



## Correlation of Ora Test and Caries Assessment Spectrum and Treatment Index (CAST) to Evaluate Caries Activity in 5-to-8-Year-Old Children

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Article Info	ABSTRACT
<p><b>Article type:</b> Original Article</p>	<p><b>Objectives:</b> Dental caries is a common chronic disease amongst children and are typically evaluated using the DMFT/deft index (decayed, missing, and filled teeth for permanent dentition/decayed, extracted, and filled teeth for primary dentition). To address the limitations associated with these indices, alternative assessment tools such as the Caries Assessment Spectrum and Treatment (CAST) index and Ora test have been developed. These methods aim to estimate caries activity within the oral cavity more accurately. The objective of our study was to evaluate and correlate caries activity in 5-to-8-year-old children using Ora test and CAST index.</p>
<p><b>Article History:</b> Received: 30 Apr. 2022 Accepted: 23 Sep 2022 Published: 21 May 2023</p>	<p><b>Materials and Methods:</b> Thirty schoolchildren between the ages of 5 and 8 years were selected and allocated into two groups (n=15) with DMFT/deft scores of &lt;5 (group A) and &gt;5 (group B). Two separate blinded examiners administered the assessments by first determining CAST scoring, which was followed by Ora test. Statistical analysis was performed using Pearson correlation test and significance was set at <math>P \leq 0.05</math>.</p>
<p><b>* Corresponding author:</b> Department of Pediatric and Preventive Dentistry, Terna Dental College, Navi Mumbai, Maharashtra, India  Email: <a href="mailto:pedotdc@gmail.com">pedotdc@gmail.com</a></p>	<p><b>Results:</b> The mean time for color change of Ora test, was <math>118.53 \pm 23.28</math> minutes in group A and <math>53.33 \pm 15.07</math> minutes in group B. CAST severity scores were <math>3.67 \pm 2.08</math> and <math>15.7 \pm 9.70</math> for groups A and B, respectively. Time taken for color change in Ora test and CAST scores showed a significant negative linear relationship (<math>P=0.039</math>).</p> <p><b>Conclusion:</b> Based on the negative correlation between CAST scores and Ora test, it may be postulated that microbial activity is directly related to caries activity in 5-to-8-year-old children.</p> <p><b>Keywords:</b> Dental Caries Activity Tests; Dental Caries; Pediatric Dentistry</p>

- **Cite this article as:** Kamble A, Katge F, Poojari M, Bhanushali P, Patil D, Nanavati K. Correlation of Ora Test with Caries Assessment Spectrum and Treatment Index (CAST) to Evaluate Caries Activity in 5-to-8-Year-Old Children. *Front Dent.* 2023;20:17.

### INTRODUCTION

The oral cavity is a site of constant battle between teeth and bacteria and thus, teeth are always at risk of developing caries. Due to the multifactorial etiology of dental caries, dentists are constantly in search of simpler caries detection methods for early treatment, in order to avoid extensive restorative therapy for the child [1]. The initial step towards understanding the

characteristics of dental caries is done by measuring this disease amongst different populations [2]. DMFT or deft index (Decayed, Missing or Extracted and Filled teeth) is one of the most common and oldest indices used for this purpose in permanent and primary teeth, respectively. Many newer systems have been proposed like the International Caries Detection and Assessment System (ICDAS),

PUFA index (Pulp Ulcer Fistula and Abscess), Significant Caries (SiC) index, and specific caries index (SiC) [3].

Both, DMFT and ICDAS do not cover all the stages of caries progression as a continuum. Also, DMFT does not include lesions located within enamel which has led to the underestimation of disease prevalence [4]. ICDAS distinguishes the three stages of enamel lesions, which could overcome the drawbacks of the DMFT index. However, ICDAS requires the use of compressed air to desiccate tooth surfaces and each tooth needs to be investigated two to three times. This makes ICDAS time-consuming and relatively expensive. PUFA on the other hand was initially met with interest, although its major disadvantage was that it covered only a small portion of the different stages of caries and was merely complementary to DMFT or ICDAS [5].

The complexity of ICDAS, limitations of PUFA and difficulty to compare the outcomes with DMFT led to the introduction of a new tool named as "Caries assessment spectrum and treatment index (CAST)". CAST plays a significant role in diagnosing the initial reversible stages of the development of dental caries. Frencken et al [6] in 2011, developed the CAST index, which categorizes all the codes of the caries process according to increasing order of severity.

The prediction of the clinical course of caries and the individual's risk can be assessed with the help of evidence regarding total amount of bacteria and composition of oral flora [2]. Rosenberg et al [7] in 1989 developed the Ora test (OT), which is a cost-effective, non-invasive, simple chairside test and requires less time for estimating the oral microbial count. The main principle of Ora test is that the microorganisms which are present in expectorated milk-samples utilize oxygen. This in turn causes reduction of methylene blue which reflects the metabolism of aerobic organisms [7]. It is in agreement with the 'ecological plaque hypothesis' proposed by Marsh [8] which states that 'if there are imbalances in the resident oral microflora, plaque-mediated diseases are generated'. The plaque becomes more anaerobic in nature as it

begins to accumulate and continues its maturation on the tooth surfaces. This results in oxygen consumption by aerobic microorganisms which is a major criterion for progression of anaerobic growth conditions for microorganisms.

The Ora test procedure was based on the principle of whole mouth rinsing with sterile milk, which is an ideal medium as it efficiently displaces microorganisms. It is non-toxic in nature which offers an excellent medium for ensuing metabolism and is also voluntarily accepted by most children [9]. The chairside test can be used as a diagnostic tool for the dental surgeon. The results can also help the dentist to reinforce motivation, plaque control and manage child behavior [10].

The rationale of this study is to find a tool which will be useful in identifying groups with an increased risk of caries development and can be applicable at both individual and community levels. In this study we aimed to evaluate and correlate caries activity in 5 to 8-year-old children using Ora test and CAST.

## MATERIALS AND METHODS

The present cross-sectional study was approved by the Institutional Review Board's Ethics Committee (TDC/EC/13/2020). All procedures conducted on human participants were in accordance with the ethical guidelines of this Committee [11], as well as the Helsinki [12] declaration (1964) and its later amendments. A detailed informed consent, both in English and local language was signed from all the participant's parents or guardians before the start of the study. Any allergy or intolerance to milk in the participants were asked.

Sample size was determined based on the results of a previous study through G\* power software (version 3.0.10) [13]. The level of significance ( $\alpha$  error) was set at 5% (0.05), power of study ( $1-\beta$ ) at 80% (0.8). The total sample size was calculated to 15 per group. Thirty healthy children were randomly selected from the outpatient department of Pediatric and Preventive Dentistry. A total of 30 participants were allocated into two blocks (15, each), group A with DMFT <5 and group B with DMFT >5.

Children aged between 5 and 8 years with no systemic or local diseases and a DMFT/deft score of more than 1, were included in the study. Participants with a recent history of antibiotic usage, at least for the past month, and those with removable prosthesis, fixed prosthesis and any orthodontic appliances as well as children with special health-care needs or with any oral habits such as mouth breathing, were excluded from the study.

Both CAST procedure as well as Ora test were carried out on the same child in a single dental sitting. For dental examination, children were made to sit comfortably on a dental chair. Initially, CAST scoring was performed by one examiner followed by the Ora test procedure, which was done by a second examiner blinded to the CAST scores.

Examination of all children for CAST scoring, was performed in a supine position under an appropriate light source from the dental chair. A validated and structured form based on that described by Frencken et al [6] was used (Table 1). Before scoring, teeth were dried with a sterile cotton roll or gauze. A sterilized mouth mirror and a 0.5mm ball-ended periodontal probe were used as diagnostic instruments (Figure 1). CAST severity scores were calculated for each participant and were classified as mild (0–1.25), moderate (1.25–6.75) and severe (>6.75) [14].



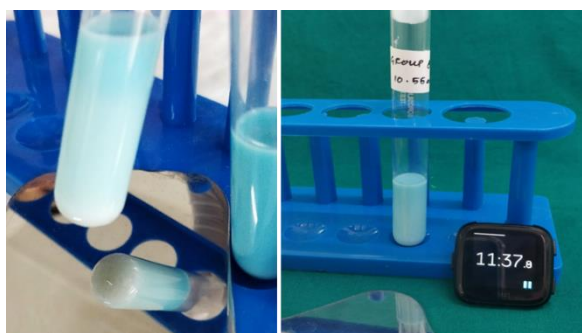
**Fig. 1.** Clinical examination for determination of Caries Assessment Spectrum and Treatment Index (CAST) scores

**Table 1.** Caries Assessment Spectrum and Treatment Index (CAST) index codes

Codes	Area Involved
0	Sound
1	Sealed
2	Restored
3	Distinct visual change in enamel
4	Internal caries-related discoloration in dentine
5	Distinct cavitation into dentine
6	Involvement of pulp chamber
7	Abscess/Fistula
8	Lost (due to caries)
9	Dose not match with any of the other categories
A	Absent

For the Ora test method, the children were asked to refrain from consumption of food or drink (except water), 90 minutes prior to the procedure. All subjects vigorously rinsed their mouths with 10ml pasteurized cow milk for 30 seconds. The expectorated milk was first dispensed in a sterile beaker and 3ml was immediately transferred to a test tube using a disposable syringe. These test tubes already contained 0.12ml of 0.1% methylene blue (CZTL Methylene Blue, MB Cure, India). The test tube containing the expectorated milk and methylene blue were mixed thoroughly and positioned vertically on a stand. Any change in color was observed under natural light. Color change from blue to white at the bottom of the tube was detected at five-minute interval using a mirror. The time taken for the initial change in color forming a ring of about 6mm was recorded [7] (Figure 2).

For statistical analysis, Pearson correlation test was used to compare the CAST severity scores and the time taken for the color change related to Ora test. The significance value was set at  $P \leq 0.05$ .



**Fig. 2.** Clinical procedures for performing Ora Test. Detection of color change at the bottom of the test tube using a mirror (left) and recording the time taken for initiation of color change from blue to white (right)

## RESULTS

The age of the children in both groups ranged from 5 to 8 years (mean age, 6.5 years) and there were 16 girls and 14 boys (total, 30). Table 2 shows the Ora test values and CAST

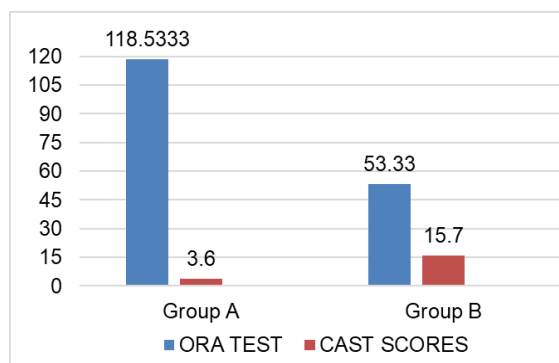
severity scores for group A (DMFT scores < 5) and group B (DMFT scores >5).

The mean time taken for the color change in Ora test was  $118.53 \pm 23.28$  minutes in group A and  $53.33 \pm 15.07$  minutes in group B, whereas CAST scores were  $3.67 \pm 2.08$  and  $15.70 \pm 9.70$  for group A and group B, respectively. The difference between these two groups was statistically significant ( $P < 0.05$ ) (Figure 3).

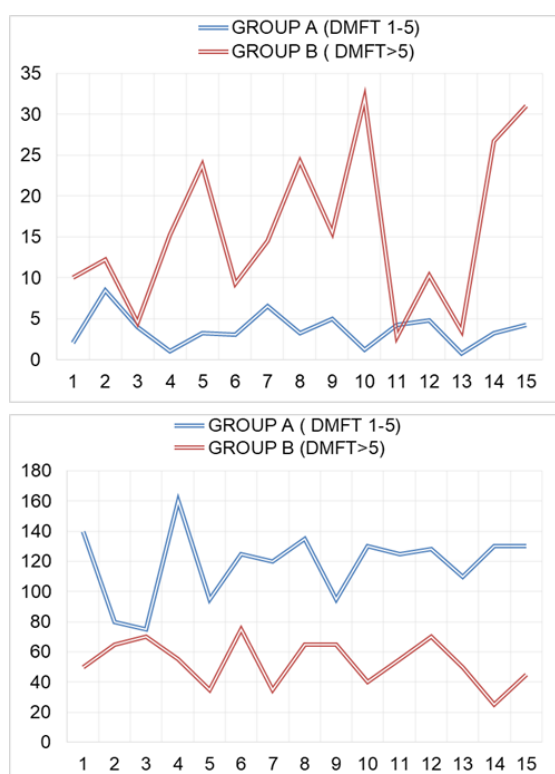
Based on our observations, when the time for color change in Ora test increased, there was a decrease in CAST scores, which demonstrates a negative relationship between the two groups ( $P = 0.039$ ). In group A- participants who had a low DMFT score, the mean time taken for color change using Ora test was  $118.53 \pm 23.28$  minutes and the CAST score was  $3.67 \pm 2.08$ . Corresponding values in group B children with relatively high DMFT scores were  $53.33 \pm 15.07$  minutes and  $15.70 \pm 9.70$ , respectively (Figure 4).

**Table 2.** Ora test values and Caries Assessment Spectrum and Treatment Index (CAST) scores for group A (DMFT <5) and group B (DMFT >5) children

Group A		Group B	
CAST severity score	Ora test (minutes)	CAST severity score	Ora test (minutes)
2 (Moderate)	140	10 (Severe)	50
6.5 (Moderate)	80	12.25 (Severe)	65
4 (Moderate)	75	4.5 (Moderate)	70
1 (Mild)	160	15.25 (Severe)	55
3.25 (Moderate)	95	23.75 (Severe)	35
3 (Moderate)	125	9.25 (Severe)	75
6.5 (Moderate)	120	14.5 (Severe)	35
3.25 (Moderate)	135	24.25 (Severe)	65
5 (Moderate)	95	15.5 (Severe)	65
1.25 (Mild)	130	32 (Severe)	40
4.25 (Moderate)	125	2.75 (Moderate)	55
4.75 (Moderate)	128	10.25 (Severe)	70
0.75 (Mild)	110	3.5 (Moderate)	50
3.25 (Moderate)	130	26.75 (Severe)	25
4.25 (Moderate)	130	31 (Severe)	45



**Fig 3.** Bar Chart showing correlation of Ora Test values and Caries Assessment Spectrum and Treatment Index (CAST) in groups A and B



**Fig. 4.** Line diagram showing correlation of Ora Test values (upper graph) and Caries Assessment Spectrum and Treatment Index (CAST) (lower graph) in groups A and B

## DISCUSSION

The rising interest in the microbiological aspect of dental caries has shifted the focus of researchers towards development of an array of innovative techniques which can make diagnosis of caries simpler and faster. Apart from the various diagnostic procedures, individual caries activity tests can aid as useful adjuncts in

making clinical decisions concerning caries [15]. Ora test was used in the present study since it is a simple, non-invasive, and chairside test. For this purpose, we used 0.1% methylene blue as suggested by Rosenberg et al [16]. The reduction of methylene blue involves the transfer of hydrogen, as there is absence of oxygen in the molecule of methylene blue. Bacterial metabolites act as hydrogen donors and the corresponding metabolism is responsible for supplying energy for the growth of microbes [17].

Our Ora test values in were  $118.53 \pm 23.28$  and were  $53.33 \pm 15.07$  minutes in groups A (DMFT: 1-5) and B (DMFT: >5), respectively. These findings were supported by the results of a previous study by Rosenberg et al [7], which confirmed the hypothesis that increased caries activity is associated with quicker color changes in milk samples, indicative of higher bacterial levels. Thus, an inference was drawn which suggested that caries activity demonstrated a positive correlation with CAST scores. A negative correlation was also seen between caries activity and time taken for color change in Ora test. Similarly, the results were also supported by findings of other studies by Patalay et al [18], Arora et al [19], Saxena et al [2], and Gainneos et al [13].

Jauhari et al [20] evaluated the effect of fluoride mouthrinse, herbal mouthrinse and oil pulling on caries activity and Streptococcus mutans count using Ora test. They found that Oratest is reliable and efficient in determining caries activity and only shows color change when the microbial activity is high. The higher the infection, the lesser will be the time required for Colour change, indicating high microbial activity. Jain et al [21] studied the effect of diet modification on salivary parameters and Ora test in high caries risk individuals and concluded that there was an increase in Oratest readings, suggesting a decrease in the caries activity, post-diet counseling. Thus, diet counseling and parental education using Ora test were proposed to alter salivary parameters and play a major role in prevention of caries.

A revised version of the Ora test was created to detect and measure the presence of



metabolically active bacteria in saliva samples. The revised version uses a resazurin-based redox indicator that reacts with bacteria when exposed to a milkshake medium, which contains protein and metabolizable sugars. The resulting reaction allows for visualization and quantification of bacteria activity. Ledder et al [22]. estimated the density of oral bacterial colonization using the resazurin milkshake test within 10 minutes and concluded that the test can serve as a sensitive semi-quantitative method for measuring oral bacteria in human oral rinse samples.

Gainneos et al [13], assessed the correlation between the International Caries Detection and Assessment System – Lesion Activity Assessment (ICDAS-LAA) and Ora test. They also evaluated the caries risk of a patient and concluded that a direct correlation exists between the two. ICDAS involves a complex procedure of data analysis which in turn makes it more challenging to present data in an easy-to-understand and -read manner. Furthermore, ICDAS entails the application of compressed air for desiccating the tooth surface, which causes financial strain on resources and use of electric supply [23]. Hence, Ora test is preferred over ICDAS-LAA for evaluation of caries activity in patients.

CAST index was utilized in the present study because it is a reliable, practical and easy-to-read reporting system. We found CAST severity scores of  $3.67 \pm 2.08$  and  $15.70 \pm 9.70$  in groups A and B, respectively. This index follows a hierarchical sequence of stages, where a less severe stage advances to a more severe one. The benefit of this sequence of stages is that it provides a better perception of severity of dental caries. In other words, the higher the CAST score, the worse the condition [24]. CAST index can be used in routine clinical practice as it consumes less chairside time and aids in determining the status of caries activity. Additionally, its outcomes could be used as a patient motivational tool and also to govern patient compliance with other treatment regimens [13].

The validation of CAST has been analyzed in various countries. Its reproducibility was “substantial” to “almost perfect” in deciduous

dentition of two- to six-year-old and of six- to nine-year-old children [25]. Castro et al [26]. did a comparative evaluation of CAST, ICDAS and DMF in epidemiological surveys and concluded that the mean time taken for assessment of the DMFT index, ICDAS, and CAST in an older population was 3.8, 8.9, and 4.7 minutes, respectively. Thus, CAST index can be used as an alternative to ICDAS for reducing chairside time.

A study comparing the prevalence of incipient white spot lesions in visually impaired children using ICDAS II and CAST; concluded that CAST index described disease distribution precisely along with lesion severity, and preventive or curative needs in the examined population [27]. Two other studies [3,28] concluded that CAST index will enable health professionals to present the real depiction of preventable carious lesions to policy makers. Thus, CAST index is a useful and practical index in epidemiological surveys.

Ribeiro et al [14] highlighted the feasibility of CAST severity score and concluded that it focuses on disease severity. Based on CAST severity scores, patients were classified into mild, moderate and severe levels of dental caries. They stated that these scores will allow a superior investigation of public health information and also to authenticate the severity of the condition. Further investigation is required to assess the application of CAST severity scores in different population clusters with variable dental caries prevalence and ages [14].

Despite the fact that the current study is the first to compare Ora test and CAST index in the assessment of caries activity in children, it was limited by its relatively small sample size. Future studies are suggested to determine the effect of other parameters such as diet on oral health, salivary composition, etc.

The lack of specificity of Ora test can cause a greater chance of obtaining false positive readings. Positive findings can be seen in oral diseases such as gingivitis and other oral ailments. It is also incompetent of recognizing the source of the microorganisms. Hence this can also be considered as one of the limitations for Ora test, which would also require further research by further investigations.

## CONCLUSION

Based on our findings, caries activity was high in children with low Ora test values and in those with high CAST severity scores. We also found a significant negative correlation between Ora test and CAST scores, which indicates that lesion severity may be directly related to microbial activity.

From the results of the present study, we can conclude that both Ora test and CAST index can help in identifying children at high risk of developing caries. They are simple and easy tools which can be clinically implicated to detect caries at both individual- and community-levels.

CAST index can thus be considered as an important epidemiological tool to determine the severity of the disease. Ora test can assist to motivate patients/guardians and to reinforce a positive behavior in the child.

## CONFLICT OF INTEREST STATEMENT

None declared

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