Profiling of Antioxidant Superoxide Dismutase in Saliva of Oral Submucous Fibrosis Patients to Categorize its Diagnosis in Varying Stages

Yuthicka Sirohi, Devi Charan Shetty, Aadithya B Urs, Harish Chandra Rai
Department of Oral Pathology, ITS-Centre for Dental Studies & Research (CDSR), Ghaziabad, Uttar Pradesh, India.

Abstract

Background: Oral submucous fibrosis is a premalignant condition in Indian and South-East Asia. Role of oxidant-antioxidant in causation and progression of cancer and pre cancers is known. Reactive oxygen species are generated in the oral cavity during chewing areca nut, the major etiological agent in oral submucous fibrosis.

Objectives: To see the alterations in the salivary superoxide dismutase levels in various clinical and histopathological grades of oral submucous fibrosis.

Materials and Methods: Unstimulated saliva was collected from 25 oral submucous fibrosis patients and age and gender matched controls. The saliva was assessed for superoxide dismutase value by spectrophotometric method using assay kit (Bio Vision Catalog # K335-100). The oral submucous fibrosis cases were grouped into clinical stages and histopathological grades and superoxide dismutase values were compared in different clinical stages and histopathological grades.

Results: The superoxide dismutase levels were reduced in oral submucous fibrosis as compared to controls. A steady decline in the levels was seen as the clinical stage and histopathological grade of oral submucous fibrosis advanced.

Conclusions: Salivary superoxide dismutase levels can be alternatively used as a surrogate marker for the diagnosis of oral submucous fibrosis.

Policy message: Oral physicians should advise the pan chewers to regularly check their salivary superoxide dismutase levels so as to ease the early diagnosis of oral submucous fibrosis.

Key words: Oral submucous fibrosis, reactive oxygen species, superoxide dismutase, saliva.

Introduction

Oral submucous fibrosis (OSMF) is a chronic fibrosing disease of oral cavity and oropharynx leading to limitation in mouth opening. Its pathogenesis is not well established, but is believed to be multifactorial. The chewing of betel quid (containing areca nut, tobacco and slaked lime) has been recognized as one of the most important risk factors for oral submucous fibrosis.

Reactive oxygen species are generated in the oral cavity during chewing betel quid (areca nut) which is rich in Fe²⁺ and Cu²⁺ and raised tissue copper levels have demonstrated in oral submucous fibrosis tissues. Reactive oxygen species act by initiating lipid peroxidation while enzymatic antioxidant superoxide dismutase plays a key role in detoxification of superoxide anion radical and hence diminishes the toxic effects of this radical and other free radicals like hydrogen peroxide and hydroxyl radicals from secondary reactions.

The diagnosis and prognosis of oral submucous fibrosis is done through biopsy which is invasive, time consuming and causes psychological trauma. As oral submucous fibrosis is a precancerous condition and not a precancerous lesion, therefore, it is important to select an appropriate site for biopsy where histopathological features are present. Moreover, routine histopathological examination depends on the expertise of the examiner. Therefore, it is important that the diagnostic tests are simple, fast, less invasive and easily interpreted. They should give a reliable diagnosis, prognosis and be useful in monitoring the response to treatment.

The mean serum superoxide dismutase levels in oral submucous fibrosis patients were found to be decreased (mean superoxide dismutase 86.63±20.36 U/ml) as compared to the healthy control group (mean superoxide dismutase 1.50±0.30 U/ml). The authors correlated the results with the premalignant potential of oral submucous fibrosis.  

Corresponding Author:
Yuthicka Sirohi
Department of Oral Pathology
ITS-Centre for Dental Studies & Research (CDSR)
Ghaziabad, Uttar Pradesh, India.
Email: yuthickasirohi@gmail.com
This study was designed to measure the levels of superoxide dismutase in saliva of oral submucous fibrosis patients and hypothesize its role in pathogenesis and progression of oral submucous fibrosis.

**Materials and Methods**

Subjects clinically suspicious of having oral submucous fibrosis who were routinely visiting the OPD of ITS-Centre for Dental Studies and Research, Ghaziabad, were selected for the study. Age and gender matched healthy individuals without any habit histories were taken as controls to assess the variation in superoxide dismutase levels between oral submucous fibrosis patients and healthy individuals. Informed consent was taken from them followed by history recording. All patients were males between the ages of 18-35 years and were free of any systemic disease and had not received any therapy for oral submucous fibrosis. The patients were grouped clinically according to Ranganathan et al (2004) classification. After establishing clinical diagnosis and one hour after the food intake, patients were asked to generate saliva in their mouth and spit it in a wide test tube for 10 minutes. The saliva was immediately centrifuged at 4000 rpm for 10 minutes and the resulting supernatant was used for further biochemical analysis. A punch biopsy of the cheek was simultaneously done from an appropriate site. Histopathologically the tissue was fixed, processed and graded according to Sirsat and Pindborg (1967) classification by a panel of three oral pathologists.

Superoxide dismutase was estimated using assay kit (Bio Vision Catalog # K335-100). This kit utilizes WST-1 that produced a water soluble formazan dye upon reduction with superoxide anion. The rate of reduction with a superoxide anion was linearly related to xanthine oxidase activity, and is inhibited by superoxide dismutase. The inhibition activity of superoxide dismutase was determined by a colourimetric method using a spectrophotometer at wavelength of 450 nm.

The data was analyzed with one-way analysis of variance (ANOVA) test.

**Results**

Twenty male patients with oral mucosal fibrosis and twenty controls were included in the study. Superoxide dismutase levels in saliva were compared between patients and controls. Superoxide dismutase levels were lower in patients (0.70 U/ml) than in controls (0.98U/ml) (Figure-1).

The levels of superoxide dismutase were compared within different clinical and histopathological grades of oral submucous fibrosis and a steady decline in superoxide dismutase levels was seen with the advancement of disease (Figure-2). On application of ANOVA a statistically significant decrease in superoxide dismutase levels were observed in various clinical stages and histopathological grades of oral submucous fibrosis (Table-1 & 2).

**Table 1: Assessment of SOD levels in various clinical stages of oral submucous fibrosis.**

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>Mean SOD levels</th>
<th>SD</th>
<th>F value</th>
<th>p value of Sig (2 tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I (n=7)</td>
<td>0.8343</td>
<td>0.03409</td>
<td>92.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stage II (n=12)</td>
<td>0.6642</td>
<td>0.03965</td>
<td>92.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stage III (n=6)</td>
<td>0.46</td>
<td>0.07694</td>
<td>92.43</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 2: Assessment of SOD levels in various histopathological grades of oral submucous fibrosis.**

<table>
<thead>
<tr>
<th>Histopathologic Grade</th>
<th>Mean SOD levels</th>
<th>SD</th>
<th>F value</th>
<th>p value of Sig (2 tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early (n=8)</td>
<td>0.77</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately advanced (n=14)</td>
<td>0.64</td>
<td>0.11</td>
<td>9.767</td>
<td>0.001</td>
</tr>
<tr>
<td>Early (n=3)</td>
<td>0.46</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Superoxide dismutase levels were low in patients with oral mucosal fibrosis and a steady decrease in the levels was found with the advancement of disease in concordance with previous studies. The decline in superoxide dismutase levels showed a statistically significant correlation between the clinical stages of oral submucous fibrosis. However, no significant correlation was found between the superoxide dismutase levels and histopathological stages in the present study. The enzymatic serum superoxide dismutase levels in oral submucous fibrosis patients did not show any significant change in any stage of oral submucous fibrosis.

Oral submucous fibrosis is one of the prevalent premalignant conditions in Indian and South-East Asian population. It is easy to diagnose but difficult to manage because of its progressive nature even after cessation of the adverse habits.

The etiopathogenesis of oral submucous fibrosis is multifactorial with a very strong correlation between betel quid chewing and development of disease with a dose dependant relationship for both frequency and duration of chewing areca nut (without tobacco). Substantial amounts of reactive oxygen species like superoxide anion and hydrogen peroxide are produced in the oral cavity during chewing of areca nut and catechu at a pH > 9.5. This areca nut-induced production of reactive oxygen species is enhanced by Fe^{2+}, Fe^{3+} and Cu^{2+}, but is inhibited by Mn^{2+}. These radicals can transfer their unpaired electron to oxygen to give free superoxide which is essential in this system to prevent oxidative stress. The superoxide dismutase catalyzes the dismutation of superoxide to hydrogen peroxide.

\[
\text{O}_2^- + \text{O}_2^- \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]

The hydrogen peroxide is then removed by glutathione peroxidase or catalase and this oxidative stress, arising as a result of imbalance between free radical production and antioxidant defences causes damage to a wide range of molecular species including lipids, proteins and nucleic acids. Antioxidants like, superoxide dismutase when present in low concentrations, as compared, to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate. These antioxidants prevent lipid peroxidation, protein damage and modification of DNA bases or as chain breaking antioxidants that receive or donate electrons to the free radical with formation of stable products or as transition metal binding proteins which act by sequestering Fe or Cu, so that they are not available to drive the formation of hydroxyl radical. The serum levels of antioxidants like superoxide dismutase and catalase are decreased in oral squamous cell carcinoma and oral submucous fibrosis.

The current study sheds further light on the role of saliva and oxidative stress in the pathogenesis of oral submucous fibrosis. As saliva can be easily obtained, tested and monitored for its antioxidants, nitrosation products, oxidized DNA, proteins, etc) therefore, using this marker one can help plan an early intervention, with local therapeutic agents acting as antioxidants that can be easily applied to the mucosa.

References