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ORIGINAL RESEARCH

# Quantification of plasma fibrinogen degradation products in oral submucous fibrosis: A clinicopathologic study

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## ABSTRACT

Oral submucous fibrosis (OSMF) has a multifactorial etiology. Recent investigations have shown the role of fibrinogen degradation products (FDP) in the causation of OSMF. A study of 35 cases showed a significant linear increase of plasma FDP levels with an increase in the clinical grade. Comparison with the histological grade of OSMF showed an increase in plasma FDP levels with increase in histological grade of OSMF, but was not statistically significant. Plasma FDP is reported to be an early indicator of fibrin deposition. When the plasma FDP increases, the fibrin deposited also increases. This strengthens the finding that OSMF is primarily a change of connective tissue causing excessive deposition of fibrin. This in turn leads to restriction of mouth opening.

**Key words:** Fibrinogen degradation products, fibrin

## INTRODUCTION

Oral submucous fibrosis (OSMF) is a premalignant condition. Studies have indicated the role of excessive chili consumption, areca nut, genetic susceptibility, autoimmunity, iron and vitamin deficiency, etc., to be the underlying causes. Recently, the identification of fibrinogen degradation products (FDP) in the plasma of OSMF patients has provided a new direction for the etiopathogenesis of OSMF. In OSMF, the body in response to inflammation produces more fibrinogen and its degradation products. Also, an increased production of fibrinogen gives rise to increased production of fibrin, and FDP is an early indicator of increased fibrin deposition. With this view in mind, the present study was undertaken to assess the FDP levels in the plasma of OSMF patients.

## MATERIALS AND METHODS

The study comprised of 35 cases of OSMF and an equal number of age- and sex-matched control subjects. A detailed case history of each patient was recorded, and patients without any bleeding or clotting disorder were selected. Provisional diagnoses of OSMF were made on clinical examination and were then grouped into grade I to grade IV. For confirmation of provisional diagnosis of OSMF, study subjects were subjected to punch biopsy or scalpel biopsy. All OSMF cases were histologically graded as very early, early, moderately advanced and advanced based on the criteria established by Smith and Pindborg.<sup>[1]</sup>

Under all aseptic precautions, 2 ml of venous blood was withdrawn by venipuncture and collected in a citrate bulb. Routine hematological investigation was performed. The bulbs were allowed to stand for one hour at room temperature and then centrifuged at 4,000 rpm to separate the plasma. This plasma was quantitated for FDP levels.

Plasma FDP was quantitated by using a diagnostic kit – ‘A qualitative and semi-quantitative latex slide test for detecting cross-linked FDP in human plasma’ (Tulip Diagnostics P. Ltd., Goa, India). The quantitation is based on the principle of agglutination. The kit contains XL FDP reagent (a uniform suspension of polystyrene latex particles coated with mouse monoclonal anti-D dimer antibody), positive control (reactive with XL FDP latex reagent), negative control (nonreactive with XL FDP latex reagent) and phosphate buffer. Plasma FDP levels were quantified based on the following formula provided by the manufacturer.

$$\text{FDP level (ng/dl)} = 200 \times d$$
  
(where d = highest dilution of plasma showing agglutination)

## RESULTS

Comparison of mean plasma FDP levels and different clinical grades of 35 OSMF patients shows a statistically significant increase in the FDP levels with increase in clinical grades [Table 1]. A comparison of mean plasma FDP levels and different histological grades also shows an increase in the

**Table 1: Comparison of mean plasma FDP levels in different clinical grades of 35 OSMF patients**

Clinical grade	No. of patients	Mean value	Std. Dev	t-value	P-value
I	6	600	219	-4.87 (I v/s II)	0.000
II	16	1450	600	-6.66 (II v/s III)	0.000
III	13	4677	1660	-8.69 (III v/s I)	0.000
IV	-	-	-	-	-

mean FDP levels with increase in the histological grades, but was not statistically significant [Table 2].

Correlation of FDP levels with clinical and histopathological grades using Pearson's correlation coefficient (0.321) showed lack of correlation between various clinical and histological grades of OSMF.

## DISCUSSION

In spite of intense research, the etiology of OSMF remains a question. As the disease produces changes localized to the oral cavity, saliva may play a role in the causation of the disease. It is found that fibrin precipitating factor (FPF) is present in the saliva of OSMF patients.<sup>[2]</sup>

Phatak AG,<sup>[3]</sup> in his study of seven OSMF cases, showed that parotid duct saliva of three patients clotted both the oxalated plasma and fibrinogen. This suggests that FPF has thrombin-like behavior.<sup>[3]</sup> 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. The degradation products have different functions. The fibrinopeptides try to combat the inflammation, while FDP tries to counter the fibrin-like action of FPF and thrombin produced in the autocatalytic process.<sup>[4]</sup> Hence as the severity of the disease increases, more amount of FPF is produced and in turn more amount of FDP is produced.

Fibrinogen is converted to fibrin by the enzymatic action of thrombin, which splits fibrinopeptides A and B from the molecules, leaving fibrin monomers, which in turn rapidly polymerizes to form insoluble fibrin. In the fibrinolytic process, fibrinogen is degraded by plasmin to fragments X, Y, A, B, C, D and E, which are the fibrinogen degradation products. FDP, particularly fragment Y and to a lesser extent fragment X, are known to produce anticoagulant effect. But in

**Table 2: Comparison of mean plasma FDP levels in different histological grades of 35 OSMF patients**

Histological grade	No. of patients	Mean value	Std. Dev	t-value	P-value
I	-	-	-	-	-
II	21	1924	1774	-1.89 (II v/s III)	0.073
III	12	3267	2060	-0.30 (III v/s IV)	0.816
IV	2	4000	3394	-0.85 (IV v/s II)	0.550

OSMF, no hemorrhagic manifestations are encountered.<sup>[4]</sup> So FDP is labeled here as 'molecules immunologically similar to fibrinogen' (MISFI). These molecules possess entirely different biochemical characteristics, though immunologically they are clumped together.<sup>[3]</sup>

The literature states that increase in the levels of FDP is a valuable early diagnostic sign of increased rate of fibrin deposition. Also, fibrinogen metabolism has been related to four F's, viz., fibrinogen degradation products (FDP), fibrin precipitating factor (FPF), increased fibrinogen level and fibrinogen cryoprecipitability.<sup>[4]</sup> This suggests that OSMF is primarily a disease of collagen metabolism causing excessive deposition of fibrin, which in turn leads to restriction of mouth opening.

In normal subjects, the plasma FDP levels are below the detectable levels. When the levels rise above 200 ng/dl, they are detected in the plasma. The mean plasma FDP levels in various clinical grades of OSMF were 600 ng/dl (grade I), 1,450 ng/dl (grade II) and 4,677 ng/dl (grade III). As none of the patients exhibited grade IV, levels in this grade could not be assessed. Statistical analysis showed highly significant difference between all the clinical grades of OSMF ( $P < 0.001$ ) [Table 1].

As FDP is an early diagnostic sign of fibrin deposition, the increase in its level suggests that there is increased fibrin deposition in OSMF. Thus the finding that OSMF is primarily a change of connective tissue is further strengthened. Moreover, with an increase in the clinical grade, plasma FDP levels are also increased. This suggests that as the clinical grade increases, the amount of fibrin deposited in the connective tissue also increases, thus leading to progressive restriction in mouth opening.

The mean plasma FDP levels in various histological grades of OSMF were 1,924 ng/dl (grade II), 3,267 ng/dl (grade III) and 4,000 ng/dl (grade IV). As none of the patients exhibited grade I, the plasma FDP levels in this grade could not be quantitated. Statistical analysis did not show any significant difference between the various histological grades of OSMF [Table 2].

Analysis of data using 'Pearson correlation coefficient' showed no statistical correlation between clinical and histological grades of OSMF ( $P = 1$  or  $-1$ ) [Table 3]. The histological and

**Table 3: Distribution of 35 OSMF patients according to clinical and histological grades**

Clinical grade	Histological grades				Total
	I	II	III	IV	
I	-	6	0	0	6
II	-	9	6	1	16
III	-	6	6	1	13
IV	-	-	-	-	-
Total	-	21	12	2	35

clinical grading systems use parameters independent of each other. So no positive correlation was found.

## CONCLUSION

Thirty-five clinically and histologically proven cases of OSMF were studied to find out the role of plasma FDP in the etiology of OSMF. In the present study, all OSMF cases showed an increase in the plasma FDP levels with increase in clinical and histological grades of OSMF. But there was no correlation between the clinical and histological grades of OSMF. So, a study at larger scale with well defined clinical and histological criteria needs to be conducted to identify the role of FDP in the pathogenesis of OSMF.

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