ORIGINAL ARTICLE

Plasma fibrinogen degradation products in betel nut chewers - with and without oral submucous fibrosis

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ABSTRACT

Context: Oral submucous fibrosis (OSMF) has a multifactorial etiology. Recent studies have shown that there is an increased level of fibrinogen degradation products (FDP) in plasma of OSMF patients suggesting its possible role in etiopathogenesis of OSMF. **Aims:** To detect the presence of FDP in the plasma of betel nut chewers with and without OSMF and in normal subjects without any habits, to correlate these levels with respect to the clinical and histological grading of OSMF and whether it can be used as a nonsurgical diagnostic aid in detection of suspected OSMF cases. **Materials and Methods:** Study comprised of 35 cases of betel nut chewers with OSMF, 10 patients with betel nut chewing habit but having apparently normal oral mucosa, and 10 normal patients without any habits. The patients were evaluated for plasma FDP levels. **Results:** All the betel nut chewers with OSMF showed the presence of plasma FDP. However, controls and subjects with habit, but without OSMF did not show FDP in the plasma. Spearman's rank correlation was used to find the association between the clinical and histological grades and it was not statistically significant (P = 0.910) and the correlation being 0.020. **Conclusion:** Since only those patients with OSMF have showed the presence of FDP in plasma, we suggest that our test can be utilized as a nonsurgical diagnostic aid in suspected OSMF patients.

Key words: Fibrin, fibrinogen degradation products, oral submucous fibrosis

INTRODUCTION

Oral submucous fibrosis (OSMF) is a chronic, progressive, scarring, high-risk potentially malignant disorder of the oral mucosa seen primarily in the Indian subcontinent and in southeast Asia.[1] OSMF has a multifactorial etiology. Various etiologic agents that are reported in the literature include betel nut, chillies, spicy foods, tobacco, nutritional deficiencies, autoimmunity and genetic susceptibility.[2-4] Initially patients complain of burning sensation in the mouth and in later stages, difficulty in mouth opening.[5] Microscopically, there is atrophy of epithelium and subjacent fibrosis.[6] Detection of fibrinogen degradation products (FDP) in plasma of OSMF patients has provided a new direction to describe the pathogenesis of OSMF.[7] This study was undertaken to detect the presence of FDP in the plasma of betel nut chewers with and without OSMF and in normal subjects without any habits and to correlate plasma FDP values in different clinical and histological grades of OSMF and if plasma FDP could be used as a nonsurgical diagnostic aid in early detection of OSMF.

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MATERIALS AND METHODS

The study comprised of 35 cases of betel nut chewers with clinically evident OSMF (Group A), 10 patients with betel nut chewing habit but having apparently normal oral mucosa (Group B), and 10 normal patients without deleterious habits and having normal oral mucosa (Group C). All the subjects were of similar age group (ranging from 20 to 40 years). After obtaining clearance from institutional ethical committee a detailed case history of each patient was recorded. Provisional diagnosis of OSMF was made on clinical examination, the confirmation of which was done by incisional biopsy and histopathological examination. The histological grading as given by Pindborg and Sirsat (1966) was followed for all the cases and the patients were clinically grouped, following the classification given by Ranganathan and Gauri (2006).[8]

Estimation of plasma FDP levels was done in the patients using a diagnostic kit for cross-linked fibrin degradation products (XL FDP - Tulip Diagnostics (P) Ltd; Goa, India). Two milliliter of venous blood was collected in a citrate bulb and routine hematological examination performed. Blood was then centrifuged at 4,000 rpm to separate the plasma, which was used for quantification of FDP levels. The quantification was done based on the XL FDP slide test which is based on the principle of agglutination. Agglutination in the highest plasma dilution corresponds to approximate amount of FDP level in nanogram per deciliter (ng/dl). The sensitivity of the kit used was 200 ng/dl, below which the values cannot be detected and only the values in multiples of 200 can be detected. To calculate the exact level of FDP in the plasma sample, the following formula was used.

FDP level in ng/dl = 200 × d, where d = highest dilution of plasma showing agglutination (as per the manual of Tulip Diagnostics).

RESULTS

Plasma FDP levels were detected (>200 ng/ml) in all Group A patients and the same was not detected in Group B and C patients. Since all the FDP values were same in Group A patients, no inferential statistical tests could be performed. We found that most of the Group A patients were in clinical grade II [Table 1, and Figure 1] and advanced histological grade [Table 2 and Figure 2]. We also found a trend that as clinical grade increased, more number of patients were in advanced histological grade [Table 3 and Figure 3]. Spearman's rank correlation was used to find the association between the clinical and histological grades and it was not statistically significant ($P = 0.910$) [Table 3 and Figure 3], the Spearman's rank correlation being 0.020. Statistical product and service solutions (SPSS) version 14.0 was used for computation of statistical tests.

DISCUSSION

OSMF has affected millions of individuals and is likely to reach an alarming proportion in the near future. Patients initially complain of burning sensation in the mouth on consuming spicy food. As the disease progress, the oral mucosa becomes blanched and white fibrous bands appear, leading to difficulty in mouth opening, inability to whistle and difficulty in swallowing.[9]

In spite of widespread research, the etiology of OSMF still remains multifactorial. Numerous agents like capsaicin, betel

Table 1: Number and percentage of patients in different clinical grades of oral submucous fibrosis

Clinical grade	Number of patients	Percentage of patients (%)
I	2	5.7
II	24	68.6
III	9	25.7
IV	-	-
Total	35	100

Table 2: Number and percentage of patients in different histological grades of oral submucous fibrosis

Histological grade	Number of patients	Percentage of patients (%)
Very early	-	-
Early	3	8.6
Moderately advanced	10	28.5
Advanced	22	62.9
Total	35	100

Table 3: Distribution of oral submucous fibrosis patients according to clinical and histological grades

Clinical grade	Histological grades				Total
	I	II	III	IV	
I	-	-	2	-	2
II	-	2	5	17	24
III	-	1	3	5	9
IV	-	-	-	-	
Total	-	3	10	22	35

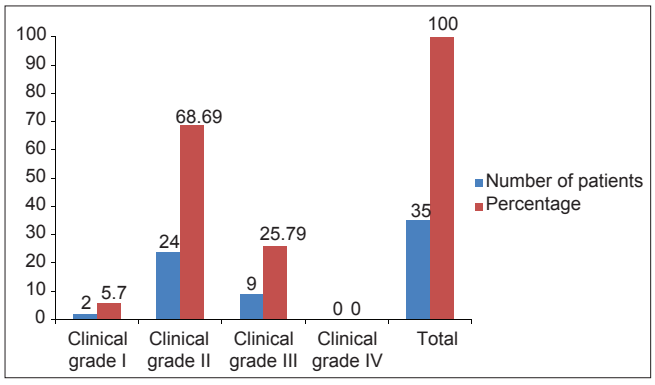

Figure 1: Number and percentage of patients in different clinical grades of oral submucous fibrosis

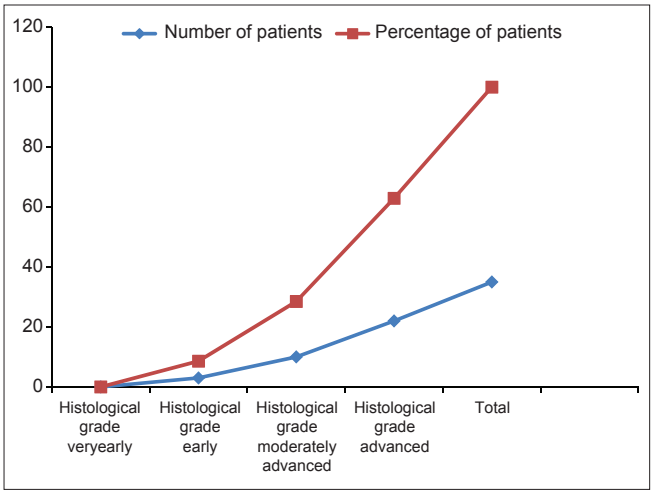

Figure 2: Number and percentage of patients in different histological grades of oral submucous fibrosis

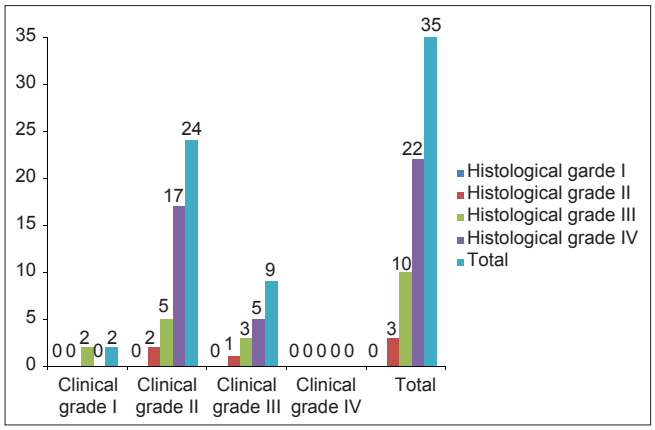

Figure 3: Distribution of oral submucous fibrosis patients according to clinical and histological grades

nut chewing, vitamin deficiency, autoimmunity, etc., have been considered as contributory factors, but no single factor has been established as being solely responsible for OSMF.[7]

Several ingredients of capsaicin have shown to produce OSMF. The preconditioned oral mucosa because of nutritional deficiency was found to be more prone for the action of different irritants leading to OSMF. However, the action of betel nut is supposed to be the major causative agent. A variety of chemicals released by betel nut chewing like arecoline and arecaidine are thought to stimulate the fibroblastic proliferation leading to fibrosis.[10] These constituents and their metabolites act as a source of continuous irritation to oral mucosa. The coarse fibers of betel nut cause mechanical irritation in addition to the chemical irritation to the oral mucosa. Over a period of time, due to persistent habit, chronic inflammation sets in at the site.[11]

Fibrinogen is an acute phase reactant which increases throughout inflammatory process. The body in response to inflammation produces more fibrinogen and its degradation products.[12] Fibrinogen is composed of three pairs of interwoven polypeptide chains, two Aα chains, two Bβ chains, and two γ chains. Both the Aα and Bβ chains have small fibrinopeptides A and B in their terminal region. Normally fibrinogen is converted to fibrin by the enzymatic action of thrombin which splits fibrinopeptides A and B from the molecule, leaving fibrin monomers which in turn, rapidly polymerize to form insoluble fibrin.[13]

Plasminogen, a zymogen is a glycoprotein which is synthesized in the liver and stored in the eosinophils. Increased concentrations of plasminogen are found in association with inflammation. In the fibrinolytic process which exists to counter-regulate the clotting mechanism, plasminogen is converted into enzyme plasmin. Plasmin degrades both fibrinogen and fibrin resulting in the appearance of essentially the same fragments for both, although Aα and Bβ chains may remain intact in fibrinogen fragments. Since fibrinogen is a single molecule, it is much more vulnerable to the action of plasmin than are the fibrin monomers that are covalently bonded to one another. The four principal degradation products are fragments X, Y, D (D-D dimer) and E.[13]

FDP have diverse functions. Fibrinopeptides aim to combat the inflammation, while FDP tries to counteract the fibrin-like action of fibrin precipitating factor (FPF) and thrombin produced in the autocatalytic process. Hence, as the severity of the disease increases, more amount of FPF is produced and in turn more amount of FDP is produced. Fragment Y and to some level fragment X, are identified to generate anticoagulant effect. But in OSMF, hemorrhagic manifestations are not encountered. Phatak, hence described FDP as "molecules immunologically similar to fibrinogen" (MISFI). Four F's, viz., FDP, FPF, increased fibrinogen level and fibrinogen cryoprecipitability are considered in fibrinogen metabolism. This suggests that OSMF is chiefly a disease of collagen metabolism causing excessive deposition of fibrin, which in turn results in restriction of mouth opening.[12]

We carried out our study to diagnose OSMF using blood plasma in order to avoid invasive biopsy procedures. Our first objective was to identify the presence of FDP in the plasma of betel nut chewers with and without OSMF and in normal subjects without any habits. FDP was not detected in plasma of Group C patients, due to the fact that the plasma FDP levels are below the detectable levels in them. Only when the levels rise above 200 ng/ml, they can be detected in the plasma. These findings are in accord with the results of Koshti and Barpande (2007) who carried out their study of detection of FDP in 35 OSMF patients and 35 normal controls.[7] As per our knowledge, ours is second study done to detect FDP in OSMF. Our criterion differs from the previous study of Koshti and Barpande (2007) in that, we have included 35 cases of betel nut chewers with and without clinical evidence of OSMF, as betel nut is considered to be one of the main etiologic factors of OSMF. Our objective was that if we can detect FDP in betel nut chewers without clinical OSMF, we can assume the patient is more likely to develop clinical manifestations of OSMF, it can act as an alarm and we can warn the patients about the likelihood of developing the disease.

Plasma FDP levels were detected (>200 ng/ml) in all betel nut chewers with OSMF. This might be because OSMF is a chronic inflammatory condition; the body in response produces more fibrinogen and its degradation products. Phatak[14] has suggested that saliva may have a role in the causation of OSMF. He detected FPF in saliva of OSMF patients and showed that parotid saliva of three OSMF patients clotted both the oxalated plasma and fibrinogen, suggesting FPF has thrombin-like behavior. He hypothesized that when this FPF encounters fibrinous exudates in the oral cavity, it rapidly clots the exudates resulting in fibrin. In response to this clotting, the body produces more fibrinogen. In an attempt to counter-regulate clotting, fibrinogen is degraded

into FDP by plasmin. He also suggested that an increase in the level of FDP is an early diagnostic sign of an increased rate of fibrin deposition.[12] Our results of detection of FDP in group A patients are in agreement with the results of Koshti and Barpande (2007) and of Phatak (1979 and 1984).[7,12,14] Hence, the hypothesis of FDP being an early diagnostic sign of fibrin deposition is supported.

Plasma FDP levels were not detected in all betel nut chewers without clinical evidence of OSMF (Group B). We have included this criterion as betel nut is supposed to be the main etiologic agent for OSMF, which was not incorporated in any of the previous studies. Since OSMF is a chronic disease and FDP played an important role in its etiopathogenesis, we expected to detect FDP in this group also, thereby attempted to know whether this can be used as a diagnostic test to predict an impending OSMF before it could manifest itself clinically. Secondly, it would have been useful in motivating and educating the patients about the present situation. However, FDP could not be detected in this group of patients which might be either because of body's strong immune reaction which has not evoked any detectable change or FDP might have been generated, but in such low levels that it could not be detected by the test kit used. Hence, we would like to suggest that a more sensitive kit that could detect extremely low levels of FDP should be used with a larger sample size before any positive statement is made.

Our second objective was to correlate the levels of plasma FDP with respect to the clinical and histological grading of OSMF. We found that the plasma FDP values were same (200-400 ng/ml) in all the clinical and histological grades of OSMF which was in contrast to the results of Koshti and Barpande (2007) who found a statistically significant increase of FDP with increase in clinical grades and an increase of FDP with increase in histological grades which was statistically insignificant.[7] This might be probably due to various antiplasmins present in plasma like α2-antiplasmin, α2-macroglobulin, α1-antitrypsin, and antithrombin-III acting on the free plasmin and not allowing it to act on the excess fibrinogen present as the grades of OSMF increased. There might be another possibility that FDP levels might be more as the clinical and histological grades increase, but the values may be in the range of 200-400 ng/ml.[13] We presume that carrying out studies with kits detecting FDP levels with less range than 200 ng or a more sensitive kit might be useful.

The association between clinical and histological grades was not statistically significant ($P = 0.910$). This might be due to the fact that the clinical and histological grading system uses parameters that are independent of each other. Clinical grades are dependent upon degree of mouth opening, degree of fibrosis, and the area affected by fibrosis; whereas, histological grades are dependent upon the nature of the collagen bundles and the degree of hyalinization of collagen. These results are in agreement with those of Koshti and Barpande (2007) and Kiran Kumar *et al*.,[7,15] However, in our study we noticed that as clinical grade increased, more number of patients were in advanced histological grade.

CONCLUSION

In the present study it was found that all patients of Group A showed the presence of plasma FDP. Though the plasma FDP was detected in all OSMF patients, the values were same in all clinical and histological grades of OSMF and the association between them was not statistically significant. Hence, we emphasize the need for a more sensitive kit which is able to detect the precise values of FDP, so that a direct correlation can be established between FDP and clinical and histological grades of OSMF. Both Groups B and C did not show plasma FDP. Hence, we are of the opinion that our test could be used as a nonsurgical diagnostic aid in suspected OSMF cases. However, the need for biopsy cannot be excluded in establishing the diagnosis of OSMF as our test does not indicate the severity of the disease. Further studies at a larger scale with well-defined clinical and histological criterion may be helpful to identify the precise role of FDP in the pathogenesis of OSMF.

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