Comparative Evaluation for Assessing Oratest as a Diagnostic Tool for Evaluation of Plaque Levels & Gingivitis

Mahasweta Joshi¹, Jayakumar⁴, Nikhil Joshi⁵

Abstract

Aims & Objectives: Periodontal disease comprises a group of inflammatory conditions of the supporting tissues of the teeth that are caused by bacteria. The present study was undertaken to evaluate whether Oratest could be used as a sensitive indicator of plaque levels and gingivitis.

Material and Methods: Hundred caries free patients visiting the out-patient department of A.E.C.S. Maaruti College of Dental Sciences and Research Centre, Bangalore were selected according to predetermined selection criteria. The study consisted of two clinical stages: Stage 1. Recording of Plaque Index (Loe, 1967) and Gingival Index (Loe & Silness 1963) in the subjects. Stage 2. Performance of the Oratest and recording the scores in the same subjects. The Oratest was performed by rinsing the mouth with sterilized UHT (Ultra high temperature) milk. About 3 ml of expectorated milk was added to a test tube containing the 0.12 ml of 0.1% methylene blue. The time required for colour change from blue to white attained at the bottom of the tube was recorded. Plaque Index, Gingival Index and Oratest scores were compared using Student t test. Pearson correlation test was applied to assess correlation between the indices and Oratest scores. Results: The results of the study showed that as age increased plaque and Gingival Indexes also increased whereas Oratest scores decreased. No significant difference between males and females was found in mean values of Plaque Index, Gingival Index and Oratest scores. Negative correlation was seen between Plaque Index and Oratest scores (r = 0.724) and also between Gingival Index and Oratest scores (r = 0.728). Conclusions: The study showed high correlation between the plaque and gingival indices and Oratest scores. This study validates Oratest as a predictable & sensitive test to assess periodontal disease.

Key words: Gingival inflammation, Microorganisms, Oratest, Plaque Index, Periodontal Diseases.

Introduction

Periodontal disease comprises of a group of inflammatory conditions of the supporting tissues of the teeth that are caused by bacteria. Our understanding of the etiology of periodontal diseases has undergone major advances in recent decades. In the mid 1900s it was believed that all bacterial species found in dental plaque were equally capable of causing disease and that periodontitis was the result of cumulative exposure to dental plaque in conjunction with a diminished host response and increased host susceptibility with age.¹

Gingivitis (inflammation of the gingiva) is the most common form of gingival disease with bacterial plaque attached to tooth surface causing inflammation along with other irritating factors which favour plaque accumulation. The most common form of gingivitis is plaque induced gingivitis. Numerous studies have shown that dental plaque accumulation induces and promotes gingivitis.²,³

One of the most commonly used indices for assessing plaque is Plaque Index (Loe, 1967), which has good validity and reliability. Disadvantage is subjectivity in estimating

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¹Lecturer, Department of Public Health Dentistry
²Reader, Department of Prosthodontics
³Department of Public Health Dentistry, Sinhgad Dental College & Hospital, Pune - 411 041, Maharashtra, India. email: sastry_sweta@yahoo.com
⁴Professor and Head, Department of Preventive and Community Dentistry, A.E.C.S. Maaruti College Of Dental Sciences & Research Centre, Bangalore - 560076, India.
⁵For assessing severity of gingivitis, the Gingival Index (Loe & Silnes, 1963) is widely used. Advantage is validity, reliability and ease of use. Disadvantage is it does not discriminate between mild & moderate as well as between moderate and severe gingivitis.⁵

Studies that have used appropriate microbiologic procedures have clearly demonstrated that the total number of bacteria, determined by microscopic counts per gram of plaque, was twice as high in periodontally diseased sites than in healthy sites.⁶,⁷ A variety of diagnostic procedures have developed to assess the microbiological aspects of periodontal diseases.

Oratest is a simple, non invasive technique which was reported by Tal and Rosenberg et. al to estimate oral microbial levels.³ In this test, the subjects rinse their mouth with sterile milk which dislodges the micro-organisms and also produces a substrate for their further metabolism. The expectorate is then added to methylene blue and color changes are observed over a period of time. This test is based on the rate of oxygen depletion by microorganisms in expectorated milk samples. 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 formation of leucomethylene blue can be easily observed because of the white color of milk.⁷,⁸ Unfortunately there is very little literature available regarding this test since it was first elucidated by Tal &
Rosenberg two decades ago. To validate a technique, more studies need to be done. The present study was conducted to confirm the validity of the study or lack of it. In the present clinical study, Oratest was used to assess periodontal disease by comparing it with Plaque Index & Gingival Index.

The objectives of the present study were to:
1. Compare Plaque Index, Gingival Index and Oratest scores with age & gender of subject
2. Compare of Plaque Index & Gingival Index with time taken for color change in Oratest
3. Correlate Plaque Index and Gingival Index with Oratest scores.

Material & Methods
Patients visiting the out-patient department of A.E.C.S. Maaruti College of Dental Sciences and Research Centre were randomly selected for the study. Ethical clearance was obtained from the ethical committee of A.E.C.S Maaruti College of Dental Sciences & Research Centre, Bangalore before the commencement of the study. The inclusion criteria for patients were:

- Patients willing to participate in the study
- Patients above 18 years of age
- Patients without dental caries
- Absence of history of antibiotic intake for the past one month
- Absence of abscess, draining sinus opening or cellulites related to oral cavity
- Patients who had eaten or drunk 90 minutes prior to the start of the study

All patients who did not satisfy the inclusion criteria were excluded and finally 50 male & 50 female patients who satisfied the inclusion criteria were included in the study. All the selected subjects completed the study.

Calibration of the investigator
The investigator was calibrated in the Department of Preventive & Community Dentistry and Department of Periodontics of A.E.C.S Maaruti College of Dental Sciences & Research Centre, Bangalore-76 for recording of plaque and Gingival Index scores. A group of 10 patients above 18 years of age visiting outpatient department of A.E.C.S Maaruti College of Dental Sciences & Research Centre were examined using a sterile mouth mirror and explorer on 4 successive days in the department of Preventive and Community Dentistry. The results were analyzed to know the diagnostic variability and the agreement for assessment was found to be 90% with a Kappa value of 0.07 for inter examiner variability.

Inform consent, demographic data and history of antibiotic intake was taken from the subject prior to the clinical examination and Oratest. The plaque levels and gingival inflammation were assessed using Plaque Index (Loe,1967) and Gingival Index (Loe and Sillness,1963). The participants were given 10 ml of ultra high temperature sterilized milk in a beaker and asked to rinse his/her mouth vigorously for 30 seconds which was timed using a digital stop watch. After 30 seconds, the subjects were asked to expectorate contents of mouth which was collected in a sterile beaker. Three ml was immediately transferred with a disposable syringe to a test tube containing 0.12ml of 0.1% methylene blue. The expectorated milk and 0.1% methylene blue was thoroughly mixed and the test tube was then sealed using a rubber cork and coded. The test tube was placed on a test tube stand with a mirror beneath the test tube to observe for color change. Time taken for the color change from blue to white within a 6mm diameter ring at the bottom of the test tube was checked at a regular interval of 5 minutes. The Oratest score is thus the time (in minutes) at which the color change from blue to white occurs at the bottom of the test tube.

The data obtained was analyzed by using SPSS version 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 (statistical software package). Student t test (Two tailed, Independent) Kruskall-Wallis ANOVA has been employed to test the significance of study parameters between the two groups of subjects. Pearson correlation has been used to find the relationship between Plaque and Gingival Index with time taken for colour change.

Results
A total of 100 caries free participants enrolled in the study in the age range of 18 years to 49 years where the mean age of the study subjects was 21.76 years (SD±5.35) of which 50 were females and 50 were males.

The mean values of the three study parameters, Plaque Index and Gingival Index time taken for color change (Oratest score) were compared between males and females and were statistically not significant (Table 1). The mean time taken for color change in this study was 51.33 minutes.

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Male</th>
<th>Female</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque Index</td>
<td>0.52 0.26</td>
<td>0.59 0.27</td>
<td>0.272</td>
</tr>
<tr>
<td>Gingival Index</td>
<td>0.39 0.23</td>
<td>0.44 0.32</td>
<td>0.439</td>
</tr>
<tr>
<td>Oratest score(Time taken for colour change in minutes)</td>
<td>50.89± 22.51</td>
<td>51.49±19.98</td>
<td>0.897</td>
</tr>
</tbody>
</table>

Table 1: Comparison of Plaque Index, Gingival Index and Oratest scores with gender
As age increased, Plaque Index and Gingival Index increased significantly and oral test scores decreased significantly (p<0.001). The mean time taken for colour change in this study was 51.33 minutes (Table 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age in years</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Up to 20 years</td>
<td>21-25 years</td>
</tr>
<tr>
<td>Plaque Index</td>
<td>0.56±0.22</td>
<td>0.51±0.26</td>
</tr>
<tr>
<td>Gingival Index</td>
<td>0.38±0.22</td>
<td>0.36±0.27</td>
</tr>
<tr>
<td>Time taken for colour change</td>
<td>50.46±17.04</td>
<td>59.48±24.22</td>
</tr>
</tbody>
</table>

Negative correlation was observed between Plaque Index and time taken for color change (r = 0.724) which was significant.

Discussion

Periodontal disease causes loss of more teeth in adult than does any other disease. Studies have shown that in periodontal diseases there is an overall increase in the microorganisms. Plaque found at diseased sites, suggest that the total bacterial load is much greater than at healthy sites. Direct correlation has been observed between the amounts of bacterial debris as determined by oral hygiene index scores with severity of gingivitis as determined by gingivitis index scores.

Various techniques are available to detect and distinguish different bacteria, and also to detect bacterial species numbers with varying selectivity and sensitivity. Some of these methods are darkfield or phase contrast microscopy, culture techniques, immunological assays like ELISA (enzyme-linked immunosorbent assays), DNA probes and enzyme based assays like BANA (benzyl arginine naphthylamide). These techniques have proved difficult to use because of the variability and unreliability of these methods. Main disadvantages of periodontal diagnostic test systems using bacterial markers are: polymicrobial nature of the disease, most are not predictive of disease activity, the site to sample needs to be known, technical problems associated with the sampling and culturing processes, detection of bacteria only being searched for and some are sent away to a special laboratory. Also some of the chair side tests like DNA probe systems are very expensive.

Hence new methods of diagnosing periodontal diseases are required, which are simple, inexpensive, less time consuming and do not require either the use of sophisticated equipment nor specialized personnel for assessing treatment needs and improving the oral health of the community. Oratest is one such diagnostic tests to estimate oral microbial levels, which was developed by Tal and Rosenberg.

In the present study, correlation was sought between Oratest scores and commonly used clinical parameters i.e. Plaque Index and Gingival Index. Statistically significant negative correlation was seen between Plaque Index and Oratest scores (r = 0.724) and also between Gingival Index and Oratest scores (r = 0.728). These findings are consistent with findings in study conducted by Tal and Rosenberg which also indicated that the two parameters were related i.e. in general higher the Oratest scores, the lower the Plaque Index and lower the Gingival Index scores. The possible explanation may be that plaque contains more number of microorganisms (twice as high) in periodontally diseased sites than in healthy sites. The bacterial load is increased with increase in severity of gingivitis.
The present study revealed that as the age increased, plaque levels and severity of gingivitis increased, whereas Oratest scores decreased which was found to be highly significant \((p<0.001)\). However no such correlation was found in the study conducted by Tal and Rosenberg.\(^1\)

A strong association has been described between plaque & periodontal disease in its initial stages.\(^2\) However there is no clear evidence to suggest that propagation of periodontal disease is linked to length of time plaque is present (the age effect).\(^3\) In this study, the relation between age and plaque index was significant. The increase in plaque scores as age increased may reflect the cumulative effect of plaque accumulation as age increases.

Various studies have indicated that as age increases the incidence of periodontal disease (including gingivitis) increases.\(^4,5\) But the cause for this was previously believed to be because age was considered as a marker for periodontal disease.\(^6\) But recent studies suggest the increased susceptibility of periodontal disease as age increases is due to cumulative effect of prolonged exposure to true risk factors and broader changes in the immune system.\(^12-15\) In the present study, significant relation was found between age and gingivitis index. The significant increase in gingival scores as age increased (except between age groups <20 years & 21-25 years and between age groups 26-30 years & >30 years) can be attributed again to the progressive accumulation of plaque over years and probably indicates changes in immune system. Direct correlation between amount of bacterial debris and severity of gingivitis is well established.\(^7\) As bacterial debris is a risk factor for periodontal disease, its monitoring becomes critical. This is the biggest advantage of the Oratest. The Oratest scores decreased significantly as age increased. There was a significant difference in Oratest scores between different age groups. The significant decrease in Oratest scores as age increased is more in line with that of gingival scores than plaque scores. The reasons for this may be that Oratest scores are more consistent with bacterial load that with presence/absence of plaque.

The result of present study revealed no statistical difference in the Plaque Index, Gingival Index and Oratest scores between men and women. However, in many previous studies, women have fared better than men when plaque accumulation and gingivitis were assessed.\(^16\) The mean time taken for color change in this study was 51.33 minutes, which was much less than the mean time of 178 minutes seen in the study conducted by Tal and Rosenberg et al.\(^7\) The exact reason for such finding needs to be further explored.

The data of the present study suggests that volunteers with high plaque levels and increased severity of gingival inflammation almost invariably yielded relatively rapid color changes. But there is no clear cut demarcation to distinguish patients with the disease and those without the disease, or between those with mild to severe periodontal disease.

The sample size of the present study is too small to base any epidemiological reasoning to these findings. However, this highlights the need to conducted further studies to establish or refute such findings. A thorough search found only the abovementioned study which used Oratest to evaluate plaque and gingival disease. There were few studies which used Oratest as a marker for caries activity,\(^8,17,18\) monitor denture hygiene\(^9\) and estimation of oral microbial levels\(^7\) but these are not in context of the present study.

The advantages of this study are: it is simple, inexpensive, non-invasive, less time consuming, reproducible and requiring no trained personnel. It can also be used to monitor mouth rinse regime, denture hygiene, gingival inflammation, plaque levels & anticipate the onset of caries. It is also a good educational & motivational tool for patients, school &
community dental health programs. Vehicle of the test (milk) is non-toxic. The disadvantages of this technique include: Its lack of specificity, as it does not identify the source of microorganisms and also positive observations are obtained in gingivitis & other oral ailments. It does not identify a specific group of organism in a specific disease. It cannot accurately differentiate between healthy state & between initial and progressive carious lesion.8,20

Though present study proves that oratest reflects the oral microbial levels, sensitivity and efficacy of the technique and ease of conducting this technique in a school and community setting as well as patient compliance has to be validated by further studies for its universal application.

Conclusion
The Oratest is a potential whole mouth diagnostic test to measure microbial load in intraoral diseases of epidemiological importance like periodontal disease and caries. More studies need to be conducted to validate the efficacy of this technique. The lack of sufficient studies to evaluate Ora test is the main reason why this is not universally acceptable in spite of its advantages and potential. Future scope lies in establishing the sensitivity of the technique, improving its specificity and establishing a grading system for this method so that results obtained using this technique may be universally standardized.

Acknowledgement
We would like to thank Dr. Suresh. K. P. (Ph.D.) Scientist (Biostatistics), National Institute of Animal Nutrition and Physiology, Bangalore for providing statistical assistance for the study

References

How to cite this Article: Joshi M, Jayakumar, Joshi N. Comparative evaluation for assessing oratest as a diagnostic tool for evaluation of plaque levels and gingivitis. J Dent Allied Sci 2012;1(2):52-56.

Source of Support: Nil.  Conflict of Interest: None Declared.