SEMINAR NO. 7
TOPIC: CARIES ACTIVITY TESTS

**INDEX:**



**INTRODUCTION:**

It is difficult to change the dietary habits of children or adults. Many patients cannot adhere to dietary advice or may be dishonest about their indiscretions. Therefore, dentists may need to determine the degree of cooperation he/she is getting from a patient in a dietary control program.

A clinical examination predicts neither caries activity nor indicates a patient's susceptibility to dental caries. This can be done through Caries Activity/Susceptibility Tests. Caries activity is determined by bacterial flora, local substrate (diet), the host (tooth and salivary environment) and time.

Caries activity tests tend to measure only 1 parameter; therefore, interpretation of a test is more reliable when coupled with a good clinical assessment. Currently, no single test is sufficiently accurate or reliable, confirming that there is no one characteristic or set of characteristics that is highly predictive of susceptibility.

Caries activity:

- refers to the increment of active lesions (old and recurrent lesions) over a stated period of time; it is a measure of the speed of progression of a carious lesion
- it is the sum of new carious lesions and the enlargement of existing cavities during a certain time
- in its broadest sense, caries activity can be *defined* as the speed with which teeth are destroyed by caries

Caries susceptibility:

- refers to the inherent tendency/propensity of the host and target tissue, the tooth, to be afflicted by the caries process
- refers to the susceptibility/resistance of a tooth to a caries-producing environment

Caries Activity Tests (CAT):

- measure the degrees to which the local environment challenge (e.g., dietary effect on microbial growth and metabolism) favours the probability of carious lesions

Most tests measure an aspect of acid production (e.g., Snyder test) or resistance to it (e.g., buffering test) or the combined effect of both (Fosdick and Dewar tests).

**PRINCIPAL MICRO-ORGANISMS INVOLVED IN DENTAL CARIES:**

1. Streptococcus mutans: correlated to etiology of initial *enamel* and root surface lesions

2. Lactobacillus: in existing carious lesions; isolated from *dentin* in both coronal and root caries lesions

3. Actinomyces naeslundii: predominant part of polymicrobial etiology for *root* caries (including Capnocytophaga and Prevotella species)

**THE REASONS WHY A CARIES ACTIVITY TEST FACILITATES THE CLINICAL MANAGEMENT OF PATIENTS:**

- To determine the need and extent of personalized preventive measures
- To serve as an index of the success of therapeutic measures
- To motivate and monitor the effectiveness of education programs relating to dietary and oral hygiene procedures
- To manage the progress of restorative procedures
- To identify high-risk groups and individuals

**IDEAL REQUISITES OF A CARIES ACTIVITY TEST: (as given by SNYDER)**

- Should have maximum correlation between predicted and actual caries development
- Should have reliability and validity, i.e., the test must be consistently accurate and reproducible
- Should have simplicity with regard to technical procedures and skills required
- Results should be obtained rapidly (within hours or a few days)
- Should have measurement of mechanisms involved in caries process
- Should be inexpensive, non-invasive, easy to evaluate and applicable to any clinical setting

**A CARIES-PREDICTIVE TEST SHOULD POSSESS:**

- Validity – with a minimum of false positive or false negative results
- Reliability – the results should be reproducible

- Feasibility – the tests should lend themselves to use in public health programs or clinical trials (because they are inexpensive, noninvasive and easy to use by semiskilled personnel)

**CARIES ACTIVITY TESTING IS ESSENTIAL TO:**

- Establish an initial baseline level of cariogenic pathogens as a basis for future evaluation and preventive dentistry counselling
- Ensure a low level of caries activity before starting any extensive restorative procedure
- Monitor patient behaviour towards reducing the no. of S. mutans and Lactobacilli as parts of counselling to curtail sucrose intake

**DIFFERENT CARIES ACTIVITY TESTS:**

1. Lactobacillus Colony Count Test: (lab test)

It was introduced by Hadley in 1933 and popularized by Jay. It was the 1st caries susceptibility test to be generally used. For many years, it was used by dentists as an aid in determining current dental caries susceptibility and in predicting future dental caries incidence; however, present use is limited to research studies.

Principle – estimates the number of acidogenic and aciduric bacteria in patient's saliva by counting the number of colonies appearing on tomato peptone agar plates (pH 5.0) after inoculation with a sample of saliva

Basis – of the test is a selective media favouring the growth of aciduric lactobacilli

Procedure

- Immediately after rinsing, the patient chews a small piece of paraffin. Saliva accumulated in the following 3-min period (5-10 ml) is collected in a sterile container and shaken for 2 mins to mix it
- Saliva sample diluted to 1:10 dilution by pipetting 1 ml of the saliva sample into a 9 ml tube of sterile saline solution and shaken
- It is again diluted (1:100) by pipetting 1 ml of the 1:10 dilution into another 9 ml tube of sterile saline solution and mixed thoroughly
- 0.4 ml of each dilution is spread on the surface of an agar plate containing 20ml of cooked liquefied agar (Rogosa's SL agar plate), incubated for 3-4 days at 37ºc and the number of lactobacillus colonies that develop are counted using a colony counter with bright lights and a large magnifying glass or the Quebec counter
- The number of lactobacilli/ml saliva is calculated by multiplying the number of colonies on the plate by the dilution factor of its inoculums

Lactobacillus colony counts in saliva as related to caries susceptibility:

| No. of organisms/cc | Symbolic designation | Degree of caries activity suggested |
|---|---|---|
| 0-1000 | ± | Little or none |
| 1000-5000 or <10,000 | + | Slight |
| 5000-10,000 or <100,000 | ++ | Moderate |
| More than 10,000 or >1,000,000 | +++ or ++++ | Marked |

Advantages

- Simple to carry out
- Useful as a screening test for caries activity in large groups
- Useful for monitoring the effectiveness of restorative dentistry and care completion

Disadvantages

- Inaccurate for predicting onset of caries
- Does not completely exclude growth of other relatively aciduric organisms
- Counts involving single individuals are not as reliable
- Counting is a tedious procedure
- Test takes a few minutes to perform, but the results are not available for several days
- Not a simple test; it is expensive, requires complex equipment and trained personnel

Dentocult test: (chairside dipslide test for lactobacilli)
It was given by Larmas in 1975.

Procedure – consists of running undiluted saliva over a dip slide covered with slightly modified Rogosa agar which is incubated at 35-37ºc for 4 days; the growth is compared with a standard illustration. Correlation of 65-90% is found between the lactobacilli count and caries experience.

Colony density is classified as 1000, 10000, 100000 or 1000000 aciduric organisms/ml saliva. Counts of more than 10,000 CFU/ml of saliva are taken as high; counts of less than 1000 CFU/ml of saliva are taken as low.

Advantages

- Simple technique, reasonable cost, the results are easy to read

- Can also be used in field studies

Indications for use

- For pre-selection of patients for yearly or half-yearly check-ups in communities
- Important educational aid for motivation and dietary counselling among patients
- It is a control of the efficacy of dietary counselling

*Different dip slide tests for determination of various causes of caries:*

- **Dentocult-LB** → for lactobacilli
- **Caries screen/Strip mutans** → for S. mutans
- **Dentobuff strip** → for buffer capacity
- **Proflow** → for flow rate determinations
- **Oricult** → reduced salivary flow or medically compromised patient

Oricult-N test:

It is used for measuring oral yeast infection.

Indications

- To determine presence of yeast infection in oral cavity even before clinical signs; for confirmation of clinical findings
- To confirm hyposalivation status of a patient
- To determine effectiveness of antifungal therapy
- To provide an indicator of medical compromise in a patient
- Fungi are aciduric and their presence is a reflection of an acidic environment, also favouring the development of dental caries

Dentobuff method:

Procedure – a dip stick coated with chemical indicators is immersed in saliva, the colour produced being indicative of the saliva's capacity to buffer acids and bases.

It is one of the best indicators of caries susceptibility because it reveals the host response; a high buffer capacity gives resistance to caries process and can even compensate for active caries habits.

Low buffer capacity indicates

- Reduced salivary flow rate
- Reduced host response to cariogenic agents
- Possible malnutrition and pregnancy (transient, may give rise to false positive results)

2. Colorimetric Snyder Test: (chairside)
It was devised by Snyder in 1951.

Principle – measures ability of salivary microorganisms to form organic acids from a carbohydrate medium which contains an indicator dye Bromcresol green which changes colour from green to yellow in the pH range of 5.4-3.8; indirectly, it is also a measure of acidogenic and aciduric bacteria.

Procedure –

- 0.2 ml stimulated saliva collected by chewing paraffin before breakfast is thoroughly mixed with 10 ml melted agar containing medium in a test tube (cooled to 50ºc), allowed to solidify and then incubated at 37ºc
- Amount of acid produced by acidogenic organisms is detected by changes in the pH indicator and compared to an uninoculated control tube against a white background after 24, 48 and 72 hours of incubation. The rate of colour change from green to yellow is indicative of the degree of caries activity
- Bluish-green colour is seen at pH 4.7-5.0; green colour at pH 4.2-4.6 and yellow colour at 4.0 or lower
- This test essentially estimates the number of both aciduric and acidogenic organisms in saliva because it relies on production of additional acid under already acidic culture condition

Colour observations in Snyder test:
Glucose agar medium with Bromcresol green indicator + 0.2 cc of saliva **is** incubated at 37ºc

24 hours → 48 hours → 72 hours

| | | |
|---|---|---|
| If yellow<br>Marked caries susceptibility | If yellow<br>Definite caries susceptibility | If yellow<br>Limited caries susceptibility |
| If green<br>Continue to incubate & observe at 48 hrs. | If green<br>Continue to incubate & observe at 72 hrs. | If green<br>Caries inactive |

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Interpretation of the Snyder Caries Activity Test:

| Caries activity | Results after incubation for 24h | 48h | 72h |
|---|---|---|---|
| Marked | positive | | |
| Moderate/definite | negative | positive | |
| Slight/limited | negative | negative | positive |
| Negative/caries-inactive | negative | negative | negative |

- Both the speed and volume of acid production are indicated; it serves as a point of reference in comparison testing for interpretation
- "Improved" result is given if slower/less colour change is seen when compared with previous results
- "Worse" result is given if faster/more colour change is seen when compared with previous results

Advantages

- Relatively simple to carry out
- Of value in assessing oral environmental cariogenic challenge
- Cost is moderate
- Only 1 tube of medium and no serial dilutions required
- A practical way to monitor patient motivation and to measure the effectiveness of the caries control program

Disadvantages

- Time consumed is more
- Sometimes, the colour changes are not so clear
- Measures acidogenic potential but is limited in predictive value because these salivary microorganisms may not be representative of those in plaque

Snyder test and its modifications use Bromcresol green as indicator (which is blue-green at pH 5.4 or above and turns yellow at pH 3.8 or less). The "critical pH" for enamel dissolution is 5.4 – 5.5. Therefore, it would be more appropriate to use an indicator such as Bromcresol purple (which is purple at pH 6.8 and yellow at pH 5.2) which is used in the RICKLES TEST.

3. The Swab Test:

It was developed by Grainger et al in 1965.

Advantage over other tests – no saliva collection is necessary; therefore, it is valuable in evaluating caries activity in very young children.

Principle involved – it is based on the same principle as Snyder's test. Oral flora sampled by swabbing the buccal surfaces of teeth with a cotton applicator, which is then incubated in the medium. Change in pH following a 48-hour incubation is read on a pH meter or by use of a colour comparator.

Interpretation

- pH 4.1 and < 4.1 = marked caries activity
- pH 4.1 - 4.4 = active
- pH 4.5 - 4.6 = slightly active
- pH 4.6 and over = caries inactive

Advantages

- of value in predicting caries increments, particularly in children with low or no previous caries experience
- no saliva collection required

4. Streptococcus mutans level in saliva:

Principle – measures the number of S. mutans colony forming units (CFU)/unit volume of saliva by culturing of the plaque samples from discrete sites (occlusal fissure/proximal area) for detecting and quantitating S. mutans colonized on teeth. Incubation is done on Mitis Salivarius Agar (MSA) – selective streptococcal medium with addition of high concentration of sucrose (20%) and 0.2 U bacitracin/ml (MSB) which suppresses the growth of most non-S.mutans colonies.

Procedure – the sample of organisms is obtained by use of tongue blades (wooden spatulas) which are then pressed against S. mutans selective MSB (Mitis Salivarius Bacitracin) Agar in special petri dishes. Agar plates are incubated at 37ºc for 48 hours in 95% at 5% $CO_2$ gas mixture.

Interpretation

- Levels of S. mutans > $10^5$/ml of saliva = unacceptable
- Colonization of a new surface does not occur readily unless level of S. mutans reaches 4.5 x $10^4$/ml for smooth surfaces and $10^3$/ml for occlusal fissures
- In unstimulated saliva collected from children who
  - -- were caries-free, S. mutans formed about 0.1% of the total viable count
  - -- had DMFS score of 5 or more, the value was about 0.85%

Advantage

- Since frequency of isolation of S. mutans is high prior to initiation of lesions as contrasted to Lactobacilli, the clinician utilizes this count as an adjunct in caries management

Disadvantages

- Difficulty of distinguishing between a carrier state and cariogenic infection
- S. mutans may constitute < 1% of total plaque flora
- S. mutans tends to be located at specific sites only
- Plates have a shelf life of only about a week, therefore not convenient for chairside tests

5. Dip-slide Method for S. mutans count:

It was devised for estimation of S. mutans levels in saliva.

Procedure – undiluted paraffin-stimulated saliva was poured on a special plastic dipslide coated with MSA (Mitis Salivarius Agar) containing 20% sucrose; agar surface was thoroughly moistened and excessive saliva was allowed to drain off. 2 discs containing 5 µg of bacitracin were placed on the agar 20mm apart. The slide was tightly screwed into a cover tube after inserting a $CO_2$ tablet and incubated at 37ºc for 48 hours in a sealed candle jar.

Evaluation

- Score 1 = low: discrete colonies which can be readily counted at 15x magnification with the total count of CFU inside the inhibition zones

< 200

- Score 2 = medium: discrete colonies and the number in the zone of inhibition > 200 at 32x magnification
- Score 3 = high: colonies are tiny and almost completely or totally cover the inhibition zone with the number of colonies uncountable even with 32x magnification

6. Salivary Buffer Capacity Test:

Principle – can be quantitated using either a pH meter or colour indicators; this test measures the number of millilitres of acid required to lower the salivary pH through an arbitrary pH interval (such as from pH 7.0 to 6.0) or the amount of acid/base necessary to bring colour indicators to their end point

Equipment – pH meter, titration equipment, 0.05 N lactic acid, 0.05 N base, paraffin, sterile glass jars containing a small amount of oil

Procedure – 10 ml of stimulated saliva is collected under oil atleast 1 hour after eating; 5 ml of this is measured into a beaker. After correcting pH meter to room temperature, the pH of saliva is adjusted to 7.0 by addition of lactic acid/base. Lactic acid is then added to sample until a pH of 6.0 is reached. The number of milliliters of lactic acid needed to reduce the pH from 7.0 to 6.0 is a measure of buffer capacity. This number can be converted to milliequivalents/l.

Evaluation – an inverse relationship is present between salivary buffering capacity and caries activity. The saliva of individuals whose mouths contain a considerable number of carious lesions frequently has a lower acid-buffering capacity than the saliva of those who are relatively caries-free.

Advantage – simple to carry out

Disadvantage – does not correlate adequately with caries activity

7. Enamel Solubility Test (Susceptibility Test):

Principle – based on the fact that when glucose is added to saliva containing powdered enamel, organic acids are formed which decalcify the enamel resulting in an increase in the amount of soluble calcium in the Saliva-Glucose-Enamel mixture; the extent of increased calcium is supposedly a direct measure of the degree of caries susceptibility

Equipment – powdered human enamel, saliva collection bottles, sterile test tubes, test tube agitation instrument, calcium determination instrument

Disadvantages

- Not generally suited for office procedures
- Test is not simple, equipment required are complex
- Personnel must be trained
- Cost is high

8. Salivary Reductase Test (Susceptibility Test):

Principle – measures the activity of the reductase enzyme present in salivary bacteria. A kit is available under trade name Treatex. Saliva is collected in a plastic container after the subject chews paraffin wax to stimulate salivary flow; it is then mixed with the dye Diazo-resorcinol. Colour changes are seen and the “caries conduciveness” reading is taken after 15 mins. No incubation procedures are required.

Evaluation

Based on the colour changes, the caries conduciveness is related to as mentioned below:

| Colour | Time | Score | Caries activity |
|---|---|---|---|
| Blue | 15 minutes | 1 | nonconducive |
| Orchid | 15 minutes | 2 | slightly conducive |
| Red | 15 minutes | 3 | moderately conducive |
| Red | immediately | 4 | highly conducive |
| Pink/White | immediately | 5 | extremely conducive |

Advantages

- No incubation is required
- Quick results

Disadvantage

- Test results vary with time after food intake and after brushing

9. Alban Test: (colorimetric)

It is a simplified substitute for the Snyder test.

Main features

- Use of a somewhat softer medium that permits the diffusion of saliva and acids without the necessity of melting the medium
- Use of a simpler sampling procedure in which the patient expectorates directly into tubes that contain the medium

Materials required to prepare the Alban test medium

- Snyder test agar, small scale to measure 60 gms, 2 liter Pyrex glass to melt medium, funnel to dispense medium into test tubes, 100 16 mm test tubes with screw caps

Procedure – 60 gms of Snyder test agar is placed in 1 liter water and the suspension brought to boil over a low flame. When thoroughly melted, the agar is distributed using about 5 ml/tube. Tubes should be autoclaved for 15 mins, allowed to cool and stored in a refrigerator. 2 tubes of Alban medium are taken from the refrigerator and the patient is asked to expectorate a small amount of saliva directly into the tubes. Volume of saliva

should be sufficient to cover the surface of the test medium. Tubes are labelled and incubated at 98.6ºF (37ºc) for up to 4 days. The tubes are observed daily for

(i) change of colour from bluish green (pH 5) to definite yellow (pH 4 or below)
(ii) the depth in the medium to which the change has occurred

Daily results collected for a 4-day period should be recorded on the patient's chart.

Scale for scoring

1. no colour change = '¾'
2. beginning colour change (from top of medium down) = '+'
3. one-half colour change (from top down) = '++'
4. three-fourths colour change (from top down) = '+++'
5. total colour change to yellow = '++++'

Following method is used for final recordings (after 72 or 96 hours of incubation)

- Readings negative for entire incubation period = "negative"
- All other readings (+, ++, +++ or ++++) = "positive"

Advantages

- Simple, can be used routinely in dental office
- Low cost
- Diagnostic value when negative results are obtained
- Motivational value (ideal for education)
- Good for indicating caries activity
- Ideal test for following patient cooperation

Disadvantages

- More armamentaria required
- Based on subjective evaluation of a colour change that is often not clear cut

*Composition of media used for Snyder and Alban tests:*

- Bacto peptone 20 gms
- Dextrose 20 gms
- Sodium chloride 5 gms
- Agar 16 gms
- Bromcresol green 0.02 gms

10. Streptococcus mutans screening test:

(A) Plaque / toothpick method:

Action – involves a simple screening of dilute plaque sample streaked on a selective culture media

Equipment – sterile toothpicks, sterile Ringer's solution (5 ml), platinum loop, Mitis Salivarius Agar (MSA) plates containing sulphadimetine

Procedure – plaque samples are collected from the gingival thirds of buccal tooth surfaces, one from each quadrant and placed in Ringer's solution. Sample is shaken till homogenized. Plaque suspension is then streaked across MSA plates. After aerobic incubation at 37ºc for 72 hours, cultures are examined and total colonies in 10 fields recorded.

This test attempts to semi-quantitatively screen the dental plaque for a specific group of caries-inducing S. mutans that has shown some relationship with subsequent dental caries experience

(B) Saliva / tongue blade method:
Action – estimates the number of S. mutans in mixed paraffin-stimulated saliva when cultured in Mitis Salivarius Bacitracin (MSB) agar
Equipment – paraffin wax, sterile tongue blades, disposable contact petri dish containing MSB agar

Procedure

- Subjects chew a piece of paraffin wax for 1 min to displace the plaque microorganisms, thereby increasing their number in saliva
- Sterile tongue blades are then rotated in patients' mouths 10 times so that both sides are thoroughly inoculated by the patients' flora; they are then pressed into a MSB agar plate in a disposable petri dish and incubated at 37ºc for 48 hours
- For field studies, plates can be put in plastic bags containing expired air, then sealed and incubated at 37ºc

Counts of more than 100 CFU by this method are proportional to greater than 10 CFU of S. mutans/ml of saliva by conventional methods.

Advantages

- A simplified and practical method for field studies; requires no transport media/dilution stops
- Was developed for use with large number of school children and avoids the necessity of collecting saliva

11. Strip Mutans Test: (chairside)
It was developed by Jensen and Bratthall in 1989.

It is based on the ability of S. mutans to grow on hard surfaces and the use of a selective broth (high sucrose concentration in combination with bacitracin). Bacitracin can be added to broth just before use, thereby prolonging the shelf-life of the test considerably compared to agar plates.

Procedure –

- bacitracin disc is taken from the vial with forceps or needle. Vial is recapped, the disc put in culture broth vial and allowed to stand for atleast 15 mins and shaken.
- Patient is given a Dentocult paraffin pellet and instructed to chew for 1 min, the stimulated saliva then swallowed or spat out.
- Test strip is removed from Strip Mutans container by only touching the square end; 2/3ds of strip is placed in the patient's mouth and rotated on the surface of the tongue about 10 times. Strip is then removed from the patient's mouth and pulled between the patient's closed lips to remove excess saliva. The strip is placed in culture vial and the cap reclosed; it is incubated for 2 days at 35ºc-37ºc. Strip is removed from vial and allowed to air dry.

Treated side (marked with line) can be examined immediately/later. After drying, strip can be stored for future reference (in plastic bag, plastic wrap, etc.). Bacitracin and high sugar content inhibit growth of all organisms except S. mutans. The S. mutans in saliva adhere to the treated side of strip in proportion to their actual amount in saliva and grow as small, dark or light blue colonies 1mm in diameter or considerably less, when growth is very dense.

12. Fosdick Calcium Dissolution Test:

Principle – this test measures the milligrams of powdered enamel dissolved in 4 hours by acid formed when the patient's saliva is mixed with glucose and powdered enamel

Procedure – saliva is stimulated by having the patient chew gum or paraffin; 25 ml of saliva is collected and part of it analyzed for calcium content. Remaining saliva is placed in an 8-inch sterile test tube with about 0.1 gm powdered human enamel, sealed and shaken for 4 hours at body temperature. It is reanalyzed for calcium content. Chewing of gum to stimulate saliva produces sugar. If paraffin used, a concentration of about 5% glucose is added. Amount of dissolution increases as the caries activity increases.

Advantage

- In limited studies, correlation reported is good

Disadvantages

- Test is not simple and requires complex equipment
- Expensive, requires trained personnel

13. Dewar Test:

Principle – similar to the Fosdick Calcium Dissolution Test. Only difference is that in this test, the final pH after 4 hours is measured instead of the amount of calcium dissolved.

Procedure – not commonly used as it has not been adequately tested for clinical correlation

14. Cariogram:

It was developed by Bratthall et al in 1996.It is a new method of illustrating interaction of factors contributing to the development of dental caries.

Pie diagram is used and it is divided into 5 sectors, in following colours:

1. Green – shows an estimation of the 'chance to avoid caries'
2. Dark blue – indicates 'diet' and is based on a combination of diet contents and frequency
3. Red – indicates 'bacteria' and is based on a combination of the amount of plaque and mutans streptococci
4. Light blue – indicates 'susceptibility' and is based on a combination of fluoride program, saliva secretion and saliva buffer capacity
5. Yellow – indicates 'circumstances' and is based on a combination of past caries experience and related disease

**LIMITATIONS OF CARIES ACTIVITY TESTS:**

- None of the tests are highly reliable as indicators of THE expected caries increments because they measure only a single parameter/variable such as acid produced/colony counts of bacterial species in the etiology of caries.
- They do not encompass factors involved in caries resistance such as fluoride exposure, enamel maturation or immune protection.
- Significant correlations have been found only between caries increments and salivary S. mutans counts and with past caries experience.
- Salivary lactobacillus counts showed some correlation with caries increments, but it was not statistically significant.
- Combining the measurements of caries prevalence, especially incipient smooth surface lesions, with the salivary S. mutans and lactobacillus counts produced a much higher correlation with caries increments.
- The best prediction of expected caries activity can be expected from the combined use of several selected tests (S. mutans count, buffer capacity, etc.)

**REFERENCES:**

1. Peter S. Essentials of preventive and community dentistry. 1st edi. New Delhi: Arya (Medi) Publishing House; 1999 and 2nd edi. New Delhi: Arya (Medi) Publishing House; 2003.

2. Finn SB. Clinical Pedodontics. 4th edi. Philadelphia: WB Saunders Company; 1991.

3. McDonald RE, Avery DR. Dentistry for the child and adolescent. 5th edi. St. Louis: CV Mosby Company; 1988 and 8th edi. St. Louis: CV Mosby Company; 1988.

4. Tandon S. Textbook of pedodontics. Hyderabad: Paras Publishing; 2001.

6. Newbrun E. Cariology. 3rd edi. Chicago: Quintessence Publishing Co.; 1989.

7. Nikiforuk G. Understanding dental caries: 2. prevention – basic and clinical aspects. Basel: Karger;1985.

8. Larmas M. Saliva and dental caries: diagnostic tests for normal dental practice. Int Dent J. 1992;42:199-208.

9. Pinelli C, Serra MC, Loffredo LCM. Efficacy of a dip slide test for mutans streptococci in caries risk assessment. Community Dent Oral Epidemiol. 2001;29:443-8.