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°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.
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 methods to control the activity of oral bacteria [3].

Bacteria in dental plaque produce acids that degrade the tooth tissues and the local reduction of pH leads to the selection of an acidic microbiota, which contributes further to lesion development. The most common bacteria associated with dental cavities are the mutants 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 genus streptococci is of great clinical interest due to their pathogenic potential. They cause a wide variety of diseases which 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 prospective bacterial colonization offers oral streptococci, an optimal environment for their growth [6]. The composition of oral microflora at different surfaces within the mouth is based on physical and biological properties like the presence of receptors for microbial adhesion, the redox potential of the site and provision of essential nutrients [7].

Microbes that were formerly associated only with oral diseases are increasingly pathogenic in general. Almost 50% of the oral microflora is constituted by oral streptococci. Bacteremia may occur after dental treatment, but also after vigorous tooth brushing especially in 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 mutans streptococci in the etiopathogenesis of dental caries, their quantification and identification is relevant for epidemiological and early intervention studies [9,10,11].

Detection and identification of S. mutans have been performed by different methods, namely microbial culture techniques, 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, mutans sanguis 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 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 prosthetic crowns

2.4 Armamentarium

Stainless steel Mouth mirror; Probe; Explorer; Tweezer (DPI, India)
HiMedia Mutans-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 (Romsons Specican, 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:

1-2 ml 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 1000 ml of distilled water.
- It was mixed well and sterilized by autoclaving at 15 lbs. pressure at 121°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°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
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°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 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    | 10 (1 finding)  |
| restorations                                     | 20 – Moderate risk |
|                                                  | 10 (2 findings)  |
|                                                  | 20 – High risk   |
| 05 (3 findings)                                  | 20 – High risk   |
| 08 (4 findings)                                  | 20 – High risk   |
| 07 (5 & more findings)                           | 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)                                   | 20 (High risk)    |
| Interproximal Restorations – 1 or more           | 18 (Moderate risk) |
| Exposed Root surfaces                            | 19 (Moderate risk) |
| Restorations with overhangs/open margins/open    | 19 (Moderate risk) |
| 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             |
|                          | 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 [KIU] | PLAQUE [KIU] |
|-------|-----|--------|----------|--------------|--------------|
| 1     | 20  | MALE   | CONTROL  | 146          | 44           |
| 2     | 21  | MALE   | CONTROL  | 118          | 22           |
| 3     | 25  | FEMALE | CONTROL  | 142          | 54           |
| 4     | 33  | 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    | 38  | MALE   | CONTROL  | 92           | 36           |
| 18    | 36  | MALE   | CONTROL  | 118          | 15           |
| 19    | 36  | MALE   | CONTROL  | 78           | 8            |
| 20    | 35  | MALE   | CONTROL  | 116          | 18           |

Table 6 showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the study group.
Table 7 showing the CFU COUNT of S.Mutans in the saliva and plaques samples among the low-risk group.

| S.No | Age | Gender | Group   | SALIVA [CFU] | PLAQUE [CFU] |
|------|-----|--------|---------|--------------|--------------|
| 21   | 46  | MALE   | Low     | 162          | 26           |
| 22   | 53  | MALE   | Low     | 66           | 15           |
| 32   | 52  | FEMALE | Low     | 96           | 38           |
| 34   | 54  | MALE   | Low     | 64           | 63           |
| 35   | 56  | MALE   | Low     | 96           | 46           |
| 36   | 18  | MALE   | Low     | 46           | 35           |
| 37   | 19  | MALE   | Low     | 45           | 25           |
| 28   | 21  | MALE   | Low     | 45           | 42           |
| 29   | 25  | FEMALE | Low     | 126          | 65           |
| 30   | 21  | FEMALE | Low     | 38           | 49           |
| 31   | 26  | FEMALE | Low     | 90           | 41           |
| 32   | 27  | FEMALE | Low     | 152          | 36           |
| 33   | 29  | FEMALE | Low     | 123          | 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   | 23  | FEMALE | Low     | 46           | 36           |
| 39   | 25  | FEMALE | Low     | 114          | 18           |
| 40   | 24  | FEMALE | Low     | 102          | 16           |

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

| S.No | Age | Gender | Group      | SALIVA [CFU] | PLAQUE [CFU] |
|------|-----|--------|------------|--------------|--------------|
| 41   | 28  | FEMALE | MODERATE  | 220          | 12           |
| 42   | 28  | FEMALE | MODERATE  | 122          | 36           |
| 43   | 29  | FEMALE | MODERATE  | 96           | 69           |
| 44   | 26  | FEMALE | MODERATE  | 86           | 26           |
| 45   | 45  | FEMALE | MODERATE  | 96           | 46           |
| 46   | 46  | FEMALE | MODERATE  | 78           | 46           |
| 47   | 24  | FEMALE | MODERATE  | 114          | 36           |
| 48   | 25  | MALE   | MODERATE  | 125          | 43           |
| 49   | 25  | 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          | 56           |
| 54   | 39  | MALE   | MODERATE  | 240          | 45           |
| 55   | 45  | MALE   | MODERATE  | 56           | 12           |
| 56   | 34  | FEMALE | MODERATE  | 86           | 11           |
| 57   | 26  | MALE   | MODERATE  | 89           | 45           |
| 58   | 25  | FEMALE | MODERATE  | 45           | 26           |
| 59   | 25  | MALE   | MODERATE  | 112          | 46           |
| 60   | 29  | FEMALE | MODERATE  | 125          | 36           |

Table 9.

| S.No | Age | Gender | Group      | SALIVA [CFU] | PLAQUE [CFU] |
|------|-----|--------|------------|--------------|--------------|
| 61   | 27  | MALE   | HIGH      | 96           | 21           |
| 62   | 35  | FEMALE | HIGH      | 176          | 80           |
| 63   | 55  | MALE   | HIGH      | 86           | 2            |
| 64   | 30  | FEMALE | HIGH      | 192          | 56           |
| 65   | 20  | MALE   | HIGH      | 206          | 18           |
| 66   | 21  | FEMALE | HIGH      | 62           | 18           |
| 67   | 15  | MALE   | HIGH      | 186          | 46           |
| 68   | 12  | FEMALE | HIGH      | 112          | 12           |
| 69   | 36  | FEMALE | HIGH      | 42           | 31           |
| 70   | 44  | FEMALE | HIGH      | 86           | 46           |
| 71   | 54  | MALE   | HIGH      | 78           | 40           |
| 72   | 54  | FEMALE | HIGH      | 65           | 12           |
| 73   | 52  | MALE   | HIGH      | 35           | 28           |
| 74   | 52  | FEMALE | HIGH      | 156          | 22           |
| 75   | 26  | MALE   | HIGH      | 112          | 36           |
| 76   | 51  | FEMALE | HIGH      | 110          | 41           |
| 77   | 52  | MALE   | HIGH      | 114          | 76           |
| 78   | 52  | FEMALE | HIGH      | 81           | 13           |
| 79   | 52  | MALE   | HIGH      | 34           | 36           |
| 80   | 42  | FEMALE | HIGH      | 164          | 21           |
|      |     |        |           | 103.8        | 27.32        |
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.

| V2                   | Sum of Squares | df | Mean Square | F         | Slg.  |
|----------------------|----------------|----|-------------|-----------|-------|
| Between Groups       | 7265.837       | 3  | 2421.946    | 1.107     | .352  |
| Within Groups        | 166279.550     | 76 | 2167.869    |           |       |
| Total                | 173945.387     | 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

|                           | Sum of Squares | df  | Mean Square | F     | Sig. |
|---------------------------|----------------|-----|-------------|-------|------|
| Between Groups            | 922.100        | 3   | 307.367     | .746  | .528 |
| Within Groups             | 31307.100      | 76  | 411.036     |       |      |
| 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

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 | N  | Mean  | Std. Deviation | Std. Error Mean |
|------------------|----|-------|----------------|-----------------|
| VAR00002         | 20 | 29.350 | 17.72383       | 3.96317         |
| 3.00             | 20 | 37.300 | 21.12863       | 4.72457         |

Independent Samples Test

Levene's Test for Equality of Variances

|            | F     | Sig.  | t     | df   |
|------------|-------|-------|-------|------|
| Equal variances assumed | .200  | .850  | -1.289| 30   |
| Equal variances not assumed |       |       | -1.289| 30.884 |

Independent Samples Test

|                      | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Mean |
|----------------------|-----------------|-----------------|-----------------------|------------------------------------|
| Equal variances assumed | .205            | -7.95000        | 0.16671               | -20.43385                          |
| Equal variances not assumed | .205            | -7.95000        | 0.16671               | -20.44626                          |

Independent Samples Test

|                      |                  |                  |                     |                                  |
|----------------------|------------------|------------------|--------------------|----------------------------------|
| Equal variances assumed | 4.53385         |                  |                     |                                  |
| Equal variances not assumed | 4.54026         |                  |                     |                                  |

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 |
|------------------|
|                 |
| VAR100003 | N  | Mean   | Std. Deviation | Std. Error Mean |
| VAR00002   | 20 | 29.3900 | 17.72338       | 3.69317         |
| 2.00       | 20 | 35.0000 | 15.90851       | 3.57440         |

Independent Samples Test

Levene's Test for Equality of Variances

|         | F     | Sig. |
|---------|-------|------|
| VAR00002| .556  | .461 |

Independent Samples Test

t-test for Equality of Means

|                   | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower |
|-------------------|-----------------|-----------------|-----------------------|-------|
| VAR00002          | .227            | -6.55000        | 5.33715               | -17.35449 |

Independent Samples Test

95% Confidence Interval of the Mean

|                   | Upper           |
|-------------------|-----------------|
| VAR00002          | 4.25449         |

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 |
| 2.00 | 20 | 35.6000 | 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 | \n| | \n| |  

**Independent Samples Test**

| t-test for Equality of Means |
|-----------------------------|
| Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Mean Lower |
| VAR00002 | Equal variances assumed | .771 | -1.95000 | 6.6024 | -15.43295 |
| Equal variances not assumed | \n| | \n| |  

**Independent Samples Test**

| t-test for Equality of Means |
|-----------------------------|
| 95% Confidence Interval of the Mean Upper |
| VAR00002 | Equal variances assumed | 11.53295 |
| Equal variances not assumed | \n| | \n| |  

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 |
| 2.00            | 20 | 35.9000    | 15.99651       | 3.57469         |
| 3.00            | 20 | 37.3000    | 21.12863       | 4.72457         |

Independent Samples Test

| F | Sig. | t | df |
|---|------|---|----|
| 1.159 | .289 | -.236 | 38 |

Independent Samples Test

| t-test for Equality of Means |
|------------------------------|
| Sig. (2-tailed) | Mean Difference | Std. Error Difference |
| 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 |
|------------------------------|
| Equal variances assumed 10.50357 |
| 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).
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 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.

B] SALIVA

Table 18. Comparison between control and high-risk groups

| Group Statistics |  |  |  |  |
|------------------|------------------|------------------|------------------|------------------|
| V3 | N | Mean | Std. Deviation | Std. Error Mean |
| 1 | 20 | 87.9000 | 45.59220 | 9.74069 |
| 2 | 20 | 108.8000 | 52.90069 | 11.82005 |

**Independent Samples Test**

Levene’s Test for Equality of Variances

| V3 | F | Sig. | df |
| V2 | .604 | .410 | 38 |
| | Equal variances assumed | | |
| | Equal variances not assumed | | |

| Independent Samples Test | t-test for Equality of Means | 95% Confidence Interval of the Difference |
|--------------------------|-----------------------------|----------------------------------------|
| V2 | Sig (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence interval of the difference |
| Equal variances assumed | .130 | -.20.9000 | 15.31057 | -51.90076 |
| Equal variances not assumed | .181 | -.20.9000 | 15.31057 | -51.90076 |

| Independent Samples Test | t-test for Equality of Means |
|--------------------------|-----------------------------|
| V2 | Sig (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence interval of the difference |
| Equal variances assumed | 10.10276 | | | |
| 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 | V2  | Mean   | Std. Deviation | Std. Error Mean |
|------------------|-----|--------|----------------|-----------------|
| V2 1             | 20  | 87.9000| 43.56230       | 9.74083         |
| V2 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                       | .011        | .916       | -.685  | 38 |
| 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 Interval of the Lower |
| V2                       | .497                  | -9.75000        | 14.22825            | -38.55358                       |
| Equal variances assumed  | .497                  | -9.75000        | 14.22825            | -38.55729                       |
| Equal variances not assumed | .497   | 14.22825    | -38.55729                       |

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 |
| 1    | 20 | 51.90000 | 43.56230 | 8.74033 |
| 3    | 20 | 112.00000 | 43.68548 | 8.76837 |

Independent Samples Test

Levene's Test for Equality of Variances

|        | F    | Sig. | t   | df   |
|--------|------|------|-----|------|
| V2     | .145 | .706 | -1.747 | 38   |

Independent Samples Test

Hest for Equality of Means

|        | Sig (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Lower |
|--------|----------------|-----------------|-----------------------|----------------------|
| V2     | .088           | -24.10000       | 13.78510              | -62.02672            |

Independent Samples Test

Hest for Equality of Means

|        | 95% Confidence Interval of the ... |
|--------|-----------------------------------|
| Upper  |                                   |
| V2     | 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 |
|---------------------------|----|----|-------|----------------|-----------------|
|                           | 2  | 20 | 97.6500 | 46.38089       | 10.37108        |
|                           | 4  | 20 | 108.8000 | 52.86089       | 11.62005        |

**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 |

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   | 97.6500 | 46.38069 | 10.37108 |
|      | 3   | 112.0000 | 43.68548 | 9.78837 |

Independent Samples Test

Levene's Test for Equality of Variances

| F   | Sig. |
|-----|------|
| .209 | .650 |

Equal variances assumed

| t     | df    |
|-------|-------|
| -1.007 | 38    |

Equal variances not assumed

| t     | df    |
|-------|-------|
| -1.007 | 37.865 |

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 |
|------------------|----|-----|-------|----------------|-----------------|
|                  | 3  | 20  | 112.0000 | 43.68548        | 9.70837         |
|                  | 4  | 20  | 108.6000 | 52.80920        | 11.82005        |

Independent Samples Test

| Levene's Test for Equality of Variance | t-test for Equality of Means |
|----------------------------------------|-----------------------------|
| F          | Sig. | t       | df |
| V3         | Equal variances assumed     | 1.262 | .265 | 209 | 38     |
|            | Equal variances not assumed |      |     |     |       |

Independent Samples Test

| t-test for Equality of Means |
|-------------------------------|
| Sig. (2-tailed) | Mean Difference | Std. Error Difference | 85% Confidence Interval of the Mean |
|------------------|------------------|------------------------|------------------------------------|
| V3               | 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 |
|-------------------------------|
| Sig. (2-tailed) | Mean Difference | 85% Confidence Interval of the Mean |
|------------------|------------------|------------------------------------|
| V3               | 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 3 reveals the comparison of p values of CFU in saliva among different caries risk groups in our study using T-test.

Table 24.

| Caries Risk Group   | CFU/ml 3 | CFU/ml 4 |
|---------------------|----------|----------|
| Control group       | 3.5×10^5 | 4.5×10^5 |
| Low caries risk     | 3.9×10^5 | 4.4×10^5 |
| Moderate caries risk| 4×10^5   | 4.4×10^5 |
| High caries risk    | 4.4×10^5 | 4.4×10^5 |

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 multifactorial 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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