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Caries Risk Assessment and Detection of Streptococcus Mutans Count in Plaque and Saliva Using Mutans-Sanguis Agar

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

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 outpatients of dental clinics in Surendera Dental College, Sriganganagar, Rajasthan. The age group of the participants ranged from 16-60 years.
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 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 colonies were counted with a digital colony counter.
The tabulated data were subjected to statistical analysis using ANOVA and t-test with SPSS.
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

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even though the mean value showed an increase. 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.

1. 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 S.mutans 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 said that Risk is a probability that an event will occur. Young 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 [15,16].

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.

2. MATERIALS AND METHODS

2.1 Source of Data

Samples were collected from outpatients of dental clinics in Surendera Dental College, 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.

2.2 Inclusion Criteria

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

2.3 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

2.4 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)

2.5 Method

Using ADA caries risk assessment as a standard. The patients was 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]

Our participant age group ranged from 16-60 years. The examination, caries risk assessment, plaque and saliva sample collection was performed by two trained examiners. As we had used only saliva and plaque samples, it did not have any rejection requests by the participants.

Clinical examination was performed in a routine dental chair under normal lighting conditions. No specific instructions or conditions were given for saliva and plaque collection as relevant exclusion criteria were applied before participant selection.

Xerostomia assessment was based on the patient history of dryness, mucosal changes and salivary characteristics. Plaque collection was performed in the buccal surfaces of premolar and molar teeth without any drying.

2.6 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 T-test were performed for statistical significance with the cut-off p value of 0.05.

Fig. 1. Collection of saliva from patient in sterile container

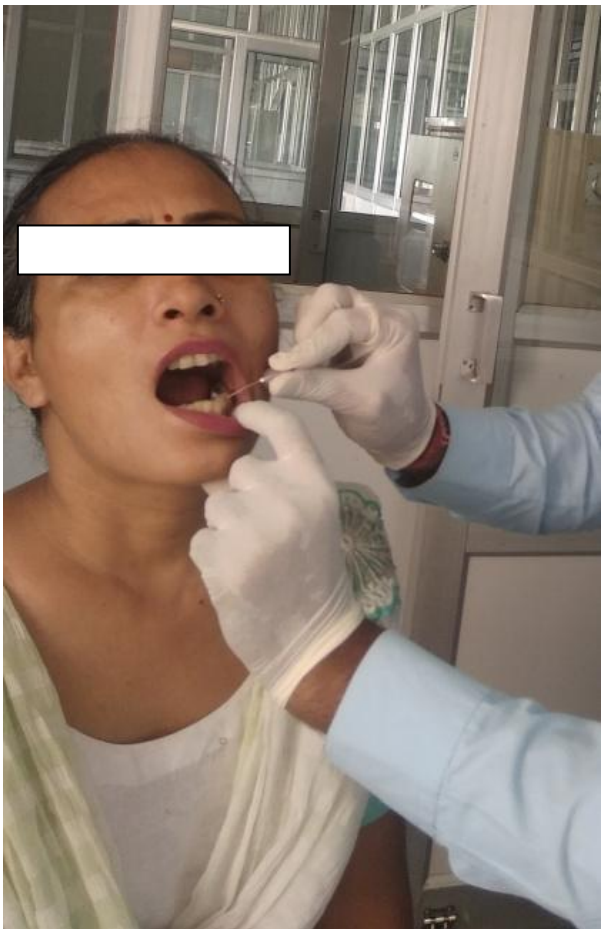


Fig. 2. Showing collection of plaque from patients mouth with sterile needle

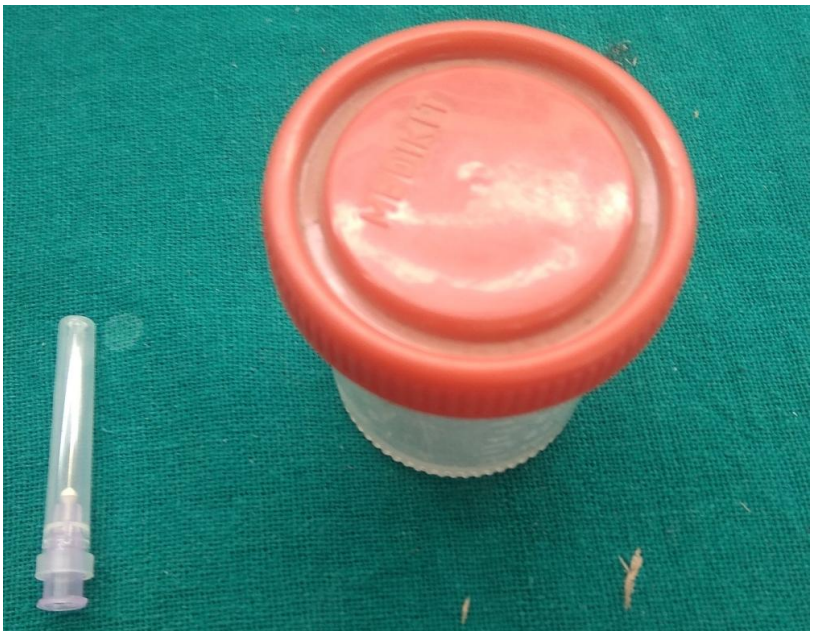


Fig. 3. Picture showing collected plaque and saliva samples

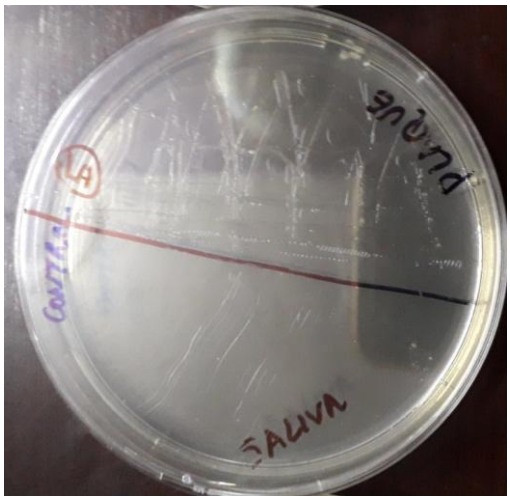


Fig. 4. Hi media Petri plates showing the CFU in control group

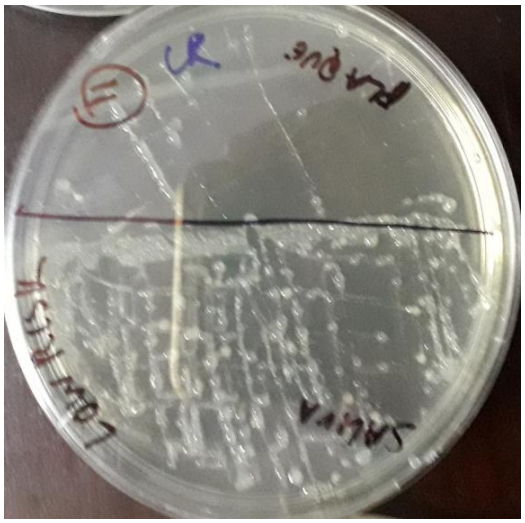


Fig. 5. Hi media Petri plates showing CFU in low caries risk group

Fig. 6. Hi media petri plates showing CFU in moderate caries risk individuals

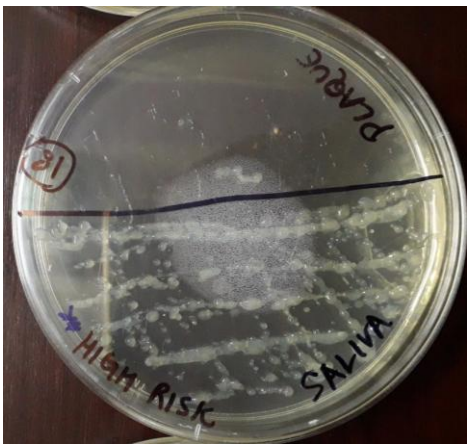


Fig. 7. Hi media Petri plates showing CFU in high caries risk individuals

3. RESULTS

The present study was conducted in the outpatients of dental clinics of Sriganganagar, Rajasthan. 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 using SPSS.

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.

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 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.

Table 3 represents the contributing conditions of caries risk assessment form.

Table 4 showing the distribution of general health conditions and clinical conditions of caries risk assessment form.

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 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 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 6.

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

Table 7.

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

Table 8.

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

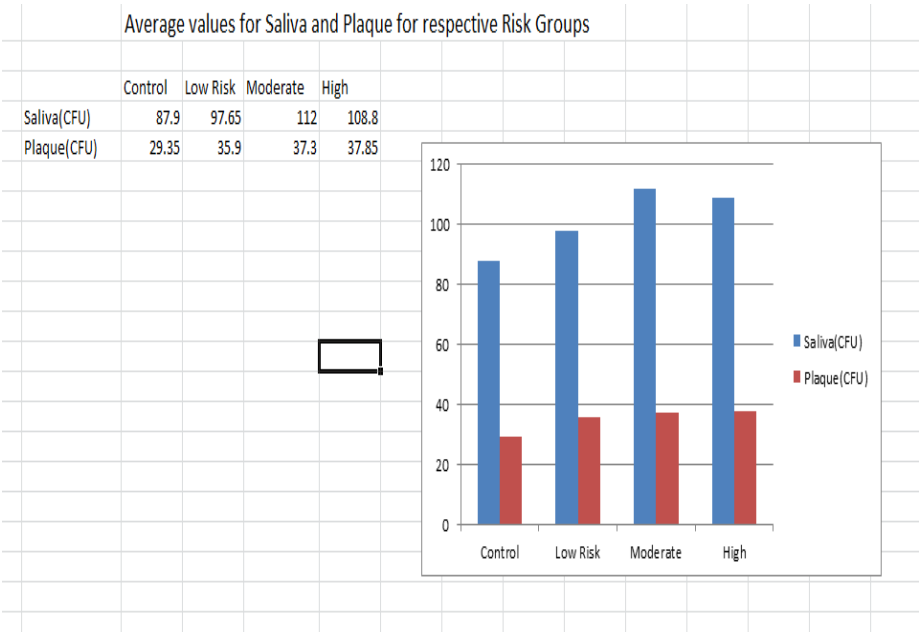
Table 8 showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the moderate-risk group.

Table 9.

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

Table 6 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 7 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 8 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 9 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 1.



Graph 1. 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).

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

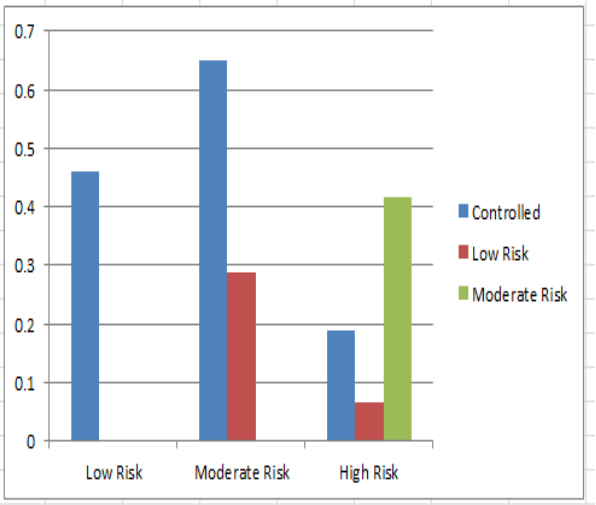
		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
VAR00002	Equal variances assumed	.941	-.55000	7.34180	-15.41269
	Equal variances not assumed	.941	-.55000	7.34180	-15.42710

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).

	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 2.

Graph 2 reveals the comparison of p values of CFU in plaque in between different caries risk groups in our study using T-test.

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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

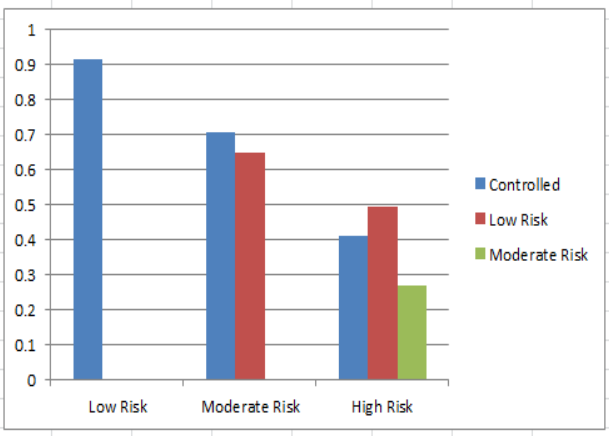
		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower
V2	Equal variances assumed	.836	3.20000	15.33410	-27.84227
	Equal variances not assumed	.836	3.20000	15.33410	-27.87848

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).

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 3.

Graph 3 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.

4. 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 [17]. The carious process is driven by a diet high in fermentable carbohydrates, suboptimal oral hygiene and elevated numbers of virulent, cariogenic bacteria [18]. Dental caries was identified as a silent epidemic two decades ago. Hence, dental care providers should focus on disease prevention and strategize to address the aetiology of dental caries [19]. 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 [18].

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 [16].

Other contributing factors include deep pits and fissures, salivary factors and socio-economic status [18,20]. Suneja et al. [19] 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.

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 [19].

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 [21]. 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. 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 [18].

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 [16-24].

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 [24]. The study protocol was ethically approved and the written

informed consent was obtained from the selected participants.

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 [24]. 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 [23]. 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].

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 [23].

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 [16].

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 [15,26].

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) [21,26].

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 [22].

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 [24-26].

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 [24-26].

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.

5. CONCLUSION

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.

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.

DISCLAIMER

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 litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Written informed consent was obtained from the selected participants.

ETHICAL APPROVAL

It was an analytical study and ethical clearance was obtained from the institutional ethical committee. Institutional ethical clearance was obtained vide SDCRI/IEC/2018/016.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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