Correlation of Presence of Salivary Fibrin Producing Factor with Plasma Fibrinogen Level in Oral Submucous Fibrosis, Arecanut Chewers and Normal Subjects

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ABSTRACT
Oral submucous fibrosis (OSMF) is a chronic disease of the oral mucosa caused by arecanut, however, it is not understood why all arecanut chewers are not the sufferers. Further, maximum changes are seen in areas of oral mucosa bathed in saliva raising the suspicion that some salivary factors influence the disease process. One such suggested factor is fibrin producing factor (FPF). Plasma and saliva samples of OSMF patients (n = 50), arecanut chewers without OSMF (n = 50) and healthy individuals (n = 50) were subjected for estimation of plasma fibrinogen level and presence of salivary FPF. In 43 OSMF patients with positive salivary FPF, there was increase in plasma fibrinogen level as compared to the healthy individuals, arecanut chewers and OSMF patients as a whole. In FPF negative OSMF cases (n = 7) average plasma fibrinogen level was very less as compared to OSMF patients as a whole. From our findings it can be established that increased plasma fibrinogen level and presence of salivary FPF have some role in pathogenesis of OSMF.

Keywords: Oral submucous fibrosis, Salivary fibrin producing factor, Plasma fibrinogen, Arecanut.

INTRODUCTION
Oral submucous fibrosis was described by Schwartz in 1952 among five Indian females living in Kenya and he coined the term atrophia idiopathica (trophica) mucosae oris. Several other descriptive terms have been attributed; submucosal fibrosis of palate and pillars, diffuse oral submucous fibrosis idiopathic scleroderma of the mouth, idiopathic palatal fibrosis and sclerosing stomatitis. It is characterized by a juxtaepithelial inflammatory reaction followed by fibroelastic change in the lamina propria and associated epithelial atrophy. This leads to a restricted mouth opening, resulting as trismus leading to restriction of food consumption, difficulty in maintaining oral health as well as impairs the ability to speak. The involvement of mucosa progresses into the anterior as well as posterior faucial pillars and soft palate. If the fibrosis extends into the esophagus, the patient may experience progressive dysphagia. Moreover, at any stage of the OSMF the overlying epithelium may become the site of nonspecific ulceration, dysplastic change or malignant transformation. The incidence of malignant changes in oral submucous fibrosis has been reported to be from 3 to 7%.

The etiology of the disease over the intervening years was thought to be multifactorial and several agents have been implicated, including the consumption of large amounts of chillies, spicy food, tobacco lime, betel leaf, fennel, nutritional deficiency, genetic predisposition and autoimmune disease. Conclusive evidence now exists indicating that OSMF is caused by chronic irritation of mucosa with arecanut, a masticatory substance used predominantly by peoples of South and South-East Asian ethnicity.

Although arecanut is attributed to cause OSMF, it is not understood why all arecanut chewers do not suffer from this disease. It is also observed that maximum changes are seen in areas of oral cavity bathed with saliva which raises the suspicion that some other factors in saliva of the patients may influence the disease process.

Phatak AG described a thrombin-like fibrinogen producing factor (FPF) in saliva of the patients suffering from OSMF. He suggested that FPF enters into the submucous zone of the oral cavity and acts on the available fibrinogen resulting in local fibrosis. There is a possibility that FPF present in the saliva...
may be directly mitogenic to fibroblasts or by acting on the diffused fibrinogen may induce the fibrin formation.

As there is higher prevalence of OSMF in Indian subcontinent, it is important to study the extent of the presence of FPF in the saliva, in normal individuals and patients suffering from OSMF. It may help to show that those individuals having positive FPF in saliva may be more susceptible or have higher incidence of OSMF. Moreover, depending on the presence of this factor it would also be possible to predict the development of OSMF in any person who continues to take arecanut or other irritants.

This study was aimed at the correlation of plasma fibrinogen and FPF in saliva that might help to pinpoint the etiology of OSMF and in future may help to evolve some lasting treatment for this dreaded disease.

MATERIALS AND METHODS

Fifty OSMF patients were selected for the estimation of presence of FPF in saliva and the level of plasma fibrinogen (group I). Other two groups comprised of arecanut chewers (group II, n = 50) and control group of healthy individual (group III, n = 50). Individuals with the habit of arecanut chewing without any oral changes for OSMF were included in group II. Those subjects that had absolutely no habit of chewing arecanut in any form and free of any oral lesion were included in group III. A detailed case history was recorded along with careful intraoral examination for all individuals. Morning sample of the saliva was collected from all individuals without using a salivary stimulant and about 10 ml saliva centrifuged to remove the particulate matter. Plasma samples were collected after centrifugation of oxalated blood and stored in a refrigerator.

Detection of FPF in Saliva

Different combinations of plasma and saliva were dispensed in test tubes for detection of clot formation. All the test tubes were incubated at 37°C and observed at the interval of 2, 4 and 24 hours for the clot formation (Table 1). The procedure was performed for all the individuals in three groups.

Estimation of Plasma Fibrinogen

Plasma fibrinogen was estimated by the King’s method (1956). One milliliter of oxalated plasma was added to 1 ml of 2.5 gm% calcium chloride and 14 ml of distilled water and incubated for 1 hour at 37°C for the clot formation. Clot so formed was removed with a glass rod, washed with distilled water, digested and treated with biuret reagent and read on colorimeter using a green filter.5

OBSERVATIONS AND RESULTS

The average age in OSMF group, arecanut chewers group and normal group was 30.4, 31.44 and 33.34 years respectively. In the present study, majority of the patients suffering from OSMF were found to be in grade II (46%) followed by grade III (44%) and 10% were in grade I.

Salivary FPF was present in 43 OSMF patients (86%) whereas seven cases were found to be negative. In group II, six cases (12%) were detected positive for FPF and 44 cases were negative. FPF was not detected in saliva of group III (Table 2).

The mean plasma fibrinogen level in group I, II and III was 429.5 mg% ± 93.36, 251 mg% ± 38.71 and 238.48 mg% ± 46.23 respectively. In normal individuals, average plasma fibrinogen level in males (n = 47, 94%) was 240.95 mg% ± 36.62 and 233.33 mg% ± 51.37 in females (n = 3, 6%). In group II, average plasma fibrinogen level in males (n = 41, 82%) was found to be 250.60 mg% ± 40, whereas in females (n = 9, 18%) it was 255.55 mg% ± 38.71. In group I, the average plasma fibrinogen level was 429.8 mg% ± 91.60 and 427.77 mg% ± 100.99 in males (n = 41, 82%) and females (n = 9, 18%) respectively (Table 3).

The average plasma fibrinogen level was 455 mg% ± 67.82, 434.78 mg% ± 85.57 and 418.18 mg% ± 103.95 in grade I, grade II and grade III of OSMF respectively (Table 4).

In group II, average plasma fibrinogen level in salivary FPF positive cases (n = 6) was 320.83 mg% ± 17.17 and in 44 salivary FPF negative cases it was 242.61 mg% ± 30.42. In group I, average plasma fibrinogen level in positive salivary FPF cases (43 cases) was 447.09 mg% ± 83.21 whereas in FPF negative cases (n = 7) it was 325 mg% ± 80.17. Average plasma fibrinogen level in various grades of OSMF positive and negative FPF cases is given in Table 5.

DISCUSSION

In group I, FPF in saliva was found in 43 patients (86%) out of which, 35 patients (70%) were males and eight patients (16%) were females. Out of 41 males, 35 male patients (85.37%) had positive FPF and out of nine females, eight patients (88.89%) had positive FPF in saliva.
In group II, FPF in saliva was found in six patients (12%), out of which five patients (83.3%) were males and one patient (16.66%) was female. Out of 41 males, five patients (12.19%) had positive FPF in saliva and out of nine females, one patient (11.11%) was positive for FPF in saliva.

In the present study, detection of FPF in males and females of both group I (M:F = 4.37:1) and group II (M:F = 5:1) is very similar which shows that gender does not play any role in production of FPF in saliva.

Phatak AG5 had detected FPF in saliva of all the seven patients (100%) suffering from OSMF. However, in our study considerably larger sample (n = 43) was found to be positive for presence of salivary FPF positive indicating major role of FPF in OSMF. On this basis, it can be suggested that presence of FPF in saliva can be a useful marker in susceptibility for development of FPF in saliva.

Phatak AG6 further detected FPF in the saliva collected from parotid duct by cannulation. This saliva clotted both the oxalated plasma and the fibrinogen manufactured by Diagen, England, that suggests FPF in saliva has thrombin like behavior. This finding further supports to the theory that there are certain biochemical changes in saliva which are causing OSMF as maximal changes are noticed in the areas of oral mucosa which remain in contact with saliva for longer time.

**PLASMA FIBRINOGEN LEVEL**

It was observed that as compared to group III there is some rise of plasma fibrinogen level in arecanut chewers but it is not very significant. However, in OSMF patients the average plasma fibrinogen level was significantly elevated.

Phatak AG6 suggested that FPF enters into submucous zone of oral mucosa and acts on the diffused fibrinogen, inducing the fibrin formation and local fibrosis in later stages. Richardson7 stated that the fibrinogen and fibrin degradation products are chemotactic to a leukocyte which stimulates the fibroblastic activity and subsequent deposition of collagen in the connective tissue. Therefore, it can be concluded that diffused plasma fibrinogen in submucous tissues reacting with FPF or some such substance in saliva may be responsible for causation of OSMF.

Average plasma fibrinogen was obtained for various grades of OSMF individually and for males and females in each grade (Table 4). It was observed that although there is no significant difference in the plasma fibrinogen level in all grades of OSMF, but contrary to the expectation mean plasma fibrinogen level in grade I patients was more than grade II and grade III patients and mean plasma fibrinogen level in grade II patients was more than grade III patients. Therefore, it can be concluded that the plasma fibrinogen level does not affect the severity of the disease and increase in plasma fibrinogen level in grade I may be due to active phase of the disease.

Out of five patients in grade I, three patients (60%) had positive salivary fibrin producing factor and two patients (40%) had salivary negative fibrin producing factor. The average plasma fibrinogen level in positive salivary FPF cases (3 cases) was 491.66 mg% ± 57.37 whereas in negative salivary FPF cases (2 cases) it was 400 mg% ± 50. Out of 23 patients in grade II, 20 patients (87%) were positive and three patients (13%) were negative for salivary FPF. Out of 22 patients in grade III, 20 patients (90.9%) were positive for salivary FPF (Table 5).

From the above findings it was observed that in positive salivary FPF cases, there is no significant difference in plasma fibrinogen level between different groups but in negative FPF cases the average plasma fibrinogen level were less than in

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**Table 3: Plasma fibrinogen levels in OSMF, arecanut chewers and normal individuals**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma fibrinogen levels (mg% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
</tr>
<tr>
<td>OSMF</td>
<td>429.87 mg% ± 91.60</td>
</tr>
<tr>
<td>Arecanut chewer</td>
<td>250.60 mg% ± 40</td>
</tr>
<tr>
<td>Normal</td>
<td>240.95 mg% ± 36.62</td>
</tr>
</tbody>
</table>

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**Table 4: Plasma fibrinogen level and FPF in various clinical grades of OSMF**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Males (n)</th>
<th>Females (n)</th>
<th>Total (n)</th>
<th>Plasma fibrinogen level</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>04</td>
<td>01</td>
<td>05</td>
<td>443.75 mg% ± 71.53</td>
</tr>
<tr>
<td>II</td>
<td>21</td>
<td>02</td>
<td>23</td>
<td>433.33 mg% ± 88.07</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>06</td>
<td>22</td>
<td>421.87 mg% ± 99.55</td>
</tr>
</tbody>
</table>

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**Table 5: Average plasma fibrinogen level in FPF positive and negative cases of various clinical grades of OSMF**

<table>
<thead>
<tr>
<th>Grades</th>
<th>FPF positive patients (plasma fibrinogen level)</th>
<th>FPF negative patients (plasma fibrinogen level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>03 (491.66 mg% ± 57.37)</td>
<td>02 (400 mg% ± 50)</td>
</tr>
<tr>
<td>Grade II</td>
<td>03 (443.75 mg% ± 80.57)</td>
<td>03 (341.66 mg% ± 51.37)</td>
</tr>
<tr>
<td>Grade III</td>
<td>02 (442.5 mg% ± 90.86)</td>
<td>02 (225 mg%)</td>
</tr>
</tbody>
</table>
patients with positive FPF cases. In grade III cases with negative salivary FPF, the average plasma fibrinogen level was much less than in grade I and II patients having negative FPF.

Average plasma fibrinogen level in normal individuals was 238.48 mg% ± 46.23 and they were FPF negative. In group II, average plasma fibrinogen level in six positive FPF cases (12%) was 320.83 mg% ± 17.17 whereas in FPF negative cases (n = 44, 88%), average plasma fibrinogen level was 242.63 mg% ± 30.42. In group I, 43 cases (86%) were positive for FPF in saliva and average plasma fibrinogen level was found to be 447.09 mg% ± 83.21, whereas in seven (14%) FPF negative cases it was 325 mg% ± 80.17.

From the above findings it was observed that in arecanut chewer group the mean plasma fibrinogen level in patients with positive salivary FPF (six cases) was more as compared to group III (238.48 mg% ± 46.23) and FPF negative arecanut chewers as well as arecanut chewers group as a whole. In arecanut chewer patients with negative FPF, average plasma fibrinogen level was within normal range.

In 43 OSMF patients with positive salivary FPF, there was increase in plasma fibrinogen level as compared to the levels in group III, group II and group I (n = 50) as a whole. Whereas in seven FPF negative cases average plasma fibrinogen level was very less (325 mg% ± 80.17) as compared to group I as a whole (429.5 mg%). It was further observed that average plasma fibrinogen level in FPF negative cases of OSMF is comparable with the average plasma fibrinogen level of arecanut chewer group with positive salivary FPF. From the study it can be accomplished that there is definite relation between the presence of salivary FPF and increased fibrinogen level in OSMF, but it is difficult to establish an exact relationship.

From the results of this study and other material available on the subject it appears that the hypothesis of pathogenesis of OSMF put forward by Phatak5,6 and others is questionable, following hypothesis may answer some unanswered questions. The excessive arecanut chewing may stimulate the collagen synthesis by fibroblasts but its tannin content increases the resistance of collagen to degradation which enhances the fibrosis, which in turn induces raised plasma fibrinogen level. Raised plasma fibrinogen level somehow induces FPF in saliva, presence of which causes OSMF. It can be safely stated that patients with raised plasma fibrinogen level with positive FPF are at greater risk of OSMF as compared to patients with normal fibrinogen level with negative FPF.

The development of severe OSMF in absence of FPF but raised plasma fibrinogen level in the present study may be due to presence of some factor similar to FPF in saliva which has not been defected so far. Isolation of that factor may further clarify the pathogenesis which may help to find out some simple method of treatment for this dreaded disease which is eluding till date.

On this basis, it can be stated that six positive salivary FPF cases in arecanut chewers group with increased plasma fibrinogen level than normal are more susceptible to develop OSMF, although the disease is not apparent clinically at present. They must be advised to discontinue arecanut chewing habit at the earliest.

CONCLUSION

The present study suggests that arecanut is responsible for increased plasma fibrinogen level and subsequent appearance of FPF in saliva. From our findings it can be established that increased plasma fibrinogen level and presence of salivary FPF have some role in pathogenesis of OSMF. Further studies are required to identify the exact role of arecanut in causing presence of salivary FPF in OSMF. This parameter can be very useful in educating and assisting the patient in discontinuation of the arecanut habit aiming towards the prevention of the disease.

REFERENCES