Original Article

Estimation of plasma fibrinogen degradation products in oral submucous fibrosis: A clinico-pathological study

Reshma V. J, Shihab Anwar A[1], Abdulla Mufeed, Roshni A[2]

Department of Oral Medicine and Radiology, MES Dental College, Perinthalmanna, [1]Department of Pedodontics and Preventive Dentistry, Educare Institute of Dental Sciences, Malappuram, [2]Department of Oral and MaxilloFacial Surgery, MES Dental College, Perinthalmanna, Kerala

Corresponding author (email: <vj.resh@gmail.com>)
Dr. Reshma V. J, Department of Oral Medicine and Radiology, MES Dental College, Malappuram, Kerala, India.

Abstract

Background: Oral submucous fibrosis (OSF) is a disease of the oral mucosa characterized by excessive accumulation of subepithelial collagen, thereby resulting in severe limitation of mouth opening. In OSF, in response to inflammation, the body produces more fibrinogen and its degradation products. The plasma fibrinogen degradation products (FDP) have been reported to be early indicators of fibrin deposition. The present study was intended to ascertain the role of FDP in OSF. **Materials and Methods:** A total of 40 subjects were included in the study. The subjects for the present study were selected from the Department of Oral Medicine and Radiology. The subjects were divided into two groups. The study group comprised 24 subjects diagnosed clinically and histopathologically as OSF and were further divided into three clinical and histological stages of OSF. The control group comprised 16 age- and gender-matched healthy individuals. Five milliliters of venous blood was drawn from the antecubital fossa of all the participants. The blood samples were centrifuged at 1000 rpm for 5 min to separate plasma, and the plasma FDP levels were assessed. **Results and Conclusion:** There was a significant difference in the plasma FDP levels between the study group and the control group. There was a significant linear increase of plasma FDP levels with an increase in severity of the clinical stage of OSF. Comparison with the histopathological grades of OSF also showed an increase in FDP levels with higher grades of OSF and there was a good correlation between the clinical staging and the histopathological grading of OSF.

Key words: *Fibrin, fibrin split products, fibrinogen, fibrinogen degradation products, oral submucous fibrosis*

INTRODUCTION

Oral submucous fibrosis (OSF) is a chronic debilitating disease of the oral cavity characterized by inflammation and progressive fibrosis of the submucosal tissues resulting in marked rigidity and an eventual inability to open the mouth.[1] The disease is predominantly seen in people of Asian descent. Worldwide estimates of OSF indicate that 2.5 million people are affected.[2] It is estimated that as many as 5 million young Indians are suffering from this precancerous condition. The buccal mucosa is the most commonly involved site, but any part of the oral cavity as well as the pharynx can be involved.[3] Data from recent epidemiological studies provide overwhelming evidence that areca nut is the main etiological factor for OSF. The potential

Access this article online	
Quick Response Code:	Website: www.jispcd.org
	DOI: 10.4103/2231-0762.161760

How to cite this article: Reshma VJ, Anwar AS, Mufeed A, Roshni A. Estimation of plasma fibrinogen degradation products in oral submucous fibrosis: A clinico-pathological study. J Int Soc Prevent Communit Dent 2015;5:309-13.

for malignant transformation of OSF is considered to be high at a rate of 7.6%[4,5] The disease affects individuals of all ages and both sexes with mild female predominance.[6] The rate varies from 0.2 to 2.3% in males and from 1.2 to 4.57% in females in the Indian subcontinent.[7]

Fibrinogen degradation products (FDP), also known as fibrin split products, are components of the blood produced by clot degeneration. In normal individuals, the range of plasma FDP is below detectable levels and when the levels rise above 200 ng/ml, they are detected in the plasma.[8] In OSF, in response to inflammation, the body produces more fibrinogen, thereby leading to increased levels of FDP.[8,9]

The plasma FDP is said to be an early indicator of fibrin deposition.[8] As OSF is a potentially malignant condition, determination of FDP levels may further help to assess the progression of the disease, which in turn helps to plan appropriate management, thus improving the quality of life of the patients. This study aims to determine the levels of plasma FDP in various clinical stages of OSF.

Objectives of the study

- To determine the levels of plasma FDP in subjects with OSF and to compare with age- and sex-matched controls
- To assess the relationship between various clinical stages of OSF and plasma FDP levels
- To assess the relationship between various histological grades of OSF and plasma FDP levels.

MATERIALS AND METHODS

The study was conducted in the outpatient Department of Oral Medicine and Radiology of D.A.P.M. R.V. Dental College, Bangalore. A total of 40 subjects were included in the study and they were divided into two groups. The study group consisted of 24 subjects diagnosed clinically and histopathologically with OSF. The study group was further divided into three clinical stages based on the clinical staging criteria given by Mathur and Jha:[10]
Stage I- Eight subjects with OSF
Stage II- Nine subjects with OSF
Stage III- Seven subjects with OSF.

The control group consisted of 16 age- and sex-matched healthy individuals with no systemic illness.

Exclusion criteria

- Patients with known systemic diseases such as bleeding and clotting disorders, collagen diseases like scleroderma, thromboembolic disorders
- Pregnant and lactating women.

Prior to conducting the study, the subjects were explained the need for the study and a written consent was taken from them. A thorough case history was elicited, which included a detailed medical and habit history. Clinical examination of the oral cavity was performed for all the subjects in both study and control groups. In the study group, the signs and symptoms of OSF were observed and the clinical staging was done based on the criteria given by Mathur and Jha. Then, the subjects in the study group were subjected to incisional/punch biopsy; the tissue specimens were confirmed histopathologically as OSF and histological grading was done based on the criteria given by Pindborg and Sirsat.[11] From the study group and control group subjects, 5 ml of venous blood was drawn from the antecubital fossa under aseptic conditions. The blood samples were centrifuged at 1000 rpm for 5 min to separate plasma which was collected in a plastic vial and transported under cold cycle for evaluation of FDP using semi-quantitative method. The latex slide test was prepared using test kits supplied by Tulip Diagnostics (P) Ltd (Goa, India). which helps to detect cross-linked FDP in human plasma.

Contents of the kit

FDP latex reagent, positive control, negative control, Phosphate buffered saline.

FDP slide test for detection of cross-linked FDP is based on the principle of agglutination. The test specimen (plasma) is mixed with FDP latex reagent. The sensitivity of the reagent is ≈ 200 ng/ml, below which the samples are negative and above which the samples give a positive agglutination reaction. Using PBS buffer solution, serial dilutions of the plasma samples as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 were prepared (i.e. 10 µl of plasma was pipetted into the vial and diluted with 20 µl of PBS and so on). On the glass slides, the markings 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128 were made. The mixture of plasma and buffer was pipetted and a drop of each dilution was placed on the corresponding marking on the slide. One drop of FDP latex reagent was taken in the pipette and added to each drop of diluted plasma specimen on the slide. The slides were rocked gently back and

forth, observing for agglutination macroscopically for 3 min. The agglutination was observed at one or more of the following markings: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128. The highest dilution of plasma at which agglutination was seen was taken as the d value.

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, the following formula was used:

D dimer level (ng/ml) =200 × d,

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

Kruskal–Wallis test, Mann–Whitney test, and the Chi-square test were used for statistical analysis.

RESULTS

Plasma FDP levels in study and control groups

The plasma FDP levels were assessed for the study and control groups. In the study group, the FDP levels for Stage I OSF ranged from 400 to 800 ng/ml, with the mean value being 600 ng/ml ± 213.81. FDP levels for Stage II OSF ranged from 1600 to 3200 ng/ml with a mean value of 2133.33 ng/ml ± 800, and FDP levels for Stage III OSF ranged from 12,800 to 25,600 ng/ml and the mean FDP level was 18,285.7 ng/ml ± 6841.89 [Table 1].

The FDP levels were zero for all the subjects in the control group [Table 1]. This indicates that the FDP levels were not detectable in the plasma of subjects in the control group.

Correlation of FDP levels with the various clinical stages of OSF

The FDP levels were correlated with the various clinical stages of OSF and it was observed that the mean FDP levels were highest in Stage III OSF subjects, followed by Stage II OSF and Stage I OSF subjects, respectively. The difference in FDP levels between the three clinical stages of OSF was found to be statistically significant ($P < 0.001$) [Table 2].

In order to assess the significant statistical difference between the FDP levels and various clinical stages of OSF, Mann–Whitney test was used, and it was found that the difference in FDP levels between Stage I OSF and Stage II OSF was statistically significant ($P < 0.001$). Also, the difference in FDP levels was found to be statistically significant between Stage I OSF and Stage III OSF ($P < 0.01$) as well as between Stage II OSF and Stage III OSF ($P < 0.01$) [Table 3].

Histopathological grading of OSF

Histopathological grading was done for subjects in the study group. Among the 24 subjects, 10 subjects (42%) could be categorized as grade I OSF, 13 subjects (54%) as grade II OSF, and 1 subject (4%) as grade III OSF [Graph 1].

Correlation of clinical staging of OSF with histopathological grading of OSF

In the study group, the clinical staging of OSF was correlated with the histopathological grading of OSF. For testing significance of the association, Chi-square test was used. Only grade I OSF and grade II OSF were taken into account, as there was only one subject with grade III OSF. It was observed that there was a statistically significant association between the clinical stage of OSF and the histopathological grade of OSF ($P < 0.05$). It was also observed that among the clinical Stage I OSF subjects, more number of samples was associated with histopathological grade I OSF and among the clinical Stage III OSF subjects, more number of samples was associated with histopathological grade II OSF [Table 4].

Table 1: Mean values of FDP between the study and control groups

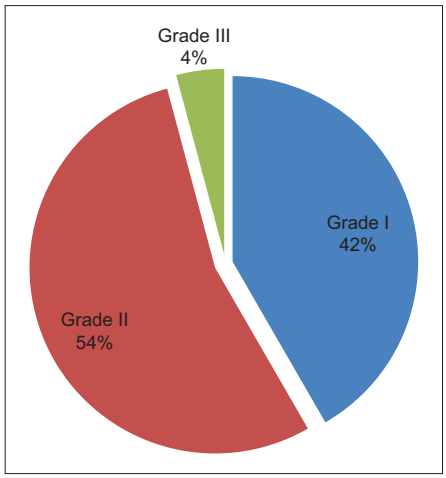
Clinical stage	FDP values (ng/ml)
	Mean
Stage I	600
Stage II	2133.33
Stage III	18,285.71
Controls	0

FDP=Fibrinogen degradation products

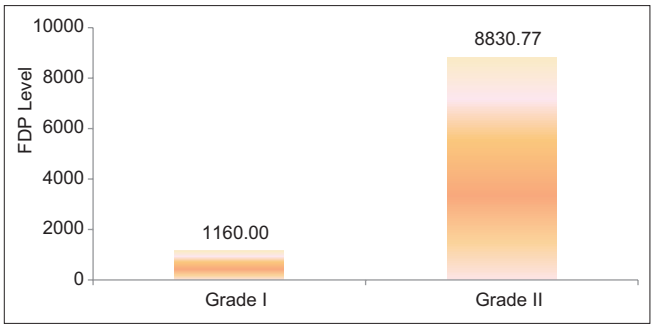
Table 2: Correlation of FDP levels with the various clinical stages of OSF

Clinical stage	Mean	Standard deviation	Median	Min.	Max.	Kruskal-Wallis chi-square	*P*
Stage I	600.00	213.81	600	400	800	21.069	<0.001*
Stage II	2133.33	800.00	1600	1600	3200		
Stage III	18,285.71	6841.89	12,800	12,800	25,600		

Shows p value is statistically significant. FDP=Fibrinogen degradation products, OSF=Oral submucous fibrosis



Graph 1: Sample distribution according to the histological grade



Graph 2: Mean FDP levels in each histopathological grade

Table 3: Comparison of clinical stages and FDP levels using Mann-Whitney test

Stage I	Stage II	Mean difference	Z	*P*
Stage I	Stage II	−1533.33	−3.597	<0.001*
	Stage III	−17,685.71	−3.343	0.001*
Stage II	Stage III	−16,152.38	−3.472	0.001*

Shows p value is statistically significant. FDP=Fibrinogen degradation products

Table 4: Correlation of clinical staging of OSF with histopathological grading of OSF

Clinical stage	Histological grade			Total	χ^2	*P*
	Grade I	Grade II	Grade III			
Stage I	6	2	0	8	7.853	0.020*
Stage II	4	5	0	9		
Stage III	0	6	1	7		
Total	10	13	1	24		

Shows p value is statistically significant. OSF=Oral submucous fibrosis

Correlation of FDP levels with histopathological grading of OSF

In the study group, the FDP levels were correlated with the histopathological grades of OSF. Comparison between grade I and grade II OSF was carried out using Mann–Whitney test, as there was only one subject with grade III OSF. It was observed that grade II OSF showed higher mean FDP levels as compared to grade I OSF and the difference in FDP level between the two groups was found to be statistically significant ($P < 0.01$) [Table 5, Graph 2].

DISCUSSION

The clinical staging of OSF and histopathological grading of OSF were compared and a significant association was observed between them. It was also observed that among Stage I OSF subjects, more number of samples was associated with grade I OSF and among Stage III OSF subjects, more number of samples was associated with grade II OSF. Contrary to our study findings, an earlier study by Koshti and Barpande showed no positive correlation between the clinical stages of OSF and the histological grades of OSF.[8]

The study group subjects showed a linear increase in FDP levels with increase in clinical stage of OSF. For Stage I OSF subjects, the FDP levels ranged from 400 to 800 ng/ml with an average of 600 ng/ml; for Stage II OSF subjects, the FDP levels ranged from 1600 to 3200 ng/ml with a mean of 2133.33 ng/ml; and for Stage III OSF subjects, the FDP levels ranged from 12,800 to 25,600 ng/ml with an average of 18,285.71 ng/ml. There was a strong correlation between the clinical stages of OSF and the FDP levels, and the difference in FDP levels between the three clinical stages of OSF was found to be statistically significant. It was found that the difference in FDP levels between Stage I OSF and Stage II OSF was statistically significant. Also, the difference in FDP levels was found to be statistically significant between Stage I OSF and Stage III OSF as well as between Stage II OSF and Stage III OSF. Previous studies have reported that in OSF, in response to inflammation, the body produces more fibrinogen and its degradation products and that there is a linear increase of plasma FDP levels with an increase in the clinical stage of OSF.[8,9]

In the control group, the plasma FDP levels were below the detectable levels. A previous study has reported that the plasma FDP levels are below the detectable levels in healthy individuals.[8]

The histological grades of OSF were compared with the FDP levels. The results showed that the difference in FDP levels between grade I OSF and grade II OSF was statistically significant and that grade II OSF showed a higher mean FDP score compared to grade I OSF. On the contrary, in a previous study, the mean plasma FDP levels were compared with the histological grades of

Table 5: Correlation of FDP levels with histopathological grading of OSF

Histological grade	Mean	Standard deviation	Median	Mean difference	Z	*P*
Grade I	1160.00	873.31	873	−7670.769	−2.683	0.007*
Grade II	8830.77	9042.80	9043			

Shows p value is statistically significant. FDP=Fibrinogen degradation products, OSF=Oral submucous fibrosis

OSF and there was no significant difference in the FDP levels between the various histological grades of OSF.[8]

As FDP is an early diagnostic sign of fibrin deposition, increase in FDP levels suggests that there is increased fibrin deposition in OSF. Thus, the finding that OSF is primarily a change of connective tissue is further strengthened. Moreover, with an increase in the clinical stage of OSF, plasma FDP levels are also increased. This suggests that as the clinical stage of OSF increases, the amount of fibrin deposited in the connective tissue also increases, thus leading to progressive restriction in mouth opening. The findings observed in the present study indicate that the FDP levels vary significantly depending on the clinical staging and the histopathological grading of OSF. Thus, determination of FDP levels may help in assessing the prognosis and treatment outcome of OSF patients.

CONCLUSION

FDP are components of the blood produced by clot degeneration. In normal subjects, the plasma FDP levels are below the detectable levels. When the levels rise above 200 ng/ml, they are detected in the plasma. In OSF, in response to inflammation, the body produces more fibrinogen and its degradation products. In the present study, the levels of FDP were assessed and compared between the study group and the control group, and also, the correlation between the various clinical stages of OSF and histopathological grades of OSF with plasma FDP levels was assessed.

The following conclusions were drawn from the present study:

- There was a significant difference in the plasma FDP levels between the study group and the control group
- There was significant linear increase of plasma FDP levels with an increase in clinical stage of OSF
- Comparison with the histopathological grades of OSF showed an increase in FDP levels with increase in histopathological grades of OSF
- There was good correlation between the clinical staging and the histopathological grading of OSF.

Plasma FDP levels may be influenced by various factors such as inflammatory conditions, exercise, anxiety, and stress. An attempt to minimize the influence of these parameters on the study sample may help in achieving more definitive results. A larger sample size which would be a better representation of the population would also facilitate in obtaining more conclusive results.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Cox SC, Walker DM. Oral Submucous fibrosis. A review. Aust Dent J 1996;41:294-9.
2. Gupta S, Manjunath SM, Jawanda MK, Bharti A. Quantification of plasma fibrinogen degradation products in Areca nut chewers with and without oral submucous fibrosis. J Clin Diagn Res 2014;8:ZC27-30.
3. Paissat DK. Oral Submucous fibrosis. Int J Oral Surg 1981;10:307-12.
4. Sunita ND. Oral Submucous fibrosis: Review on etiopathogenesis. J Canc Sci Ther 2009;1:72-7.
5. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral Submucous fibrosis: Review on aetiology and pathogenesis. Oral Oncol 2006;42:561-8.
6. Kumar A, Bagewadi A, Keluskar V, Singh M. Efficacy of lycopene in the management of oral submucous fibrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:207-13.
7. Aziz SR. Oral submucous fibrosis: An unusual disease. J N J Dent Assoc 1997;68:17-9.
8. Koshti SS, Barpande S. Quantification of plasma fibrinogen degradation products in oral submucous fibrosis: A clinicopathologic study. J Oral Maxillofac Pathol 2007;11:48-50.
9. Kadani M, Satish BN, Maharudrappa B, Prashant KM, Hugar D, Allad U, *et al*. Evaluation of plasma fibrinogen degradation products and total serum protein concentration in oral submucous fibrosis. J Clin Diagn Res 2014;8:ZC54-7.
10. Mathur RM, Jha T. Normal oral flexibility-A guideline for SMF cases. J Indian Dent Assoc 1993;64:139-43.
11. More CB, Gupta S, Joshi J, Varma SN. Classification system for oral submucous fibrosis. J Indian Acad Oral Med Radiol 2012;24:24-9.