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Dentistry Section

Evaluation of Plasma Fibrinogen Degradation Products and Total Serum Protein Concentration in Oral Submucous Fibrosis

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ABSTRACT

Background: Oral submucous fibrosis (OSMF) is a potentially malignant disorder with a multifactorial etiology. Malnutrition is a major problem for the inhabitants of most countries where OSMF is prevalent. Recently, a new direction in the etiopathogenesis was provided by the identification of fibrinogen degradation products (FDP) in the plasma of OSMF patients.

Aims and Objectives: To assess the role of FDP in the etiology of OSMF and to correlate with the nutritional status by evaluating the total serum protein level. The study also determines to evaluate the correlation between the levels of plasma FDP with respect to the staging and grading of OSMF. Correlation between the levels of Total Serum Protein (TSP) with respect to the staging and grading of OSMF was also evaluated.

Materials and Methods: The study included 30 cases clinically and histopathologically diagnosed as oral submucous fibrosis. The FDP levels were assessed using both qualitative and semi quantitative method as supplied by 'Tulip Diagnostics (P) Ltd. Total Serum Protein (TSP) estimation was done by Biuret method using Liquixx Protein kit by Erba, Manheim.

Results: The study indicates that in qualitative assessment of FDP only 14 subjects showed the presence of FDP levels>200ng/ml. In semiquantitative assessment there is no significant association between varying clinical stages and histopathological grades and FDP levels. Total serum Protein level showed a marginal increase in all subjects. The study revealed a positive correlation between FDP and TSP in all OSMF subjects.

Conclusion: A larger sample size which would be a better representation of the population and the use of different methods which have higher sensitivities and specificities to evaluate FDP level and detailed fractional analysis of protein along with immunoglobulin profiling would facilitate in attaining more conclusive results.

Keywords: Oral submucous fibrosis, Fibrinogen degradation products, Fibrinogen, Fibrin, Fibrin split products, Total Serum Protein

INTRODUCTION

World Health Organization (WHO) in the year 1978 had proposed that in oral cavity the clinical presentations which were recognized as precancerous were classified into two broad groups, as precancerous lesions and precancerous conditions. Recently at a work shop coordinated by World Health Organization (WHO), the term "Potentially Malignant Disorders" (PMD), was recommended to refer to precancer as it conveys that not all disorders described under this term may transform to cancer rather that there is a family of morphological alterations amongst which some may have an increased potential for malignant transformation. Potentially malignant disorders of the oral mucosa are also indicators of risk of likely future malignancies elsewhere in (clinically normal appearing) oral mucosa and not only site specific predictors [1-3].

In South-East Asia and other Asian countries especially in the Indian subcontinent, the usage of smokeless tobacco in different forms is very popular. The habit usually involves chewing of a betel quid (areca nut, betel leaf, tobacco and slaked lime), has led to the development of a generalized fibrosis of the oral soft tissues in a large proportion of users; a condition called oral submucous fibrosis (OSMF) [4-7].

According to Pindborg JJ et al., and WHO "OSMF is an 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 a juxtaepithelial inflammatory reaction, followed by a fibroelastic change of the lamina propria, with epithelial atrophy, leading to stiffness of the oral mucosa causing trismus and inability to eat" [8].

OSMF has a multifactorial etiology. Various studies have indicated that areca nut chewing, excessive consumption of chillies, autoimmunity, genetic susceptibility, iron, protein and vitamin deficiency, etc., are the underlying causes for OSMF [9-11].

Recently, a new direction in the etiopathogenesis was provided by the identification of fibrinogen degradation products (FDP) in the plasma of OSMF patients. In oral submucous fibrosis, excessive fibrinogen is produced in the body in response to inflammation which in turn leads to increased level of fibrinogen degradation products. The presence of these fibrinogen degradation products in the plasma are thought to be the markers for increased deposition of fibrin which in turn leads to restricted mouth opening [12,13].

Malnutrition is a major problem for the inhabitants of most countries where OSMF is prevalent. Several investigators have reported vitamin, iron and protein deficiencies among OSMF patients [6,7,9,10,14].

Keeping this view in mind, the present study was undertaken to ascertain the role of FDP and total serum protein in etiopathogenesis of OSMF.

MATERIALS AND METHODS [12,13]

The study comprised of 30 subjects that were clinically and histopathologically diagnosed as oral submucous fibrosis. Prior to conducting the study approval from the ethical committee was obtained. An informed consent was obtained from all the patients selected for the study. A detailed case history was recorded and a thorough clinical examination was carried out for subjects in the study group. Healthy subjects with no systemic complications

were considered for the study. Subjects with temporomandibular joint (TMJ) disorders were excluded from the study. The study group comprised of individuals between age ranges of 18 to 45 years and all subjects were males. The study was a single blinded study. Provisional diagnosis of OSMF was made on the basis of clinical examination. Control group was not considered since the patients were clinically diagnosed as OSMF patients. The patients were clinically grouped as stage 1, stage 2, stage 3 and stage 4 according to the classification given by Ranganathan K et al., [15]. For confirmation of the provisional diagnosis, study group subjects were subjected to incisional/punch biopsy and histopatholgically examined. All OSMF subjects were graded histologically as very early, early, moderately advanced and advanced based on criteria established by Pindborg JJ and Sirsat SM [15].

Under all aseptic precautions, 5 ml of venous blood was withdrawn by venipuncture, from this; 1.8 ml blood was collected in the vacutainer tube containing 0.2 ml of dipotassium EDTA to separate plasma for assessment of FDP levels. The remaining 3 ml of blood sample was collected in plain plastic vacutainer to separate serum for evaluation of total serum protein concentration. The vacutainer tubes containing blood sample was centrifuged at 4000 rpm for 5 minutes. The plasma from EDTA vacutainers was separated and collected in a plastic vial and used to assess FDP level. The serum was separated from the plain vacutainer and was used to estimate total protein level.

Plasma FDP levels was assessed using 'A qualitative and semi-quantitative latex slide test for detecting cross-linked FDP in plasma' (Tulip Diagnostics P. Ltd., Goa, India.) The test 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 reagent), negative control (non-reactive with XL FDP latex reagent) and phosphate buffer. Plasma FDP levels were qualitated to detect FDP levels> 200ng/ml and then were subjected to semi quantitative assessment based on the following formula provided by the manufacturer.

D dimer level (ng/ml) = 200 X d

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

Total serum Protein estimation was done using Liquixx. Total Protein kit supplied by Erba, Manheim which uses Biuret method. The kit contains a biuret reagent and protein standard. The reagents are ready to use. The estimation of total serum protein was done using the following formula.

Total protein (g/dl) = (Absorbance of standard) X (concentration of standard (g/dl)

Statistical Package for the Social science (SPSS Inc, Chicago) software packages were used for data entry and analysis. Chi-square test, Student t-test and Karl Pearsons correlation were used in the present study.

RESULTS

In all the 30 subjects of OSMF only 14 subjects showed positive for agglutination indicating plasma FDP levels>200ng/ml in Qualitative assessment [Table/Fig-1,2]. Chi-square value was <5.99 for p=0.05 which is statistically not significant. While comparing clinical stages and histopathological grades with the plasma FDP levels, in semi-quantitative assessment, the Chi-square value was found to be <18.31 for p=0.05 and test of significance was found to be <1.75 which indicates that there is no statistically significant association with the increasing in severity of OSMF to plasma FDP levels [Table/Fig-3-6]. Stage IV in clinical staging and histopathological grading did not show any representation.

Total serum protein levels were marginally increased in all the OSMF subjects with increased inclination in the range of 7-9g/dl [Table/Fig-7 & 8]. Chi-square value was <5.99 for p=0.05 and test of significance was found to be <1.75 which indicates that there was no association between total serum protein concentration with various clinical stages and histopathological grades of OSMF. A positive Karl Pearson correlation of 0.2747 was observed in the study between plasma FDP levels and total serum protein concentration.

Clinical Staging	Fdp Level Qualitative		
	Negative	Positive	Total
STAGE I	02	02	04
Stage II	09	04	13
Stage III	05	08	13
Total	16	14	30
Chi-Square Value	2.49 (<5.99 for p=0.05) No Association		

[Table/Fig-1]: Number of Cases According to Clinical Staging and FDP Level Qualitative

Histopathological Grading	Fdp Level Qualitative		
	Negative	Positive	Total
Grade I	04	02	06
Grade II	08	06	14
Grade III	04	06	10
Total	16	14	30
Chi-Square Value	1.22 (<5.99 for p=0.05) No Association		

[Table/Fig-2]: Number of Cases According to Histopathological Grading and FDP Level Qualitative

Clinical Staging	Fdp Level d Value						
	<200	>200	400	800	1600	3200	Total
Stage I	02	00	02	00	00	00	04
Stage II	09	02	01	00	01	00	13
Stage III	05	02	03	02	00	01	13
Total	16	04	06	02	01	01	30
Chi-Square Value	9.72 (<18.31 for p=0.05) No Association						

[Table/Fig-3]: Number of Cases According to Clinical Staging and FDP Level D Value: Semiquantitative

Clinical Stages	No of Patients	Mean Value	Std. Dev	t-Value
I	4	400	0	0.39 (I v/s II)
II	13	600	673.30	0.36 (II v/s III)
III	13	800	997.14	0.54 (I v/s III)

[Table/Fig-4]: Comparison of Mean Plasma FDP Levels in Different Clinical stages of 30 OSMF Patients
't' Value < 1.75 Shows No Significant Difference

Histopathological Grading	Fdp Level D Value						
	<200	>200	400	800	1600	3200	Total
Grade I	04	00	02	00	00	00	06
Grade II	08	03	02	01	00	00	14
Grade III	04	01	02	01	01	01	10
Total	16	04	06	02	01	01	30
Chi-Square Value	7.48(<18.31 FOR P=0.05) NO ASSOCIATION						

[Table/Fig-5]: Number of Cases According to Histopathological Grading and FDP Level d Value: Semi-quantitative

Histopathological Grades	No of Patients	Mean Value	Std. Dev	t-Value
I	6	400	0	0.19(I v/s II)
II	14	366.67	233.81	1.54(II v/s III)
III	10	1100	1143.68	0.82(I v/s III)

[Table/Fig-6]: Comparison of Mean Plasma FDP Levels in Different Histopathological Grades of 30 OSMF Patients
t-Value < 1.75 Shows No Significant Difference

Clinical Staging	Total Serum Protein					
	5-7	7-9	9-11	Mean	Sd	t-Value
STAGE I	01	03	00	7.83	0.59	0.09 (I v/s II)
STAGE II	01	12	00	7.93	0.69	0.69(II v/s III)
STAGE III	02	08	03	8.20	1.21	0.47(I v/s III)
TOTAL	04	23	03			

[Table/Fig-7]: Comparison of Total Serum Protein Concentration Level in Different Clinical Stages of 30 OSMF Patients.
Chi-Square Value is 2.49(<5.99 For p=0.05) No Association

Histopathological Grades	Total Serum Protein					
	5-7	7-9	9-11	Mean	Sd	t-Value
GRADE I	01	05	00	7.74	1.04	1.12(I v/s II)
GRADE II	02	11	01	8.19	0.65	0.41(II v/s III)
GRADE III	01	07	02	8.03	1.20	0.45(I v/s III)
TOTAL	04	23	03			

[Table/Fig-8]: Comparison of Total Serum Protein Concentration Level in Different Histopathological Grades of 30 Osmf Patients
Chi-square Value Is 2.49(<5.99 For P=0.05) No Association and 't' Value < 1.75 Shows No Significant Difference.
Karl Pearson Correlation Between Plasma FDP Level D Value and Total Serum Protein Is 0.2747

DISCUSSION

Ever since Schwartz described the condition in 1952, there has been extensive research work in understanding the exact etio-pathogenesis and modulating various treatment modalities for management of OSMF subjects. The prevalence of OSMF has been on the rise, which at present is attributed to the use of areca nut and its products. The lack of awareness, aggressive marketing strategies of these products and failure to impart primary preventive measures have also contributed to a considerable increase in the incidence of OSMF among the rural and urban younger population. During inflammatory process, fibrinogen which is an acute phase reactant increases and in response to the inflammation, the body produces more fibrinogen and its degradation products [16,17].

Fibrin degradation products (FDPs), also known as fibrin split products, are components of the blood, produced by clot degeneration. Fibrinogen is converted into fibrin by the enzymatic action of thrombin, which splits fibrinopeptides A and B from the molecule leaving fibrin monomer, which in turn rapidly polymerizes to form insoluble fibrin [12-14].

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 [12,17].The most notable subtype of fibrin degradation products is the D-dimer. Fibrinogen metabolism is related to increased fibrinogen levels, fibrinogen cyro-precipitability, fibrin degradation products and fibrin precipitating (producing) factor. Based on these four factors a possible hypothesis regarding the mechanism of OSMF has been described. It states that the parotid saliva contains a coagulant or procoagulant which is designated as fibrin producing factor (FPF). When this FPF interacts with the fibrinous exudates in the oral cavity, it promptly clots the fibrinous exudates. This reaction is accelerated by calcium ion, though it is not entirely calcium dependent. After the formation of clot, the ensuing thrombin perpetuates the process. In response to this fibrous deposition in the inflammatory area, the body produces more fibrinogen and its degradation products. The fibrinopeptides tries to combat inflammation, while FDP counteracts the thrombin like action of FPF and the thrombin produced in the autocatalytic process. Therefore, plasma FDP could be used as a diagnostic aid in suspected OSMF cases without surgery/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, cost effective, and produces rapid results [12-14,17,18].

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. Studies have shown that increase in the FDP levels is a valuable early diagnostic indicator of increased rate of fibrin deposition. Hence, determination of FDP levels may further help in the assessment of prognosis and treatment outcome in OSMF [12,13].

Malnutrition is another major problem for the inhabitants of most countries where OSMF is prevalent. Deficiencies of vitamins, proteins and iron have been implicated as being of etiological importance in OSMF. Despite many researches and observations the actual role played by these factors remains unclear as these micronutrients may also be deficient in control subjects without OSMF [14].

In the present study, in qualitative assessment 14 subjects showed presence of FDP levels more than 200 ng/mg and 16 subjects showed negative response to the presence of FDP level. Contrary to our present study, a previous study done by Pathak AG, Koshti and Barpande and Gharat et al., Kiran et al., showed presence of FDP in all OSMF subjects considered for the study [12,13,17,19].

The probable reasons for undetectable FDP levels could be because of the method employed by the kit to detect FDP levels. Latex agglutination slide test method used in the present study, which according to previous studies lacks sensitivity to detect minimal rise of FDP levels which results in lower sensitivity [20].

Studies done by Gupta PK et al., to evaluate the diagnostic ability of whole blood D- dimer and Plasma D-dimer resulted in sensitivity of 66.7% for plasma FDP latex method as compared the sensitivity of 82.8% for whole blood D-dimer method [21].

Similar results have been in studies done by Nisio et al, that indicate a sensitivity of 69% for qualitative and 85% for semiquantitative assessment using latex agglutination method to detect FDP levels in deep vein thrombosis [20].

Studies by Wakai et al., accord the statement that D-dimer assays especially qualitative latex agglutination assays that are not 100% sensitive, limiting their use as a single screening test [22].

In contrast to these studies, studies done by Rathi et al., indicate that latex method has 100% sensitivity to detect D-dimer levels in suspected pulmonary thromboembolism at high altitude [23].

Very recently studies done by Gharat et al., to quantitatively estimate serum fibrinogen degradation product levels in oral premalignant and malignant lesion resulted in rise in FDP levels in all the 25 subjects considered for the study [19].

D-Dimer assays detect plasmin mediated FDPs which contain cross linked D fragments in whole blood or plasma. D-Dimer assays which are available currently for clinical usage includes, manual immunochromatographic assays, manual immunoagglutination assays, automated ELISA systems, microtitre plate (ELISA) systems, immunofiltration assays and latex enhanced photometric immunoassays. All these types of assays use monoclonal antibodies that are directed against the epitomes on the D-dimer fragment which are absent on fibrinogen, fibrin and also on non cross linked fragments of fibrin. D-Dimer moiety of different molecular weight may be formed depending on the extent or degree of lysis of XL-Fg (cross linked fibrin). Variable results with different assays within individual patients are as a result of variation in reactivity of different D-dimer monoclonal antibodies to these different molecular weight species. In addition to this some monoclonal antibodies may react with non cross linked degradation products of fibrin or fibrinogen. Plasmin is neutralised by $\alpha 2$ antiplasmin thereby restricting its fibrinogenolytic activity and localizing the fibrinolysis on the fibrin clot. This could influence the presence of FDP levels in plasma. This also could be the reason for undetectable FDP levels in the present study [13,22].

In the semiquantitative assessment of FDP level with regard to clinical staging and histopathological grading did not show any

association with increasing severity of OSMF. More number of samples presented in the range of 200-400 ng/ml which signifies an increase in FDP levels but without any association with varying clinical stages and histopathological grades of OSMF. In agreement to the present study, studies done by Gharat et al., to evaluate serum FDP levels in premalignant lesions like leukoplakia and OSMF and malignant lesion also indicated that there is no appreciable increase in serum FDP levels in OSMF subjects with mean±SD=6.08±1.59. Contrary to our study, studies done by Phatak AG[17], shows a FDP value of 80 microgrammes per ml. as compared to the mean normal level of serum FDP in resting adult subjects is 4.7-2.3. This indicates a significant elevated FDP value.

Estimation of total protein concentration yielded majority of samples in the range of 7-9 g/dl in both clinically and histopathologically graded subjects of OSMF. In comparison to the normal reference value of 6.0 – 8.0 g/dl our study shows a marginal increase in the protein level with chi-square value of 2.49 for p=0.05 with students t-value <1.71 showing no significant difference with varying clinical staging or histopathological grading of patients with OSMF. Studies done by Anuradha et al have revealed a significant rise in total protein level with decrease in A/G (albumin/globulin) ratio due to increase in globulin fraction of protein [24]. Patidar and Rajendran et al., have shown a significant decrease in total protein level in clinically and histopathologically confirmed cases of OSMF [25]. The findings observed in the present study indicate that the FDP levels and total serum protein concentration vary independently with the varying clinical staging and histopathologic grading of OSMF. The plasma FDP kit used in the present study does not detect the minimal rise of plasma FDP levels. Therefore, the usage of FDP kits with higher sensitivity and specificity is required to assess FDP levels in OSMF subjects. A scope for further study with different FDP assessment kits in larger statistically significant population remains open to asses FDP level in OSMF subjects. A detailed analysis of FDP levels in different age groups, sex and ethnicity using different FDP assessment kits keeps gates opened for further study. Assessment of FDP levels can act as an affordable and easily available adjunct method in the diagnosis of OSMF. A detailed protein and immunoglobulin profiling would provide a better understanding in understanding the role of FDP and total serum protein in the etiopathogenesis of OSMF.

CONCLUSION

Thirty clinically and histopathologically proven cases of OSMF were studied to find out the role of plasma FDP and total serum protein concentration in the etiology of OSMF. From the present study following conclusions can be drawn, Qualitative method of estimation for FDP levels did not show association between the presence of FDP levels and OSMF. Semi-quantitative method of plasma FDP estimation did not show association between the increases of FDP levels with the increasing in severity of OSMF. Total serum protein levels showed a marginal increase in the OSMF individuals considered for the study. There was a positive correlation seen between the presence of FDP levels and total protein concentration in OSMF study group.

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