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ORIGINAL RESEARCH

Milk, a Simple Tool to detect Caries Activity: Oratest

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ABSTRACT

Caries activity tests are based on the concept of a specific odontopathic infection, the principle causative organism being *Streptococci mutans*. The Oratest is found to be a simple chair side, less time-consuming, and inexpensive caries activity test. This test was performed on 60 school-going children to estimate the efficacy of the test. High statistical significance was found when the means of control and test group were compared (p = 0.001).

Keywords: Caries, Caries activity test, Methylene blue, Oratest.

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INTRODUCTION

Information about the total amount of bacteria and the composition of the oral flora may aid in the assessment of an individual's risk and prediction of the clinical course. Studies on caries activity are aimed at finding relevant microorganisms. But to date, the ideal method to evaluate in terms of sensitivity, specialization, and reliability has not been found. However, in routine clinical practice, these caries activity tests require specially prepared culture media and laboratory facilities for incubation and expensive kits to perform them.[1] Unfortunately, many of these caries activity tests require extensive work-up time and additional equipments.

Rosenberg et al[2] in 1989 developed Oratest, a simple, economical, noninvasive, and less time-consuming test for estimating the oral microbial level. This study was designed to estimate the efficiency of the simple Oratest, as a measure of oral microbial levels in school-going children.

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Principle of Oratest

Oratest is based on the rate of oxygen depletion by microorganisms. Under aerobic conditions, the bacterial enzyme, aerobic dehydrogenase transfers electrons or protons to oxygen. Once oxygen gets utilized by the aerobic organisms and an anaerobic environment is attained, methylene blue (redox indicator) acts as an electron acceptor and gets reduced to leucomethylene blue. The metabolic activity of the aerobic microorganism is reflected by the reduction of methylene blue to leucomethylene blue. The test is based on rinsing the mouth with sterile milk, which dislodges the microorganisms and also produces a substrate for their further metabolism. The formation of leucomethylene blue can be easily observed because of the white color of milk.[3]

MATERIALS AND METHODS

The study was conducted on 60 children who were randomly selected from those who reported to the Department of Pedodontics and Preventive Dentistry. The study was conducted after obtaining the ethical clearance and parent consent.

Sample Selection

The selected sample of children was divided into two groups. Group I (control group) consisted of 30 children free of caries, gingivitis, and other oral ailments, with plaque scores of zero as per the Quigley–Hein Index (Plaque Index), and group II (test group) consisted of 30 children with dental caries. Children in group II were further divided into A, B, and C subgroups according to the DEFT and DMFT of 2–5, 6–10, and 11–15 respectively.

Criteria for Selection of Test Group

- Dental caries involving one or more teeth
- Gingival index score of zero
- Absence of abscess, draining sinus, or cellulitis
- Absence of history of antibiotic for the past 1 month

Armamentariums (Fig. 1)

- Sterile beakers
- Sterilized milk (double-toned cow milk, 3% fat, pH 6.5)
- Screw cap test tubes
- 0.1% aqueous solution of methylene blue

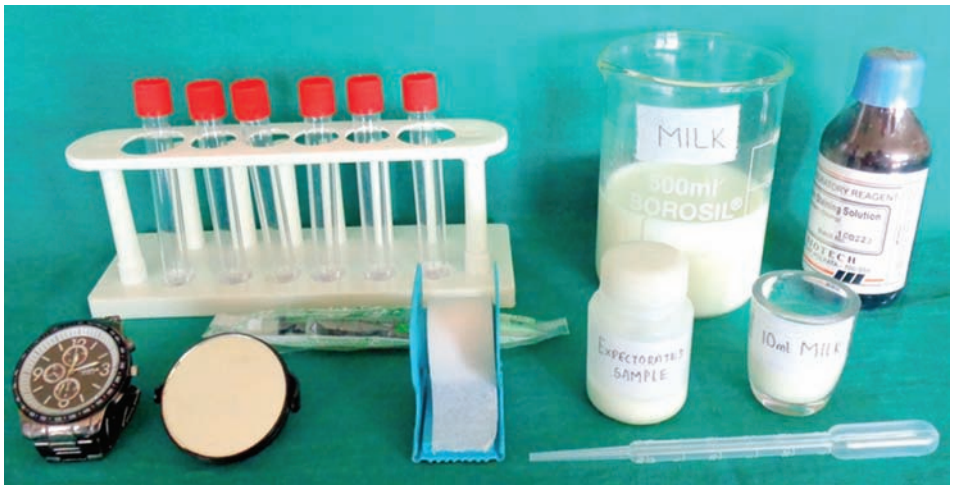


Fig. 1: Armamentarium

Fig. 2: Rinsing with milk for 30 seconds

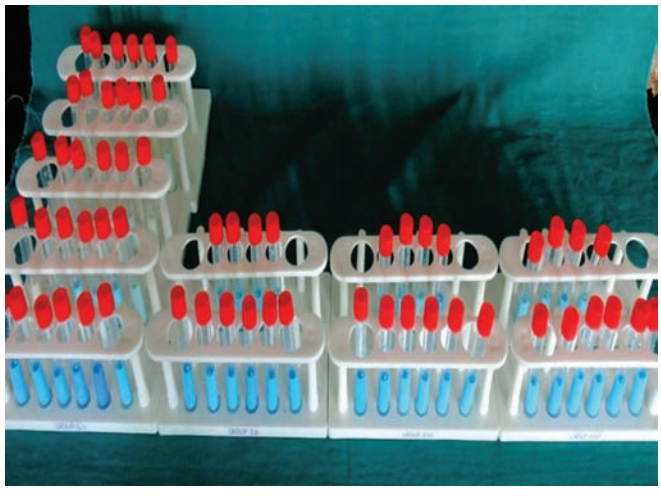


Fig. 3: Expectorated samples at the beginning of observation

- 10 mL disposable syringes
- Mirror, stopwatch, and test tube stand

Method

The children were asked to rinse their mouth vigorously for 30 seconds with 10 mL of ultra-high-temperature sterilized cow's milk (Fig. 2). The expectorate was collected in a sterile beaker, and 3 mL of this was immediately transferred with a disposable syringe to a screw cap test tube, which contained 0.12 mL of 0.1% methylene blue. (0.1% methylene blue was obtained by mixing 100 mg of methylene blue in 100 mL of distilled water). The expectorated milk and methylene blue was thoroughly mixed and the test tube was placed on a stand in a well-illuminated area. A mirror was used to detect any color change (blue to white) in the bottom of the test tube every 5 minutes. The time taken for the initiation of the color change within a 6 mm ring was recorded (Figs 3 and 4). The data were collected and analyzed using Statistical Package for the Social Sciences (SPSS) version 7. The tests used were chi-square test, Mann–Whitney "U" test, analysis of variance (ANOVA), and Spearman's rank correlation.

Fig. 4: Color change of the samples at the end of observation

OBSERVATIONS AND RESULTS

The mean time for the color change of methylene blue was 258 ± 28 minutes for the control group and 74 ± 37 minutes for the test group. Comparison between the means of these two groups was found to be statistically significant (p = 0.001).

The test group was divided into three subgroups according to the DEFT/DMFT values. Subgroups A, B, and C had a caries score of 1–5, 6–10, and 11–15 respectively. The maximum time for color change was observed in subgroup A, i.e., 84 ± 27 minutes with a mean caries score of 3 ± 1.07. The shortest time taken for change in color was observed in subgroup C, i.e., 63 ± 7 minutes. The subgroup B with a mean caries score of 7.91 ± 1.14 had taken a time of 76 ± 8 minutes for color change. The caries score and time taken for color change were negatively correlated for subgroups A and C ($\gamma = -0.592$ and 0.495 respectively; $p = 0.003$), whereas a positive correlation was observed in subgroup B ($\gamma = 0.172$; $p = 0.614$).

DISCUSSION

Microbiology of the oral cavity has consistently deemed to be of interest for better understanding of oral conditions associated with caries risk and gingival and periodontal disease.

The present caries activity test was carried out in 60 children from the Department of Pedodontics and Preventive Dentistry. The basis of the test was to rinse the whole mouth with sterile milk, which was thought to be a suitable vehicle as it is readily available and acceptable, effectively dislodges microorganisms, is nontoxic, and provides an excellent medium for subsequent metabolism.[4]

The time taken for the color change from blue to white after addition of methylene blue determined the caries activity. For the control group, the time taken for color change was 258 + 28 minutes and 74 + 37 minutes for the test group. The mean of both groups was statistically significant ($p = 0.001$), which lead to prove the fact that higher the infection (i.e., increased caries score), lesser the time taken for the change in the color of the expectorate (Table 1 and Graph 1). This is in accordance with the findings of Rosenberg et al.[2]

In group II, the minimum time taken for the color change (i.e., 63 + 7 minutes) was in children with a caries score of 11 to 15, and the maximum time taken for the color change (i.e., 84 + 27 minutes) was in children with a caries score of 1 to 5 (Table 2 and Graph 2). Similar findings were reported by Bhasin et al,[4] Saxena et al,[5] and Patalay et al.[6]

The concept of Oratest has given positive observation in care of gingivitis, periodontitis, and dental caries.[4] Hence, this simple chair side test can be used as a diagnostic tool as the results are easily visualized by the parent and child and the results can help the dentist or practitioner to reinforce motivation, plaque control, and behavior. The only drawback of this test is the lack of specificity (i.e., identifying the causative organism).

CONCLUSION

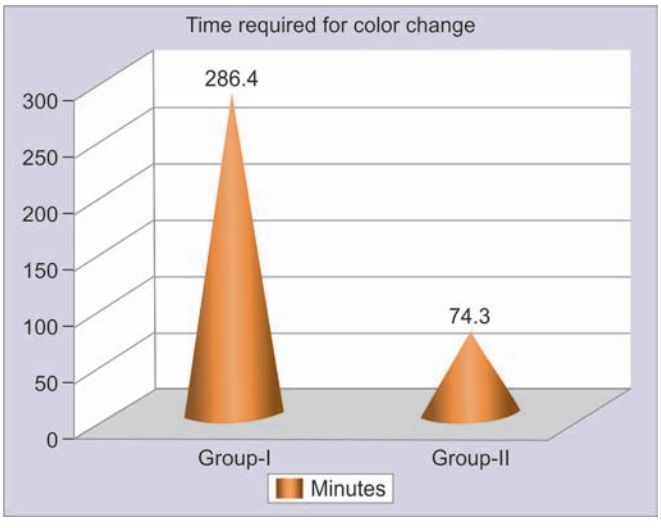
Oratest, a simple caries activity test, helps in identification of high-risk groups and thereby easy management by determining the need and extent of customized preventive and therapeutic measures. It also serves as an index to check for the effectiveness of treatment instilled.

Table 1: Average time for change in color in groups I and II

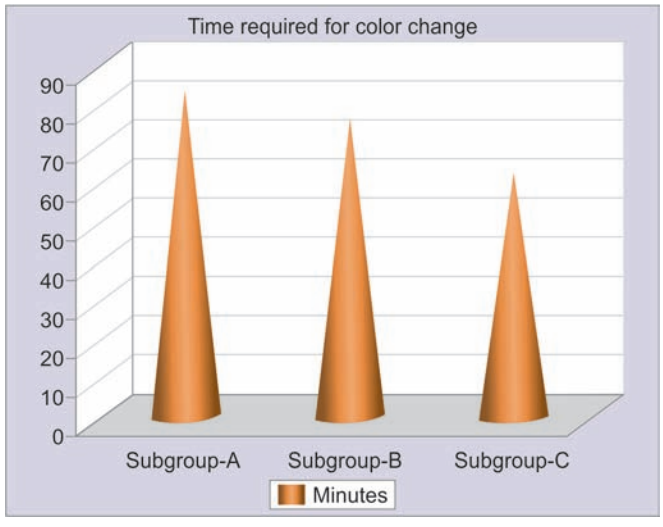
Sl. no.	*Groups*	*Mean time (min)*	*p-value*
1	I	286.4	0.001
2	II	74.3	

Table 2: Mean time for change in color in subgroups A, B, and C

Sl. no.	*Subgroup*	*Mean time (min)*	*p-value*
1	A	63	0.003
2	B	74	
3	C	84	



Graph 1: Average time for changing the color in groups I and II



Graph 2: Mean time for change in color in subgroups A, B, and C

This is an inexpensive test that can be utilized in mass community dental health-based programs.

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