-economic factors, fluoride exposure, medical factors, salivary factors and clinical factors.

Pathological factors are as follows - Dietary factors include frequent between-meal snacking, prolonged night-time or at-will breastfeeding/bottle feeding of an infant, multiple sugar exposures through the day, infant ready availability of cariogenic snacks. Socio-economic factors include high caries risk in siblings/parents, children from deprived/immigrant backgrounds, high maternal MS levels, low dental aspirations. Fluoride factors include no exposure to fluoridated drinking water, no access to professionally applied topical fluorides especially when permanent molars erupt delaying post-eruptive maturation. Medical factors include special childlike compromise in the physical, medical or mental condition that may limit oral health care measures or increase caries susceptibility, salivary dysfunction caused by medications, radiation therapy or general systemic conditions, long term cariogenic medication. Salivary factors include high salivary MS and lactobacilli counts, poor salivary flow rate impeding clearance. Clinical factors include early colonization of

infant's teeth by MS, presence of dental appliances or restorations, deep retentive pits and fissures, new carious lesions or white spot lesions every 6 months.[52]

Protective factors are as follows– Dietary factors include sugar exposures limited to mealtimes, preference of non-cariogenic snacks, no deleterious bottle-feeding or breastfeeding of the infant. Socio-economic factors include good oral hygiene in parents with adequate knowledge about dental health and prevention, regular access to a well-established dental home. Fluoride factors include living in an area with community water fluoridation, presence of a continuous low concentration of free Fluoride ions around teeth especially at the time of the cariogenic acid attack and daily use of a fluoridated dentifrice. Medical factors include institution and maintenance of intensive preventive regimen for the special child, saliva substitutes and alternate sugar-free medication. Salivary factors include salivary buffers that help in acid neutralization, salivary proteins and lipids that protect the tooth surface, salivary calcium and phosphate ions that enhance remineralization and delay demineralization. Clinical factors include early sealant application in all susceptible pits and fissures, use of antibacterial compounds like xylitol, chlorhexidine, povidone-iodine, sodium bicarbonate in children with active carious lesions, measures to interfere with vertical transmission of cariogenic bacteria from mother to child.[52]

The balance among the pathologic factors, protective factors and caries disease indicators determines whether dental caries will progress, stabilize or reverse. In a clinical setting, the dentist can identify these factors with detailed medical and dental history. The clinical examination findings can determine the directional swing towards caries progression. This process of data collection is called Caries Risk Assessment and assigns the individual to a low, moderate or high risk, representing

the likelihood of a new caries development or lesion progression over a specific period in the individual patient.[53]

Zero et al concluded that no single indicator or combination of risk indicators can give a consistent prediction of caries risk across different populations and age groups. [55] The past caries experience can be a good indicator of future caries risk. Hence, we have used the ADA caries risk assessment form among our study samples to ascertain their caries risk and compare it with their MS levels in saliva and plaque.

The assessment of caries risk status of an individual is important for the preventive approach rather than restoration or extraction of affected teeth. It could be used for patient motivation and encourage them to actively cooperate in the shift from a high-caries-risk child to a low-caries-risk category in adulthood.[52]

Caries risk assessment can help the dentist in giving standard recommendations for caries prevention and treatment planning. The risk status can help in standardizing the frequency of recall visits, the need for radiographic assessment, fluoride application, guidance protocols etc.[52]

The prevalence of dental caries is declining in developed countries and increasing in developing nations. It has reached epidemic status in a few emerging economies too. This is referred to as Polarisation of caries. This rise could be attributed to lower-income, reduced awareness in oral hygiene practices, lack of dietary modifications and sugar reduction, lack of preventive programs and reluctance to oral hygiene procedures. [49]

Very few studies have highlighted the risk factors affecting dental caries. Ismail et al reported that different individual, social and community risk factors were associated with non-cavitated versus cavitated tooth surfaces. Harris et al concluded that the

prevalence and incidence of dental caries in a population was influenced by risk factors like age, sex, ethnic group, dietary patterns and oral hygiene habits.[49] Hence, The present study was conducted in the outpatient department of Surendera Dental College and Research Institute, Sriganganagar, Rajasthan, India. The study protocol was ethically approved and the written informed consent was obtained from the selected participants.

As per Keys concept in 1960, host factors like teeth and saliva, microflora and substrate – diet was responsible for dental caries. In 1982, Newburn added the new dimension of time. There are many inconsistencies among the research criteria to measure caries. WHO criteria did not differentiate between non-cavitated and cavitated lesions. ICDAS (International caries detection and assessment system) was developed in 2002 based on a systematic review of clinical caries detection systems which is now a benchmark for clinical and epidemiological research. [49] In our study, ADA caries risk assessment form was used to ascertain the caries risk of the individual participant.

Caries diagnosis is considered as a three-step process including identification of the lesion–caries detection, assessment of lesion severity and assessment of lesion activity.[58] A group of phenotypically similar but genetically different streptococcal species knows as Mutans streptococci (MS) are the main etiological agents for dental caries in humans. Caries susceptible individuals could be identified by correlating the numbers of MS and caries incidence. Mutans counts greater than 10^5 colony forming units/ml of saliva have been associated with greater caries risk. The limitation is based on the sample selection, bacterial dental plaque or saliva as the source, medium used for culture. MSB medium yields lower CFU/ml than Tryptic soy agar medium[15] Subsequently, the plaque and saliva samples were collected from each patient.

In humans, MS serotypes c, e and f are the most common etiological agents of dental caries. Matee et al have reported that low counts of highly cariogenic species can cause high caries incidence.[15] The bacterial culture was performed on Mutans-Sanguis agar. The colonies were counted after 18 hours of incubation at 37^{o}C. The S.mutans colonies were greyish-yellow in colour and those of S.sanguis were colourless.

The tabulated data were subjected to statistical analysis using ANOVA and t-test.

All six samples in the age group of 16-20 years were males. we had 34 samples in 21-30 years' age group with 24 females and 10 males.18 samples in the 31-40-year group with 9 females and 9 males.13 samples in the 41-50-year group with 11 females and 2 males. We have 9 samples in the 51-60-year group with 4 females and 5 males.11 to 60 years, with a mean age of 33.2 years. The maximum number of patients was in the age group of 21-30 years.

60% of cases were females in our study and 40% of cases were males in our study. The number of females was predominantly found to be more than males in our study.

We had 40 subjects [control group and low caries risk group each] related to fluoride exposure. 40 samples [20 low caries risk group and 20 high caries risk group] contributing to sugary foods and drinks. We had 40 samples [20 control group and 20 low caries risk] that was related to regular dental visits.

Considering the general health conditions like Special health care needs, Chemotherapy/radiation therapy, eating disorders and medications that reduce salivary flow, we did not have any case.

Ekstrom et al have reported that assessment of the depth of coronal caries, the activity of primary coronal caries lesions could be done with visual appearance, location of

the lesion and tactile sensation during probing. Plaque stagnation areas could be the occlusal surfaces of erupting teeth, groove-fossae of fully erupted teeth and other smooth tooth surfaces.[58] Considering the cavitated/non-cavitated, carious lesions or restorations, we had 10 cases with one finding, 10 cases with 2 findings – that fitted into the moderate risk category. We also had 5 cases with 3 findings, 8 cases with 4 findings and 7 cases with 5 or more findings that fitted into the high-caries-risk category.

All the 20 cases in the high risk category showed missing teeth due to caries in the past 36 months. The visible plaque was noted in 18 moderate-caries-risk patients. Unusual tooth morphology was noted in 19 moderate-caries-risk patients and 20 high-caries-risk patients. 1 or more interproximal restorations were noted in 18 moderate-caries-risk patients. Exposed root surfaces were evident in 19 moderate risk patients.19 moderate risk patients showed restorations with overhangs/open margins/open contacts with food impaction.15 moderate risk patients had dental/orthodontic appliances. 17 high-caries-risk patients showed clinical evidence of severe dry mouth/xerostomia.

In the control group, the average mean value of CFU in saliva and plaque is 87.9 and 29.35 respectively. In the low-caries-risk group, the average mean values of CFU in saliva and plaque are 97.65 and 35.9 respectively The average mean value of CFU saliva and plaque in the moderate-caries-risk group is 112 and 37.3 respectively. The average mean value of CFU saliva and plaque of the high risk category is 108.8 and 37.85 respectively.

The ANOVA comparison of the salivary CFU of four study groups reveals that the p-value was 0.352 and was not statistically significant. This implies that the salivary

CFU did not vary significantly among the controls, low risk, moderate risk and high-caries-risk groups.

The ANOVA comparison of plaque CFU among the groups yielded a p-value of 0.528 which did not statistical significance. This also implies that the plaque CFU di not vary significantly among the groups.

Sanchez-Perez et al have reported a higher yield of MS in cultures from fissure plaque samples on TSY20B medium. A higher predictive value was found for plaque rather than salivary samples. Salivary samples are easy to collect but may not be an accurate representation.[15]

The mean CFU of the plaque was 29.35 in controls and 37.85 in the high-caries-risk group. But, Independent samples T-test comparison between the control and high-caries-risk group yielded a p-value of 0.189 which was not statistically significant.

The mean CFU of the plaque was 29.35 in controls and 37.30 in the moderate-caries-risk group. But, T-test between moderate risk and controls had a p-value of 0.650 did not achieve statistical significance.

The mean CFU of the plaque was 29.35 in controls and 35.90 in the low-caries-risk group. But, T-test values between controls and the low-caries-risk group had a p-value of 0.461 which was not statistically significant.

Among plaque CFU analysis, T-test comparison between low risk and high-caries-risk groups yielded a p-value of 0.065, which was statistically insignificant. The comparison between low risk and moderate risk group had a p-value of 0.289 which was also statistically insignificant. The analysis of moderate-caries-risk and high-caries-risk groups had a p-value of 0.417 which was insignificant.

The mean CFU of saliva was 87.9 in controls and 108.8 in the high-caries-risk group. But, Independent samples T-test comparison between the control and high-caries-risk group yielded a p-value of 0.410 which was not statistically significant.

The mean CFU of saliva was 87.9 in controls and 112 in the moderate-caries-risk group. But, T-test between moderate risk and controls had a p-value of 0.706 did not achieve statistical significance.

The mean CFU of saliva was 87.9 in controls and 97.65 in the low-caries-risk group. But, T-test values between controls and the low-caries-risk group had a p-value of 0.916 which was not statistically significant.

Among salivary CFU analysis, T-test comparison between low risk and high-caries-risk groups yielded a p-value of 0.494, which was statistically insignificant. The comparison between low risk and moderate risk group had a p-value of 0.650 which was also statistically insignificant. The analysis of moderate-caries-risk and high-caries-risk groups had a p-value of 0.268 which was insignificant.

Caries prediction based on the MS count has been reported to be 7 – 20.4% by Sanchez-Perez et al, Irigoyen-Camacho et al, Vanderas et al, Russel et al and Granath et al. Lesions can develop in the absence of detectable MS. Sullivan et al have reported that initially MS free surfaces can get infected from other areas in the future, even in individuals with low bacterial counts. Other microbes can contribute to a lower pH and may coaggregate with MS. Hence, this prediction is limited by the multi-factorial nature of caries. MS count can aid in the identification of groups with high caries risk and those with little or no risk. But, they are less effective in the identification of moderate risk[15]

WHO considers 12 years of age as the global indicator age for monitoring dental caries.Schlagenhauf et al have avoided children with mixed dentition to avoid discrepancies in microbial counts. The chance to avoid caries is grouped into 3 levels – low chance 0-20% (high caries risk), 21-60% (moderate caries risk), and high chance 61-100% (low caries risk)[37]

In recent years, caries management has shifted from the traditional drill and fill surgical model to prevention and minimally invasive treatment. It is already proven that surgical extraction or restorations do not stop the carious process. Hence, Individualized patient care with a focus on prevention and patient education will become the gold standard to assess, educate and monitor the caries risk status of the patient.[43,53]

AAPD recommends the CRA tools as an important element for contemporary clinical care for infants, children and adolescents. CRA tools like Cariogram, AAPD's CRA tools, Caries Management by Risk Assessment (CAMBRA) is a valuable aid for clinicians. This CRA assessment and individualized treatment protocol is not common in Indian scenario. [49]

For a low-caries-risk patient, recall visits every 6-12 months and radiographs every 12-24 months is recommended. For the moderate-caries-risk patient, 6-month recall and annual radiographs with fluoride usage, professional fluoride application every 6 months, diet counselling and active surveillance of incipient lesions and restoration of cavitated/enlarging lesions. For the high-caries-risk patient, 3-month recall visits, radiographs every 6 months, professional topical fluoride application every 3 months, usage of xylitol and restoration of incipient, cavitated or enlarging lesions. [43]

Advances in assessment techniques will emerge with time and can be employed based on evidence of its efficiency. Dental caries risk assessment should become a routine component in dental practice. Estimation of the caries risk will help to establish the periodicity and intensity of caries management protocol.

CONCLUSION:

Many factors such as bacteria, carbohydrate diet, and host response cause initiation of dental caries and its progression. Assessment of the caries risk of individual patients is a critical component in determining an appropriate management strategy. Along with patient motivation and risk assessment successful outcome for caries management can be achieved. Hence it can be concluded that there is an association between various components of saliva and dental caries.

S.mutans are potential human odontopathogens and colonize the tooth after the eruption. However, if the colonization is delayed by colonization by other bacteria, there is the possibility that decay will not occur or its occurrence will be greatly reduced.

The paradigm change in our understanding of dental caries, its prevention and treatment make it mandatory for all dentists treating infants, children, adolescents and adults to incorporate caries risk assessment into their clinical practice. They must implement risk-based caries management protocols to make diagnostic, preventive, and restorative recommendations for their patients.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any

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