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Original Article

"Estimation of Plasma Fibrinogen Degradation Products in Oral Submucous Fibrosis: A Clinicopathologic Study"

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Abstract

Introduction: Oral submucous fibrosis (OSMF) is a potentially malignant disorder with a multifactorial etiology. Recent studies have shown that there are increased levels of fibrinogen degradation products (FDP) in the plasma of OSMF patients suggesting its possible role in etiopathogenesis.

Purpose: To assess the FDP levels in the plasma of OSMF patients in betel nut chewers and in healthy participants without any habits. To correlate the plasma levels of FDP in various clinical stages of OSMF and their role in the etiopathogenesis of the same. Further to read the significance of plasma FDP if any as a prognostic indicator in betel nut chewers without clinical OSMF.

Materials and Methods: This study included 40 cases of betel nut chewers with OSMF, 30 participants with habit of betel nut chewing, and 30 participants without the habit. All participants were evaluated for plasma FDP levels.

Results: In this study, all the participants with OSMF with habit were found to be FDP positive, there were no FDP positive cases for healthy participants without habit. Among the healthy participants with habit, 3 out of 30 were found to be FDP positive. Comparison of FDP values with clinical stages of 40 OSMF patients showed that the correlation was statistically significant.

Conclusion: About 40 cases of clinico-histopathological proven cases of OSMF were included in the present study. All OSMF cases showed an increased trend in the plasma FDP level which was found to directly correlated with the severity of the disease.

Key words: Fibrin, Fibrinogen, Fibrinogen degradation products, Oral submucous fibrosis

INTRODUCTION

Schwartz(1952)[1] described five Indian women from Kenya with a condition of the oral mucosa including the palate and pillars of the fauces, which he called "atrophia idiopathica (tropica) mucosaeoris," whereas Joshi (1953)[2] coined the same condition as oral submucous fibrosis (OSMF); however, plethora of terms are also reported to describe the aforesaid condition which includes "diffuse OSMF," "idiopathic scleroderma of the mouth," "idiopathic palatal fibrosis," "sclerosing stomatitis," and "juxta-epithelial fibrosis." Pindborg and Sirsat. (1966)[3]

According to Pindborg and Sirsat (1966),[3] "OSMF is insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxta-epithelial inflammatory reaction followed by a fibroelastic change of the lamina propria with epithelial atrophy leading to stiffness of mucosa and causing trismus and inability to eat."

Although a number of postulates such as areca nut chewing, intake of spicy foods stuffed with chilies, genetic

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predisposition, autoimmune characteristics, nutritional deficiency, and infectious agents have been reported to uncover the pathogenesis of the disease, still the credible etiology remains obscure, and therefore, mandating for extensive research in this field. Recent studies have shown that there are increased levels of fibrinogen degradation products (FDP) in the plasma of OSMF patients suggesting its possible role in etiopathogenesis.[4] With this view in mind, the present study is aimed to assess the FDP levels in the plasma of OSMF patients and their critical role in the etiopathogenesis of the same. Further to read the significance of plasma FDP as a diagnostic tool of OSMF in addition to their being labeled as a prognostic indicator in betel nut chewers without clinical OSMF.

MATERIALS AND METHODS

Total 100 participants were included in the study and divided into three groups. Group I: Comprised 40 cases of betel nut chewers with clinically evident OSMF. Group II: Comprised 30 participants with habit of betel nut chewing without OSMF. Group III: Comprised 30 participants without the habit of betel nut chewing and without OSMF. Before conducting the study, ethical committee approval was obtained.

Patients with any bleeding or clotting disorder and collagen diseases were excluded from the present study. A detailed case history of each patient was recorded. Provisional diagnoses of OSMF were made on clinical examination and were then further divided into three groups based on the clinical staging given by Ranganathan *et al.*(2001)[5-6]

For confirmation of the provisional diagnosis of OSMF, study participants were subjected to scalpel biopsy and histopathologically examined.

Collection of Sample

Under all aseptic conditions, 2 ml of venous blood was withdrawn by venipuncture and collected in a sodium citrate tube. Routine hematological investigations were performed. The tubes were allowed to stand for 1 h at room temperature and then centrifuged at 4000 rpm to separate the plasma. Then, platelet poor plasma (PPP) was prepared by centrifuging the supernatant (plasma) obtained after centrifuge (as above) for 10 min at 3700 rpm. This PPP was quantified for FDP levels.

Estimation of Plasma FDP

Plasma FDP was quantified using a diagnostic kit (TULIP XL FDP™)[7]-"a quantitative latex slide test for detecting cross-linked FDP in human plasma." XL FDP slide test for detection of cross-linked fibrin degradation products is based on the principle of agglutination. Agglutination is a positive result indicating D-dimer level above 200 ng/ml. No agglutination is a negative result indicating the absence of clinically significant D-dimer levels in the plasma specimen. Agglutination in the highest plasma dilution corresponds to the approximate amount of D-dimer level in ng/ml.

To calculate D-dimer level in ng/ml in the sample, following formula was used:

D-dimer level (ng/ml)=200×d

d = highest dilution of plasma showing agglutination during the semi-quantitative test of the sample.

Statistical Analysis

The results will be statistically analyzed using Chi-square test, analysis of variance test, and Kruskal-Wallis test.

RESULTS

Plasma FDP levels were detected >200 ng/ml in OSMF patients (Group I), and there are no FDP positive cases for participants without habit and without OSMF (Group III). Among betel nut chewers without clinical evidence of OSMF (Group II), 3 out of 30 were found to be FDP positive (Table 1 and Figure 1).

Comparison of mean plasma FDP levels and various clinical stages of 40 OSMF patients showed a statistically significant increase in the FDP levels with increase in clinical stages($P = 0.000$) (Table 2 and Figure 2).

DISCUSSION

OSMF is predominantly seen in Asian countries, with a high prevalence in India. Recent epidemiological data indicate that the number of cases of OSMF has increased exponentially from 2,50,000 in 1980 to 20,00,000 cases in 1993 justifying an alarming situation.[8] The reasons for such rapid increase of OSMF may be due to an upsurge in the popularity of commercially available areca nut preparations (pan masala) in India and an increased uptake of such preparation by young people due to easy access, effective price changes, and attractive marketing strategies.[9]

The prodromal symptoms include burning sensation in mouth on consumption of spicy food and appearance of blisters, especially in the palate. As the disease progresses, the oral mucosa becomes blanched and white fibrous bands appear, leading to difficulty in mouth opening.[10]

Although a number of postulates have been proposed in the etiopathogenesis of the OSMF, the exact causative factor (s) remained enigma. In the array of proposed hypothesis, the multifactorial origin of the disease is suggested. The role of local irritants in the form of chili, tobacco, areca nut, spicy food, alcohol, and underlying systemic disease can be speculated in view of the geographical and the ethnic distribution of the disease.

Although there is compelling evidence to implicate the habitual chewing of areca nut with the development of OSMF, there are still some cases where the incidence of OSMF was reported without the habit of areca nut chewing, and at the same time, all areca nut chewers do not necessarily develop OSMF.

Fibrinogen is an acute phase reactant which increases throughout the inflammatory process. The body in response to inflammation produces more fibrinogen and its degradation products.[4]

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.[11]

In the fibrinolytic process, fibrinogen is degraded by plasmin to fragments X, Y, A, B, C, D, and E. There are four principal fibrin degradation products called X, Y, D, and E. The most notable subtype of fibrin degradation products is the D-dimer. (Figure 3)[12-16]

Table 1: Comparison of FDP status in study groups

Groups	Number (%)		
	FDP status		Total
	Positive	Negative	
OSMF with habit	40 (100.00)	0 (0.00)	40 (100.00)
Participants without habit and without OSMF	0 (0.00)	30 (100.00)	30 (100.00)
Participants with habit and without OSMF	3 (10.00)	27 (90.00)	30 (100.00)
Total	43 (43.00)	57 (57.00)	100 (100.00)

Chi-square=88.984, *P*=0.000. FDP: Fibrinogen degradation products, OSMF: Oral submucous fibrosis

Table 2: Comparison of FDP values (ng/ml) with various clinical stages of OSMF patient

FDP value	Number (%)			
	Clinical stage			Total
	Stage I	Stage II	Sage III	
400.00	11 (100.00)	1 (4.35)	0 (0.00)	12 (30.00)
800.00	0 (0.00)	19 (82.61)	0 (0.00)	19 (47.50)
1600.00	0 (0.00)	3 (13.04)	6 (100.00)	9 (22.50)
Total	11 (100.00)	23 (100.00)	6 (100.00)	40 (100.00)

Chi-square=58.261, *P*=0.000, FDP: Fibrinogen degradation products

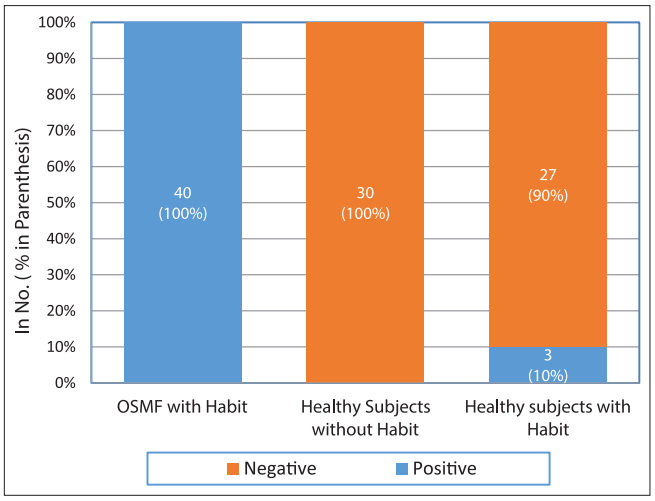

Figure 1: Comparison of fibrinogen degradation products status in study groups

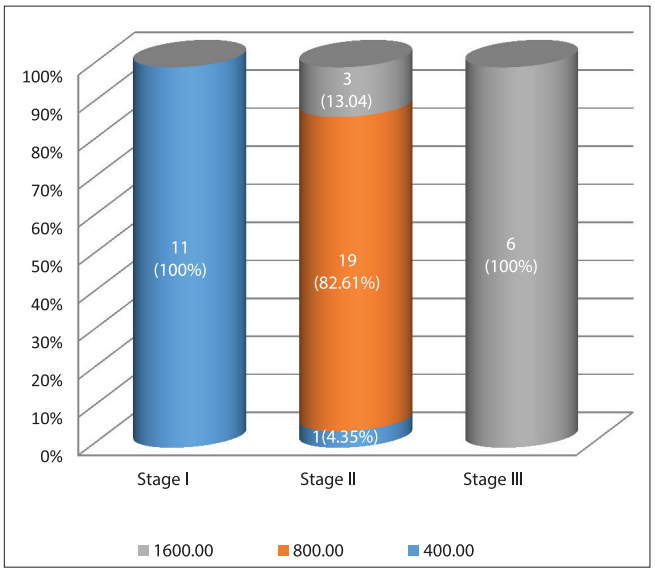

Figure 2: Comparison of fibrinogen degradation products values (ng/ml) with various clinical stages of oral submucous fibrosis patients

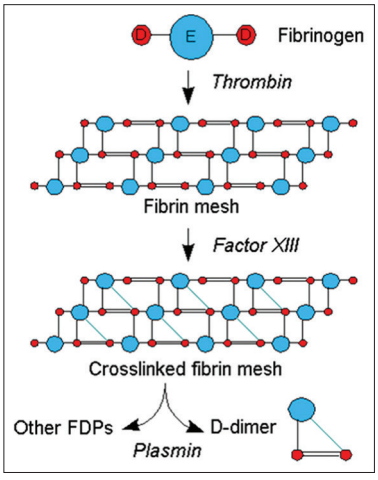

Figure 3: Formation of fibrinogen degradation products by activation of fibrinolytic and coagulation system

FDP have diverse functions. Fibrinopeptides aim to combat the inflammation while FDP tries to counteract the fibrin-like action of fibrin producing factor (FPF) and thrombin produced in the autocatalytic process. Hence, as the severity of the disease increases, more amount of FPF is produced. Fragment Y and to some level fragment X are identified to generate the anticoagulant effect. However, in OSMF, hemorrhagic manifestations are not encountered. Phatak hence described FDP as molecules immunologically similar to fibrinogen.[4]

The literature states that increase in levels of FDP is a valuable early diagnostic sign of increased rate of fibrin deposition. Furthermore, fibrinogen metabolism has been related to four F's FDP, fibrin precipitating factor (FPF), increased fibrinogen level, and fibrinogen cryoprecipitabiliy.[4]

In normal participants, the plasma FDP levels are below the detectable levels. When the levels rise above 200 ng/ml, they are detected in the plasma. Therefore, plasma FDP can be used as a diagnostic aid in suspected OSMF cases without biopsy.

Varieties of FDP assessment kits are available with varying sensitivity and specificity. The present kit was used as it is readily available, easy to use, and cost-effective and has sensitivity and specificity of 100%.[7]

Phatak[4] has suggested that saliva may have a role in the causation of OSMF. In his study of seven OSMF cases, he showed that parotid duct saliva of three patients clotted both the oxalated plasma and fibrinogen suggesting thrombin-like behavior of FPF. When this FPF encounters fibrinous exudates in the oral cavity, it promptly clots the exudate. The body in response to this clotting produces more fibrinogen and its degradation products. He also suggested that an increase in the level of FDP is an early diagnostic sign of an increased rate of fibrin deposition.[4]

The first objective of our study is to detect plasma FDP in betel nut chewers with and without clinical evidence of OSMF. In the present study, plasma FDP levels were detected in all betel nut chewers (Group I Patients) with OSMF, and this finding is in agreement to the results of the previous study done by Phatak (1984)[4], Kosthi and Barpande (2007),[12] Gharat *et al.*(2013),[17] and Kiran *et al.* (2013)[13] Hence, the hypothesis of FDP being an early diagnostic sign of fibrin deposition is supported. However, our selection criteria differ from the previous study of Kosthi and Barpande (2007),[12] in that we have included participants of betel nut chewers with and without OSMF as betel nut is considered to be the main etiologic factor of OSMF.

Plasma FDPs were detected in 3 out of 30 betel nut chewers without clinical evidence of OSMF (Group II). This could be explained by the fact that they might develop the disease in the later stage of life which might be confirmed in a prospective longitudinal study. Since OSMF is a chronic disease and FDP played an important role in its etiopathogenesis, it can be used as a diagnostic aid in suspected OSMF patients, i.e., before the clinical evidence of OSMF. Second, it would have been useful in educating the patient about his/her present situation.

FDP was not detected in Group III cases, and this finding is in accord with the results of study done by Kosthi and Barpande (2007).[12]

Our second objective is to correlate the plasma FDP levels in various clinical stages of OSMF patients (Group I). We found that with increase in the clinical stages there is increase in levels of FDP (semi-quantitative assessment), and it was statistically significant, and this is in accordance to the study done by Kosthi and Barpande (2007)[12] and Gupta *et al.* (2014)[18]

As the plasma FDPs are an early indicator of fibrin deposition, the increase in their level with the increase in clinical stage indicates that there is increased deposition of fibrin in OSMF, leading to a severe condition of the disease.

It is suggested that FPF enters into the submucosal zone of oral mucosa and acts on the diffused fibrinogen, inducing fibrin formation. Such fibrin formation stimulates the fibroblasts producing more collagen as well as production of soluble circulating fibrin monomer in plasma known as FDP.[16,19]

Further, Richardson *et al.* (1976)[20] stated that the fibrin and fibrin degradation products are chemotactic to leukocyte which stimulates the fibroblastic activity and subsequent deposition of collagen in the submucosal connective tissue. Cytokines and growth factors (transforming growth factor beta-1, platelet-derived growth factor-b, and fibroblast growth factor) produced by leukocytes may promote fibrosis by inducing proliferation of fibroblasts, upregulating collagen synthesis and downregulating collagenase production.[21]

CONCLUSION

About 40 cases of clinico-histopathological proven cases of OSMF were included in the present study to find out the role of plasma FDP in the etiology of OSMF and their significance in reading the severity of the disease.

From the present study, it could be inferred that plasma FDP has got a direct correlation with the deposition of fibrin in the extracellular matrix of the submucosal tissue. Such deposition stimulates the fibroblasts to produce more collagen leading to excess accumulation of collagen eventually causing OSMF. Such relationship of the FDP value with that of the severity of the disease could be used as a useful tool to measure the prognosis of the disease. Furthermore, plasma FDP can be used as a diagnostic aid in suspected OSMF cases without biopsy.

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