EVALUATION OF LEVELS OF PLASMA FIBRINOGEN DEGRADATION PRODUCTS IN PATIENTS WITH HABIT INDUCED ORAL LESIONS.

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LIST OF ABBREVIATIONS

Sr.No.	Abbreviations	Full Form
01	OL	Oral Leukoplakia
02	OSMF	Oral Submucous Fibrosis
03	OSCC	Oral Squamous Cell Carcinoma
04	FDP	Fibrin/Fibrinogen Degradation Product
05	TF	Tissue Factor
06	u- PA	Urokinase-Type Plasminogen Activator
07	DVT	Deep Vein Thrombosis
08	PE	Pulmonary Embolism
09	AT	Arterial Thromboembolism
10	DIC	Disseminated Intravascular Coagulation
11	EIA	Enzyme Immunoassay
12	ELISA	Enzyme Linked Immunosorbent Assay
13	TAT	Thrombin Antithrombin
14	FPF	Fibrin Precipitating Factor
15	MI	Myocardial Infarction
16	CYFRA	Cytokeratin 19 Fragment
17	ACS	Acute Coronary Syndrome
18	XL	Cross Linked
19	WDSCC	Well Differentiated Squamous Cell Carcinoma
20	MDSCC	Moderately Differentiated Squamous Cell Carcinoma
21	PDSCC	Poorly Differentiated Squamous Cell Carcinoma

Sr.No.	Abbreviations	Full Form
22	MISFI	Molecules Immunologically Similar To Fibrinogen
23	P-MISFI	Plasma- Molecules Immunologically Similar To Fibrinogen
24	S-MISFI	Serum- Molecules Immunologically Similar To Fibrinogen
25	SDPT	Serial Dilution Protamine Test
26	TSP	Total Serum Protein
27	PBS	Phosphate Buffer Solution
28	INR	International Normalized Ratio
29	MACES	Major Adverse Cardiovascular Events
30	INR	International Normalized Ratio

COLOUR PLATE

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INTRODUCTION

Habit is an act, practice, behavioral response, or custom established by frequent repetition of the same activity while substance abuse is the habit of consuming harmful psychoactive substances like tobacco, areca-nut, alcohol, ganja, bhang, afeem etc. The habit of consuming tobacco, areca-nut containing substances and alcohol is in vogue among the Indian population as it is readily available. India is the third largest producer and consumer of tobacco[1,2].

The effects of these substances on the oral mucosa range from initial mucosal changes like habit induced keratosis, quid-induced lichenoid reaction, smokers palate and tobacco associated melanosis to potentially malignant disorders like oral

leukoplakia (OL), erythroplakia and oral submucous fibrosis (OSMF) to full blown oral cancer.

Oral cancer is among the top three types of cancers in India[3]. Over 5 people in India die every hour a day because of oral cancer of which more than 90% of cases report use of tobacco products[4]. Prevalence of oral cancer is higher in males as compared to females and the incidence increases by age where the commonest age is the fifth decade of life[5]. Buccal mucosa is the most common site of cancer followed by gingiva and tongue[3]. The lifetime risk of mortality from cancer in both males and females is 61%[6].

Although the present treatment modalities have improve the quality of life of oral cancer patients however the overall survival rate of 5 years has not improved in the past decade[6]. Oral cancer is particularly dangerous because in its early stages it is usually asymptomatic and may not be noticed by the patient. In the later stages, the primary tumor invades more deeply into local structures and there is increased probability of metastasis leading to poor prognosis[7].

Thus, early detection of oral cancer is the key in fighting against oral cancer and associated death and morbidity. There are a variety of commercially available diagnostic aids and adjunctive techniques which potentially assist in the screening of healthy patients for evidence of otherwise occult cancerous change[8]. However the sensitivity and specificity of all the aids vary and a definitive diagnostic modality is difficult to suggest. Thus the histopathological examination remains the gold standard for final diagnosis which can again lengthen the time lap between diagnosis and initiation of treatment.

During the course of tumor development, quantitative changes have been shown to occur in a variety of substances in blood. These substances are collectively referred to as biochemical markers or tumor markers. This tumour marker would be useful to suspect the presence of the carcinoma at early stages. Today a plethora of tumour markers are available which can be used for screening, diagnosis, establishing prognosis and monitoring treatment. However most of the tumour markers do not have sufficient sensitivity or specificity for use in screening and cannot be detected in early stages of malignancy[9].

A specific association between cancer and the hemostatic system has been recognized for decades. Plasma fibrin/fibrinogen degradation product (FDP) has been shown to be valuable as a tumour marker in cancers like ovarian, gastrointestinal, breast, renal, colorectal, urinary bladder and various other malignancies. In these malignancies the FDP levels have been seen to correlate with cancer occurrence, stage, progression and prognosis[10]. However the available literature regarding the role of FDP levels as a diagnostic and prognostic marker in oral potentially malignant lesions and condition shows mixed school of thoughts while the studies on FDP levels in oral malignancies are very few. There is no literature which shows association of FDP levels with habit induced oral lesion. Thus this study was carried out with an aim to evaluate plasma FDP in potentially malignant, malignant and other habit induced oral lesion and overcome the shortcomings in the associated literature.

AIM AND OBJECTIVES

AIM

To evaluate levels of Plasma Fibrinogen Degradation Products (FDP) in patients with habit induced oral lesions.

OBJECTIVES

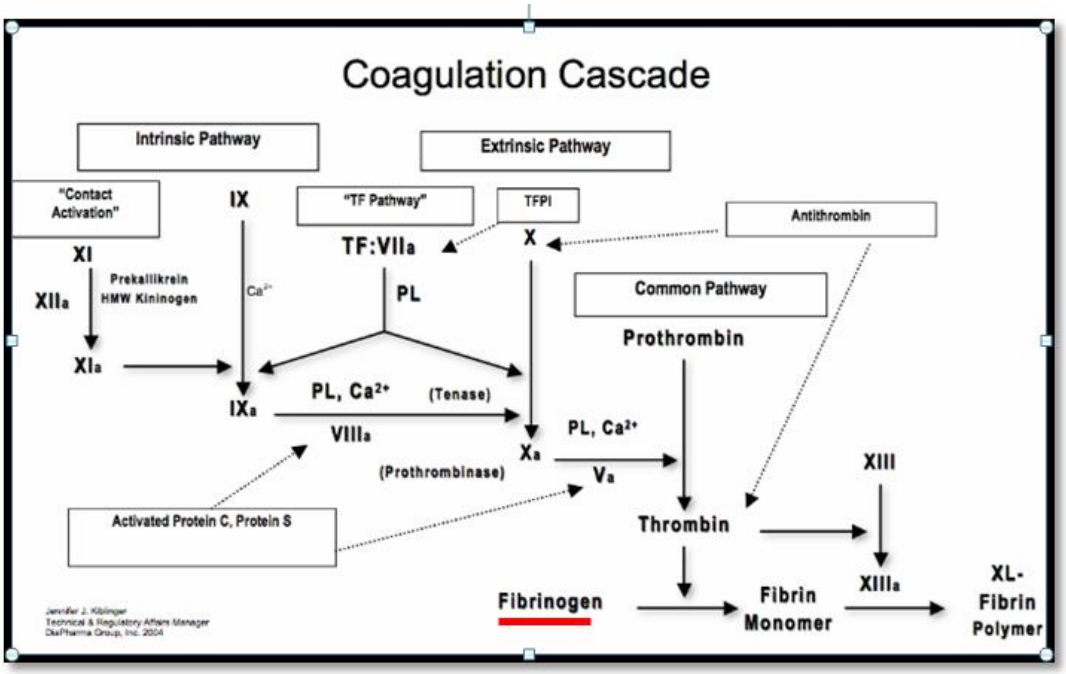
- To evaluate plasma FDP levels in normal healthy individual with normal oral mucosa and no habit.
- To evaluate plasma FDP levels in individual with habits and normal mucosa.
- To evaluate plasma FDP levels in individuals with habit and having oral lesion those are not premalignant in nature.

- To evaluate plasma FDP levels in individuals with habit and having oral submucous fibrosis.
- To evaluate the plasma FDP levels in individuals with habit and having oral leukoplakia.
- To evaluate plasma FDP levels in individuals with habit and having oral squamous cell carcinoma.
- To compare and correlate FDP levels in the above groups and to assess the reliability of FDP levels as an early diagnostic indicator.

REVIEW OF LITERATURE

Fibrinogen

Fibrinogen is a plasma protein which is factor I of coagulation cascade. It is synthesized in the hepatocytes. The plasma concentration of fibrinogen is 1.5-4.5 g/L as clottable protein and approximately 8.8 mmol/L as total protein. It is the precursor of fibrin which is important for clot formation. It plays important role in platelet aggregation by linking activated platelets. Activated platelets express integrin on their surface which binds fibrinogen. Thus, it plays a key role in haemostasis and thrombosis[11].



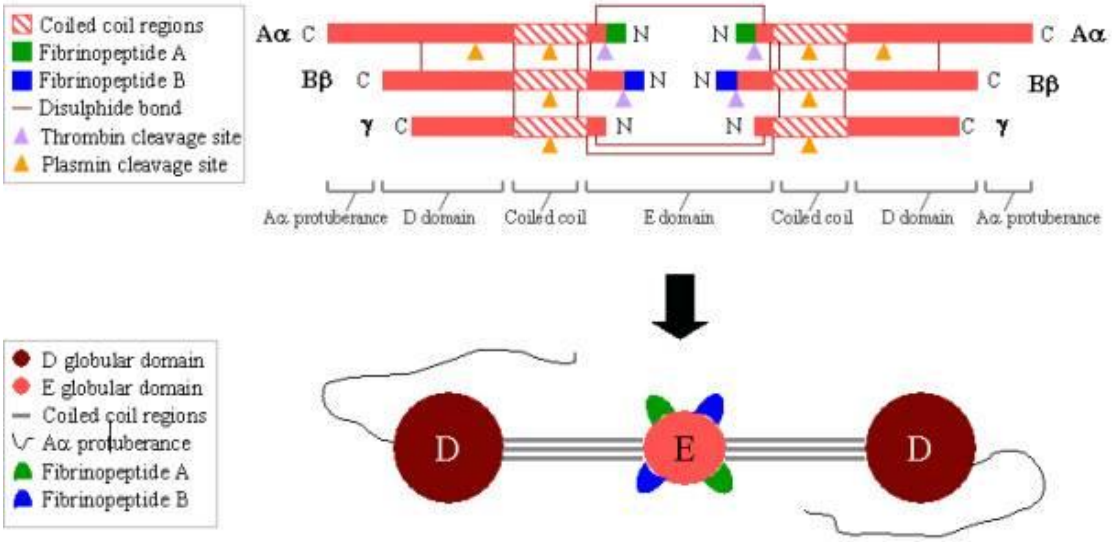
- I : Fibrinogen
- II: Prothrombin
- III: Tissue factor (TF)
- IV: Calcium (Ca)
- V: Proaccelerin
- VI : old name of Factor Va
- VII : stable factor, proconvertin
- VIII : Anti Hemophilic Factor A
- IX Antihemophilic factor B or Christmas factor
- X (Stuart-Prower factor
- XI (Plasma Thromboplastin Antecedent
- XII (Hageman factor
- XIII (Fibrin-Stabilizing Factor
- Von Willebrand factor or vWF

Role of fibrinogen in coagulation cascade [12]

Molecular structure of fibrinogen and its degradation process

Fibrinogen molecule is a 45 nm elongated structure which is heterohexameric glycoprotein. Each molecule has two outer D domain and one central E domain. There are two terminals in the molecule: N-terminal and C-terminal. These molecules are comprised of two sets of disulfide-bridged Aα, Bβ-, and γ-chains with molecular weights of 67000, 58000 and 47000 daltons respectively (molecular weight for the whole molecule of 344000 daltons). Aα, Bβ-, and γ-chains are joined

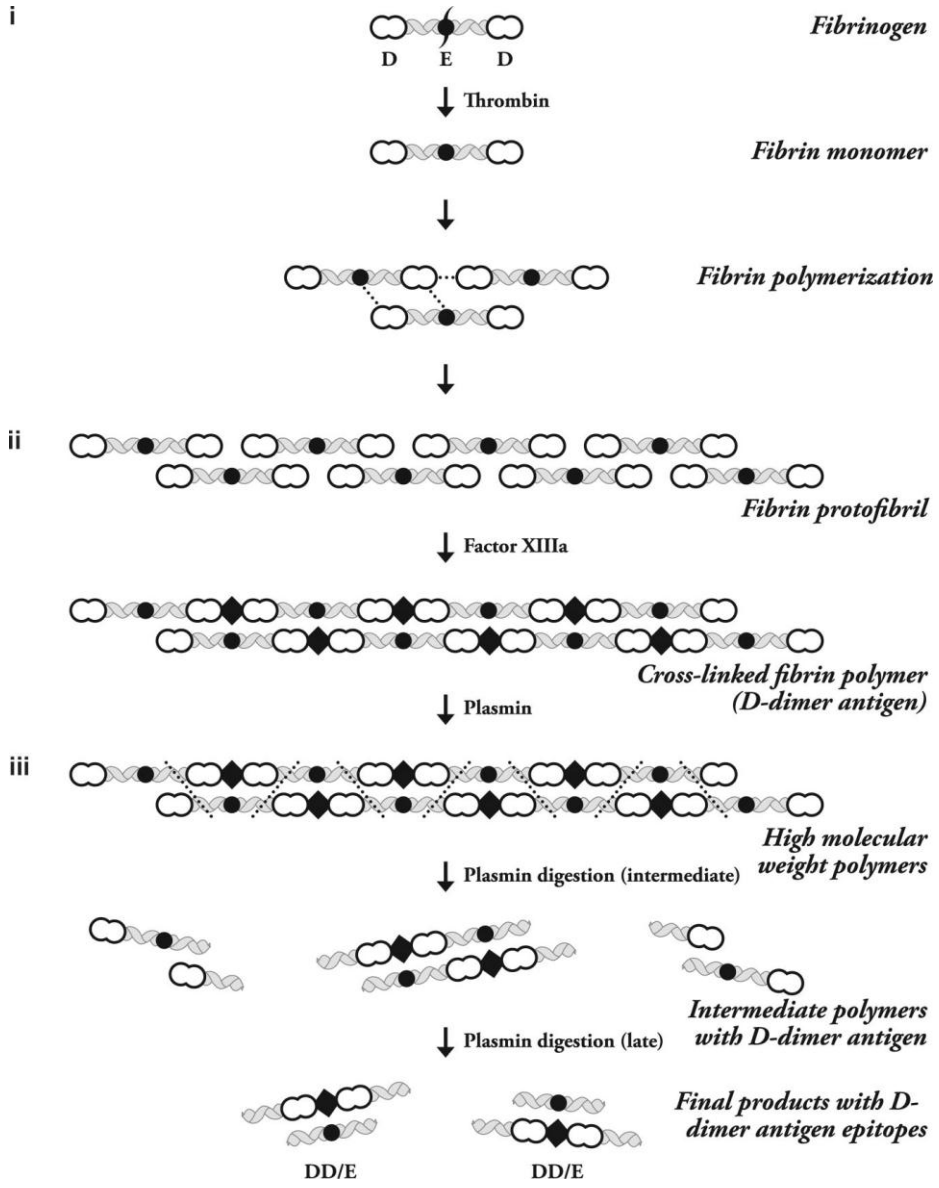
to each other in the N-terminal of E domain. Aα and Bβ chains have fibrinopeptides A and B in their terminal region[13,14,15].



Molecular structure of fibrinogen. (a) polypeptide organisation of fibrinogen; (b) domain organisation of fibrinogen[13].

There are three major steps in formation of fibrinogen degradation product formation. Firstly, fibrinogen molecule is cleaved by thrombin to produce fibrin monomers. These fibrin monomers combine with fibrinogen or fibrin to form protofibrils, held together by non-covalent forces. In the second step, a zymogen form of Factor XIII is converted to active Factor XIIIa (a transglutaminase) through the action of thrombin. Factor XIIIa covalently attaches D domains and inserts a covalent intermolecular linkage and forms cross linked fibrin polymer. In the last step, plasmin degrades fibrin at multiple sites to release fibrin degradation products. The final products by degradation of fibrin/fibrinogen are fragment D and E fragment formed from central D and E domains respectively[10,13,16].

Different type of FDPs are produced depending upon the type of substrates i.e. fibrinogen or fibrin digested by plasmin. When fibrinogen is the substrate, 'Fragments D and E' are the end products with 'Fragments X and Y' as intermediate products. When fibrin is the substrate 'D-dimer' and fragment E is the end product[17].



Steps in fibrinogen degradation[18].

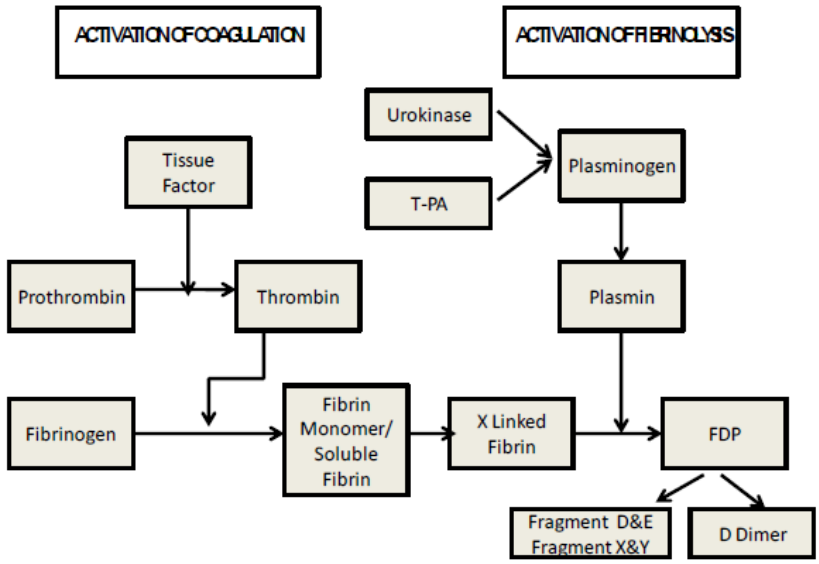
The role of FDPs in tumor

Synthesis of fibrinogen takes place mainly in the liver, but about 5–10% is formed in the megakaryocytes. As fibrinogen is an acute phase protein, its concentration is increased up to 2–10-fold in response to inflammatory agents. Though inflammation associated with cancer, is primarily responsible for the increased level of plasma fibrinogen, tumor tissue can also be the source of fibrinogen. The presence of fibrinogen within the tumor is thought to be one of the factorsnecessary for its growth and metastasis[18].

Tissue factor (TF) in normal condition is strictly regulated in order to maintain the fluidity of blood, but in cancer its expression increases in normal and neoplastic cells in response to proinflammatory factors[19]. TF is responsible for activation of coagulation cascade and induction of formation of thrombin, capable of stimulating tumor growth and metastasis.[20]Both thrombin and fibrin are proangiogenic factors and angiogenesis-dependent activation of thrombin may be dependent or independent of the blood coagulation cascade[10].

In cancer patients the coagulation pathway or the fibrinolysis pathway are activated either separately or simultaneously. Cancer has been known to elevate FDP levels through these two pathways. Due to the activation of these pathways there is rise in the levels of both TF and the urokinase-type plasminogen activator (u-PA). The TF pathway alters the extrinsic coagulation system by causing an activation of thrombin which will convert fibrinogen to fibrin and on the other hand, the u-PA pathway will activate plasmin by catalyzing its inactive precursor plasminogen into

functional plasmin. Plasmin will then degrades fibrin/ fibrinogen at multiple sites to release degradation products[12].



Formation of Fibrinogen Degradation products by activation of the Fibrinolytic and Coagulation pathway[17]

FDP IN VARIOUS GENERAL SYSTEMIC CONDITIONS AND NEOPLASM

Astedt B, Svanberg L and Nilsson IM in 1971[21] investigated F.D.P. levels in 32 malignant and 131 benign tumour by using immunochemical method. Out of 32 malignant tumour patients 23 showed FDP values above 0.5 mg/100 ml while out of 131 benign tumour only 6 showed FDP in range of 2 mg/100 ml. They concluded that absence of demonstrable F.D.P. does not exclude malignancy. However, occurrence of F.D.P. lends support to a provisional diagnosis of malignant disease and indicates further investigation. Thus examination for F.D.P. in patients with suspected malignant ovarian tumours may be of diagnostic value.

Svanberfg L Ineiam L, Pandolf and Astedt B in 1975[22] assessed the local fibrinolytic activity and vascularization in ovarian tumours and serum FDP levels. 25 patients of ovarian tumours and of 14 patients with normal ovaries were included in study. FDP levels were determined before laparotomy procedure and irradiation. On determination of fibrinolytic activity they found that in all the subjects it was confined mainly to vessels of both malignant and benign tumours. Close correlation was found between the fibrinolytic activity and the vascularity of the tumour specimen. 13 out of 14 patient with malignancy showed presence of FDP while it was absent in benign tumours. They stated that the difference in FDP occurrence in malignant and benign cases might be owing to the extent of fibrinolysis due to the invasive growth of the malignant tumours leading to easier escape of fibrinolytic activators into the bloodstream.

Chan T K and Chan V in 1978[23] evaluated the effect of venous occlusion on anti-thrombin III, plasminogen activator and fibrinogen degradation product level in normal healthy individuals. On assessing FDP levels, the basal level was significantly increased in female as compared to males after venous occlusion. There was a biphasic change in FDP level, first there was rise in levels which was suggestive of fibrinolysis and then a transient fall due to leakage of the small molecules of FDP from the vascular compartment as a result of an increase in venous pressure due to the occlusion. Thus they concluded that for assessing the in vivo fibrinolytic capacity of individual in venous occlusion a timed measurement of FDP will be helpful as an adjuvant.

Kosugi T, Takagi I, Ariga Y, Okada S, Morimitsu T, and Mihara H in 1982[24] investigated 5 parameters of the coagulation and fibrinolytic system (fibrinogen, FDP, the fibrinolytic activity of the euglobulin fraction, the antiplasmin, and the antithrombin activity) to clarify the significance of the coagulation-fibrinolysis system in cancer of the head and neck. They found significantly higher levels of fibrinogen in cancer than in healthy adults, lysis area of euglobulin was significantly greater, FDP levels in cancer were somewhat higher than those in healthy adults, although the difference could not be regarded as significant. The antiplasmin and antithrombin was significantly lower. They concluded that the fibrinolytic activity in advanced cancer is greater than in early stage cancer and this increase might reflect increased release of plasminogen activator from the tumor tissue to improve the hypercoagulable state in cancer. Thus hyperfibrinolytic and hypercoagulable state occurred concomitantly in the circulating blood in cancer of the head and neck and affects the clinical stage.

Whitaker AN et al in 1984[25] conducted a study to measure cross linked fibrin derivatives (XL-FDP) in plasma and serum of 25 healthy leucopheresis donors,7 patients with deep venous thrombosis (DVT), 14 with pulmonary embolism (PE), 4 with arterial thromboembolism (AT) and 28 with consumption coagulopathy having diagnoses characteristically associated with disseminated intravascular coagulation (DIC). An enzyme immunoassay measured XL-FDP in plasma and serum using D dimer as standard and confirmation was obtained by parallel monitoring of FDP using affinity chromatography and sodium dodecyl sulphate polyacrylamide gel electrophoresis. They found serum concentrations of FDP were increased in all cases of DIC, in 10 of 14 patients with PE and 2 of 7 patients with

DVT, contrasting with the finding of increased concentrations of XL FDP in all cases. The concentration of plasma values for XL-FDP greatly exceeded the serum values which suggest that this enzyme immunoassay can measure fibrin derivatives in plasma which are absent in serum.

Elms M et al in 1986[26] determined specific XL-FDP (D dimer) in plasma of 40 normal subjects, 19 patients with DVT, 42 patients with pulmonary embolism (PE) and 27 patients with DIC using latex agglutination test and calibrated their values by an enzyme immunoassay (EIA). They found that the entire healthy subject showed negative latex agglutination similar to EIA. All the patients showed positive EIA while 98% patients showed positive latex agglutination titers. They concluded that though the sensitivity of latex agglutination assay was less compared to enzyme immunoassay, it has provided a rapid and less elaborated test for elevated levels of plasma D dimer in patients with thrombosis.

Song K, Kim H, Park K and Kwon H in 1993[27] measured FDP in plasma in 52 patient with liver disease and 20 healthy individual to evaluate fibrinogenolytic state in liver diseases. ELISA was used to determine FDP levels in plasma of these patients. They found that out of 52 patient 19 showed elevated levels of FDP. They showed values in range of 660- 3200ng/ml while the upper limit value for healthy subjects was 580 ng/ml. They found no relation between the liver function test and FDP and concluded that though increased fibrinolysis is common in liver disease it poorly correlates with liver function.

Sato N, Takahashi H and Shibata Ain 1995[28] conducted a study to assess the frequency, clinical backgrounds, and hemostatic states of patient with dissociated

values of fibrinogen/fibrin Degradation Products and D-dimer together with other hemostatic parameter. 371 patients of different group of diseases like hematological diseases, diabetes mellitus, thrombotic diseases, liver diseases , collagen disease, DIC, acute promyelocytic leukemia, malignant lymphoma, cancer, sepsis, solid tumor and infection were included in study. On quantification of serum FDP and plasma D-dimer, the serum FDP values had positive correlation with plasma D-dimer values and both were elevated in parallel with the progression of blood coagulation and fibrinolysis. However, 11.5% of samples showed relatively lower D-dimer values than serum FDP levels, and this was considered as dissociated group. This dissociation was due to lesser extent of activation of coagulation and fibrinolysis than others. The possible reasons for dissociation between FDP and D dimer suggested by them were accelerated fibrinogenolysis, elevated soluble fibrin, accelerated fibrinogenolysis by non-plasmic proteinases and possibly false-positive FDP levels due to non clottable fibrinogen remained in the serum samples. So they suggested that simultaneous measurements of D-dimer and FDP and useful for more accurate estimation of hyperfibrinolytic states.

Okholm M, Iversen L, Thorlacius-Ussing O, Ejlersen E and Boesby Sin 1996[29] conducted a studyto correlate the preoperative plasma levels of FDP in patients with colorectal cancer to tumor stage, metastasis, and postoperative thromboembolic complications. The study group consisted of 182 patients of 40 years of age. Among them, 91 patients had colorectal cancer, 20 had colorectal adenoma and 71 patients were without neoplastic lesions in the colon or rectum. FDP was determined using sandwich-type ELISA for quantification of fibrin and fibrinogen degradation products in plasma. They found that preoperative

concentration of FDP in plasma was elevated in patients with colorectal cancer, especially in patients with metastasis which is indicative of enhanced fibrinolyric activity in plasma of these patients. 24 % of patients with colorectal cancer developed deep vein thrombosis after surgery. So they concluded that increased level of FDP in colorectal cancer patient preoperatively has more chance of developing deep vein thrombosis complicating the prognosis.

Genis AB et al in 2000[30] examined plasma D-dimer levels to determine their independent diagnostic value over the other conventional hemostatic markers (thrombinantithrombin (TAT) complexes, prothrombin fragment, activated factor VII, and fibrinogen) for the early diagnosis of acute coronary syndromes in the emergency department (ED). In patients with acute ischemic events (myocardial infarction and unstable angina) D-dimer and fibrinogen levels were significantly higher than in non-ischemic patients while there was no significant elevation of other hemostatic markers in patients with ischemic events. D-dimer level >500 microgram/L indicated an independent diagnostic value for MI which increased the diagnostic sensitivity of the electrocardiogram and history from 73% to 92%. Thus they concluded that D-dimer is a marker of substantial incremental value for the early diagnosis of acute coronary syndromes.

Oya M, Akhiyama Y, Okuyama T and Ishikawa H in 2001[31] carried out a study to examine relationship between preoperative plasma D-dimer and both pathological finding and TNM classification and also the prognostic significance of preoperative plasma D-dimer level. D dimer level was assessed preoperatively by using latex agglutination assay in 93 patient who later undergone curative resection

of colorectal cancer and 40 patients with benign colorectal disease. They found that plasma D-dimer levels were significantly higher in colorectal cancer patient than in patient with benign colorectal disease. Levels were higher when the tumor were large, deeply penetrated and with advanced TNM stage. They also found that higher preoperative level was significantly related to shorter post-operative survival rate. Thus they concluded that pre-operative D-dimer level is useful in pre-operative tumour staging and prediction of post-operative survival rate.

Khan ZM, Khan MS, Raziq F and Khattak AM in 2007[32] assessed the levels FDPs and D-dimers in 50 patients with breast carcinoma at different TNM stages and 25 normal healthy controls. On estimation of FDPs out of 50 patients, 28 patient had FDP more than 40 μg/ml and the maximum cases belonged to stage III nad. 15 patients had levels between 5-40μg/ml where maximum cases were in stage II and III. Only 7 patient had levels <5μg/ml and those were in stage II and III. Similarly, on estimation of D-dimer levels, 8 patients showed D-dimer level 1000-2000·g/ml which belonged to stage III and IV. 11 patients had levels 500-1000·g/ml present in stage III and IV. 30 had level 250-500·g/ml which were maximum in stage II and III. Thus they concluded that levels of both the FDP and D-dimers are elevated progressively in breast carcinoma, especially as in those with distant metastasis.

Ay C et al in 2012[33] determine the role of the activation of hemostasis and fibrinolysis measuring the plasma levels of D-dimer, in the prognosis of patients with various types of cancer. D-dimer levels were measured by a quantitative latex assay in 1178 cancer patients consisting of malignancies of the lung, lower gastrointestinal tract, pancreas, breast, stomach, kidney, prostate, brain and hematologic

malignancies. They found high plasma levels of D-dimer were associated with increased mortality risk. After 2 years, the probability of overall survival in cancer patients with the highest D-dimer levels of the total study population was only 30% as opposed to that of 78% in cancer patients with lower levels. Thus they concluded that the D-dimer levels are promising prognostic biomarker associated with poor overall survival in the general cancer population.

Osman S and Muddathir AR in 2013[34] conducted a study to measure plasma fibrinogen and D-dimer levels in 100 diagnosed hypertensive patient (age ranged 35-65 years) and 50 non-hypertensive individuals. The mean of plasma fibrinogen level was significantly higher in hypertensive patients (395.59 mg/dl) than in control group (354.69 mg/dl) with p value= 0.000. D-dimer concentrations have been categorized into 4 groups :< 0.1 mg/l, 0.1mg/l, 0.2 mg/l and 0.3 mg/l and they found significant difference in plasma fibrinogen level between these groups. D-dimer level was insignificantly increased in hypertensive patients when compared with control group. Thus they concluded that as fibrinogen is a major determinant of blood viscosity and involved in haemostasis and thrombosis pathway, measurement of fibrinogen level may be of some benefit in detecting thrombosis which appears to complicate the hypertension.

Sogani S and Sarakar P in 2013[35] evaluated plasma fibrinogen and plasma FDP in preeclampsia, a systemic disease characterized by hypertension, edema and proteinuria and commonest complications of pregnancy. They found that normal pregnant group showed elevated levels of fibrinogen than the normal range while average values of FDP and fibrinogen were higher for the preeclamptic pregnant

patients as compared to normal pregnancy group and significantly higher in severe preeclampsia group than in the mild preeclampsia group. They concluded that elevation in plasma fibrinogen indicate exaggerated inflammatory responses and the endothelial activation while elevation in FDP indicates the increased intravascular coagulation fibrinolytic activity in preeclampsia. Thus both are the complementary predictors explaining the severity of the disease.

So HJ, Hong SI, Lee JK, Chang YH, Kang SJ and Hong YJ in 2014[36] compared the serum fibrin-fibrinogen degradation products with cytokeratin 19 fragment (CYFRA 21-1) a well-established biomarker in patients with lung cancer. The serum samples were obtained from 193 lung cancer patients, 106 patients with benign respiratory diseases and 84 healthy controls. They found the clinical sensitivity and specificity of CYFRA 21-1 was 77 and 74% respectively while it was 86 and 75%, respectively for FDP. No correlation was found between FDP and CYFRA 21-1. However, diagnostic sensitivity can be increased to <95% by combining FDP with CYFRA 21-1. Thus they concluded that serum FDP is comparable to CYFRA 21-1 as a lung cancer biomarker and can be used in clinical practice for diagnosis.

Dastjerdi MV, Ahmari S, Alipour S and Tehranian A in 2015[37] compared FDP (D-dimer) levels as a tumor marker in patients with benign and malignant tumors of uterus, cervix and ovary. 15 patients were included in each subgroup of benign and malignant tumors. The mean FDP level was highest in malignant cervical tumors (3.9 mg/L) and lowest in benign uterine tumors (0.27 mg/L). Among malignancies, the levels were significantly higher in cervical cancer than uterine and

ovarian cancer. In malignant cervical tumors, the differences of levels between various stages weren't significant. However stages I and IV had significantly different levels of D-dimer. Thus they concluded that a correlation exist between prognosis of disease or the stages of tumors and the level of plasma D-dimer.

Gu H and Yang X in 2016[38] examined combined value of D-dimer and FDP to predict the short-term prognosis of acute coronary syndrome (ACS) patients. 126 patients were divided into 2 groups i.e. 68 cases of major adverse cardiovascular events (MACEs) and 58 cases, without MACE. The levels of D-dimer, FDP, INR, PLT and PT, were detected and compared between the two groups. The patients kept on follow-up for 1 year and the occurrence of MACE in both the groups was recorded. They found plasma levels of D-dimer and FDP in MACEs group were higher than those in control; however the differences in the indices of PLT, PT and INR were not statistically significant between two groups. The sensitivity, specificity and accuracy for the prediction were 46.6%, 78.3% and 56.8%, respectively. Thus they concluded that the combined detection of plasma D-Dimer and FDP has good predictive value for the occurrence of MACE in patients with ACS.

FDP IN ORAL POTENTIALLY MALIGNANT DISORDERS AND MALIGNANCY

Pathak A.G in 1979[39] in his case report of 25 year old male with OSMF evaluated the plasma fibrinogen, cryofibrinogen and salivary fibrin precipitating factor. They found strong fibrin precipitating factor (FPF) in the parotid saliva of patients with OSMF. Plasma fibrinogen levels were also elevated in this patient. Precipitable fibrinogen was noted in this patient. They suggested that saliva may

have a role in the causation of OSMF as they detected FPF in saliva of OSMF patients and hypothesized that FPF has thrombin-like behavior. 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 degradedinto 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.

Pathak A.G in 1984[40] detected molecules immunologically similar to fibrinogen (MISFI) in plasma and serum called plasma-MISFI (P-MISFI) and serum-MISFI (S-MISFI), respectively. Seven patients with classical OSMF undergone test for fibrin derivatives, serial dilution protamine test (SDPT), cryofibrinogen studies, fibrinogen degradation products and plasma fibrinogen. The values of plasma fibrinogen estimated were significantly lower than MISFI obtained by immunologic assay. MISFI were found to be increased in the serum of these patients. Ethanol gelation was positive in 3 of the 7 patients. The SDPT and cryofibrinogen in plasma showed delayed positivity. He suggested that OSMF can be used as human model for the study of fibrinogen and other molecules that are immunologically similar to fibrinogen. According to him, fibrinogen, fibrinogen intermediates, and FDPs deserved further scrutiny, as this might help define the etiology of OSMF which had been obscure at that time.

Pathak A.G in 1984[41] reported a 25-year-old male case of OSMF without any habit of areca nut and chewing tobacco. He was well-built and nourished,

however had iron deficiency anemia. Fibrinogen and FDP were assayed in a hemagglutination inhibition system (FDP kit Wellcome).

Plasma fibrinogen level was found to be increased to 800 mg/ml (normal 200–400 mg/ml). FDPs were also found to be elevated. Cryofibrinogen was found in heat‑oxygenated plasma and dilute oxylated plasma. No precipitate was detected in the control tubes kept at 37°C. Fibrin precipitating factor, a factor that had been described by him previously in routine saliva, was also detected in the saliva collected after parotid duct stimulation.

Ghosh M, Aroor AR and Raghavana MR in 1990[42] observed significant increase in the mean values of plasma FDP levels with the advancement of stages in oral cancer patients as compared to normal individuals.

Koshti Sand Barpande S in 2007[43] conducted a study to assess the FDP levels in the plasma of OSMF patients. The study comprised of 35 cases of OSMF and an equal number of age- and sex-matched control subjects. Plasma FDP was quantitated by using a diagnostic kit, a qualitative and semi quantitative latex agglutination slide test for detecting cross-linked FDP in human plasma. They found 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) levels in this IV grade could not be assessed as none of the patients exhibited in this grade. Thus statistically significant increase in the mean FDP levels with increase in clinical grades was seen. The mean plasma FDP levels in various histological grades were 1,924 ng/dl (grade II), 3,267 ng/dl (grade III) and 4,000 ng/dl (grade IV). An increase in the mean FDP levels with increase in the histological grades was also noted but was not statistically

significant. They concluded that 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 which in turn leads to restriction of mouth opening.

Wanjari PV, Wanjari SP, Gharote and WarhekarAM in 2011[44] aimed at the correlation of plasma fibrinogen and FPF in saliva to evaluate the etiology of OSMF. 150 patients were divided into 50 OSMF patients (group I), 50 areca nut chewers without OSMF (group II), 50 control healthy individual. Morning saliva samples were collected from all the groups. Different combinations of plasma and saliva were dispensed in test tubes for detection of clot formation. All the test tubes were incubated at 37°C and observed at the interval of 2, 4 and 24 hours for the clot formation. Plasma fibrinogen was estimated by the King's method (1956). They found salivary FPF in 86% of OSMF patients, group II 12% patient showed positive FPF and none were detected in group III. The mean plasma fibrinogen level in group I, II and III was 429.5 mg% ± 93.36, 251 mg% ± 38.71 and 238.48 mg% ± 46.23 respectively. In 43 OSMF patients with positive salivary FPF, there was increase in plasma fibrinogen level as compared to the healthy individuals, areca nut chewers and OSMF patients as a whole. In FPF negative OSMF cases average plasma fibrinogen level was very less as compared to OSMF patients as a whole. Thus they suggested increased plasma fibrinogen level and presence of salivary FPF may have some role in pathogenesis of OSMF.

Kiran G, Sekhar MS, Hunasgi S, Ahmed SA, Suri C and Krishna A in 2013[45] evaluated plasma FDP in betel nut chewers in patients with and without oral submucous fibrosis. They grouped study population into group A comprising of 35 cases of betel nut chewers with clinically evident OSMF, group B of 10 patients with betel nut chewing habit but normal oral mucosa, and group C of 10 normal patients without habits and having normal oral mucosa. Estimation of plasma FDP levels was done using a diagnostic kit for cross-linked fibrin degradation product based on the principle of agglutination. They found plasma FDP levels >200 ng/ml in all Group A patients and not with Group B and C patients. All the FDP values were same (>200 ng/ml) in Group A patients. Most of the Group A patients were in clinical grade II and advanced histological grades. As clinical grade increased, more number of patients was in advanced histological grade. They concluded that although patients of Group A showed the presence of plasma FDP, the association between them was not statistically significant and thus emphasize the need for a more sensitive kit which could be able to detect the precise values of FDP, so that a direct correlation can be established. However this test could give a quantitative assessment of FDP levels and could be used as a nonsurgical diagnostic aid in suspected OSMF cases.

Gharat L, Rathod GP and Kandalgaonkar S in 2013[17] undertook a study to evaluate the serum FDP levels in individuals without any oral lesions and those with oral premalignant and malignant lesions and determine whether this can be used as an aid in early diagnosis. The study group consisted 25 cases of leukoplakia, 25 of OSMF and 25 OSCC along with an equal number of age and sex matched control patients. The slide latex agglutination method was used for quantitative determination of presence of FDP. They found statistically significant rise in the

levels of serum FDP levels in OSCC. The mean serum FDP levels showed a rise with increase histological grades in case of well and moderately differentiated OSCC, but the increase was not statistically significant. Serum FDP levels were not significantly raised in OSMF and leukoplakia cases. The mean serum FDP levels in 25 OSCC patients also showed a statistically significant increase in FDP levels with an increase in the stage of the lesion. They conclude that a definite relationship is seen to exist between the serum levels of FDP and the stage of OSCC and the levels are seen to correlate directly with the spread of the lesion to the regional lymph nodes and distant metastases. However, unlike oral malignancies, there are no proteolytic enzymes present in premalignancies, because of absence of a tumour mass. This might explain the insignificant changes seen in premalignancies.

Kadani et al in 2014[46] assessed the role of FDP in the etiology of OSMF and correlated with the total serum protein level (TSP) and also evaluate correlation between the levels of plasma FDP and TSP to the staging and grading of OSMF. The study included 30 cases clinically and histopathologically diagnosed as oral submucous fibrosis. The FDP levels were assessed using qualitative and semi-quantitative latex slide test for detecting cross-linked FDP in plasma and TSP by Biuret method. In all the 30 subjects of OSMF only 14 subjects showed positive plasma FDP i.e levels >200ng/ml. No statistically significant association with the increasing in severity of OSMF to plasma FDP levels was seen. Total serum protein levels were marginally increased in all the OSMF subjects. There was no association between total serum protein concentration with various clinical stages and histopathological grades of OSMF. Thus they concluded that qualitative method of estimation for FDP levels do not show association between the presence of FDP

levels and OSMF. Semi-quantitative method of plasma FDP estimation do not show association between the increased FDP levels with the increasing severity of OSMF. Total serum protein levels can increase in the OSMF individuals.

Gupta S, Manjunath SM, Jawanda MK and Bharti N in 2014[47] carried out a study to quantify the plasma FDPs levels in the individuals with the habit of areca nut chewing with and without OSMF. 95 subjects were divided into three groups. Group I: 35 subjects with the habit of areca nut chewing with OSMF, Group II: 30 subjects with the habit of areca nut chewing without OSMF and Group III: 30 individuals without any habit of areca nut chewing without OSMF, (control group). Classification of OSMF was done according to More et al. Estimation of plasma FDPs was done using a diagnostic kit, Tulip XL-FDP (latex agglutination test). They found that all the groups showed highly significant differences (<.001) between their mean values. Out of 35 cases in Group I, 9 were categorized with Grade I OSMF, 4 were with Grade II OSMF, 13 were with Grade III OSMF and 9 were with Grade IV OSMF. Levels of FDPs were increased with the increase in clinical grades. This indicates that there is increase in deposition of fibrin, thus leading to increased severity of the disease characterized by restricted mouth opening. Thus they concluded that plasma FDP test can be used as early indicator of OSMF.

Sharma R, Vikey A, Jaiswal S, Bagulkar B, Bhat A and Bharadwaj Nin 2017[48] carried out a study to correlate the plasma FDP levels in subjects with carcinogenic product habit; but without clinical findings with the control group. At the same time they assessed whether these levels can be used to predict the likelihood of developing clinical manifestations of oral potentially malignant

disorders in subjects with a chronic habit of consuming carcinogenic products. The study comprised of a total of 60 subjects divided into Group A consisting of 30 subjects with habit of carcinogenic product consumption but having apparently normal oral mucosa, and Group B consisting of 30 systemically healthy controls without any deleterious habit. The elucidation of FDP levels was carried out using the automated latex-enhanced immunoturbidometric method by a diagnostic kit for cross-linked fibrin degradation products. They found that the mean FDP level in subjects with smoked tobacco consumption habit was 3005.60 mg/ml, whereas in smokeless tobacco consumption habit, a mean of 418.51 mg/ml was noted. Subjects with a mixed habit of both smoked and smokeless tobacco consumption had a mean value of 2788.32 mg/ml. The mean FDP levels in normal subjects were 44.20 mg/ml. They concluded that the plasma FDP are early diagnostic indicator of fibrin deposition and may be useful to determine the risk of development of oral potentially malignant disorders in individuals with a chronic habit of carcinogenic product consumption. They also suggested the use of the latest automated latex-enhanced immunoturbidimetric method which more reliable than the other techniques for estimation of FDP levels.

MATERIAL AND METHODS

This is a hospital based cross sectional analytical study which was initiated after getting approval from Institutional Ethics Committee and was carried out in the Department of Oral Medicine and Radiology.

Total 150 subjects were selected randomly from the departmental OPD out which 25 were normal healthy individual with no habits and 125 were with history of habits (areca nut chewing, tobacco, betel quid chewing, smoking, alcohol) for more than 1 year and were categorized into **SIX** groups, each group consisted of 25 individuals.

STUDY GROUP

- Group I : Normal healthy individual having no habit with normal oral mucosa.
- Group II : Individual with habits and normal mucosa.
- Group III : Individual with habits and having oral lesion those are not premalignant in nature (fig 7)
- Group IV : Individuals with habit and having oral submucous fibrosis (OSMF fig 8)
- Group V : Individuals with habit and having oral leukoplakia (OL fig 9)
- Group VI : Individuals with habit and having oral squamous cell carcinoma (OSCC fig 10)

Patient's selection criteria

INCLUSION CRITERIA

- Individuals who had habit of areca nut, tobacco, or alcohol for more than 1 year.
- Age above 10 years
- Individuals who were willing to participate in study.

EXCLUSION CRITERIA

- Individuals with any systemic disease and/or on any medication.
- Patient with history of treatment for premalignant and malignant conditions.
- Patient with malignancy in any other region.
- Pregnant women.
- Women in menstruation period

MATERIALS

1. Armamentarium for clinical examination (fig.1)

   - Disposable gloves
   - Stainless steel kidney tray
   - Mouth mirror, straight probe, tweezer
   - Sterile gauge piece and cotton
   - Vernier caliper

2. Armamentarium for blood sample collection (fig.2)

   - Disposable gloves
   - Tourniquet
   - Sterile gauge piece and cotton
   - Spirit
   - 2ml sterile disposable syringe with 26 gauge disposable needle
   - Sodium citrate bulb

3. Biopsy instruments (fig.3)

   - 5 ml sterile disposable syringe with 26 gaugedisposable needle.
   - 2 % lignocaine hydrochloride with 1: 80,000 arenaline.
   - A sterile biopsy tray.
   - Toluidine blue
   - BP handle no. 3 with no.15 blade
   - Biopsy punch no. 5,6,7,8
   - Sterile gauge and cotton
   - Tissue holder

- Non resorbable suture material and needle.
- Needle holder
- Formaline 10% for tissue collection

4. Centrifuge machine (fig.4)

5. Plasma FDP kit (Tulip XL FDP diagnostic kit fig.5)

REAGENTS

1. XL FDP latex reagent: A uniform suspension of polystyrene latex particles coated with mouse monoclonals Anti D dimer antibody (DD-3B6/22). The reagent is standardized to detect XL FDP ⪰ 200 ng/ml.
2. Positive control, reactive with XL FDP latex reagent.
3. Negative control, non-reactive with XL FDP latex reagent.
4. Phosphate buffer, for performing semi-quantitative test.

- Plastic slide with six reaction circles, disposable sample dispensing dropper, mixing sticks.
- Additional material added to kit: stopwatch, test tubes, high intensity direct light source.

METHODOLOGY

Clinical examination

A detailed case history of each patient was carried out with the proforma designed for recording all the relevant information and observation (Annexure I). Thorough clinical examination was carried out in a dental chair under proper illumination.

Patient with OSMF were graded clinically as per classification system given by Khanna and Andrade[49]. Patient with oral leukoplakia were classified as homogeneous or non homgeneous leukoplakia while patient with oral malignancy were categorized as per clinical TNM staging. Other miscellaneous habit induced lesions like habit induced lichenoid reaction, smoker's palate, tobacco pouch keratosis etc., were also clinically diagnosed.

Routine blood investigation was performed before carrying out biopsy procedure. Biopsy was performed in patient with OSMF, oral leukoplakia and OSCC for histopathological confirmation. 6mm punch was used for biopsy in OSMF and leukoplakia patients while incisional biopsy was performed for malignant lesion. After confirmation of diagnosis FDP test was performed.

For FDP level estimation 2 ml of venous blood was withdrawn by venipuncture under aseptic precaution and collected in a citrate bulb (fig. 11 and 12). The bulbs were allowed to stand for one hour at room temperature and then centrifuged at 4,000 rpm to separate the plasma (fig. 13). Plasma was estimated for FDP levels using Tulip XL diagnostic kit which is a qualitative and semiquantitative latex slide test for detection of cross linked fibrin degradation products in human plasma.

PRINCIPLE OF FDP TEST

FDP can be detected by XL FDP slide test and it is based on the principle of agglutination. In this test the sample (plasma) is mixed with XL FDP reagent and checked for agglutination. The sensitivity of the reagent is 200⊐ ng/ml. It will give positive agglutination reaction when the FPD levels are 200 ng/ml or above and

below this level samples are considered negative. The cross-linked fibrin degradation products, D dimer, D dimer E, and high molecular weight derivatives are all can be recognized by this kit reagent incorporating the monoclonal antibodies but the kit is not reactive to FDP- X, Y, D, and E below 20 mg/L or to fibrinogen up to 1000 mg/L.

TEST PROCEDURE

The reagents and samples used were brought to room temperature before performing the test. The samples were analyzed by qualitative method and those with positive results were subjected to semi-quantitative method.

QUALITATIVE METHOD

1. One drop of plasma specimen was put onto the plastic slide using the disposable sample dropper provided with the kit. The dropper was held exactly in vertical position to dispense the drop accurately (fig. 14).
2. One drop of XL FDP latex reagent was added adjacent to the drop of plasma specimen. Care was taken not to let the dropper tip touch the plasma specimen on the slide (fig.15).
3. Using a mixing stick, the plasma and latex reagent were uniformly mixed over the entire circle (fig.16).
4. Immediately a stopwatch was started, the slide was gently rocked, back and forth, and observation for agglutination for three minutes was done.

Interpretation of results

Agglutination is a positive result indicating D dimer level above 200 ng/ml (fig. 17.a) No agglutination is a negative result indicating absence of clinically significant D dimer levels in the plasma specimen (fig. 17.b).

SEMI QUANTITATIVE METHOD

1. Using PBS buffer solution serial dilutions of the plasma sample 1:2,1:4,1:8,1:16,1:32 and so on were prepared (fig.18)
2. Each dilutions of plasma specimen were put onto the separate reaction circles.
3. one drop of XL FDP latex reagent was added to each drop of diluted plasma specimen onto the slide.
4. Stopwatch was started, the slide was gently rocked, back and forth, observing for agglutination at three minutes (fig 19).

Interpretation of results

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.

$$\text{D dimer level (ng/ml)} = 200 \times d$$

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

STATISTICAL METHODS

The data on demographic and behavioural characteristics about habits was summarized according to scale of measurement. Age was expressed in terms of mean and standard deviation, while behavioural parameters were expressed in terms of numbers and percentage for each study group. Statistical comparison of number of cases with FDP present across the study groups was performed using Fisher's exact test. Further, the comparison among clinical TNM stage as well as histological grades of OSCC groups, OSMF stagesand type of leukoplakia was tested for statistical significance using Fisher's exact test. The habit pattern between genders was tested using Pearson's Chi-square test. All the analyses were performed using SPSS version 20.0 (IBM Corp) and R-3.0.0 and statistical significance was tested at 5% level.

The description of methods used is as below:

If $x_1, x_2, \ldots x_n$ are the observations on random variable X, then

A) **Sample mean** for a set of observations is given by

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

B) **Standard deviation** for a set of observations in given by

$$s = \sqrt{\frac{1}{(n-1)}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

where x_i = observation on each object

n = number of objects

C) Chi-square test

Let *X* and *Y* be two variables under study with *r* and *s* levels respectively; and the data on $r \times s$ levels be in the form of counts. Let the null hypothesis be that the two variables are independent. That is, knowing the levels of *X* does not help in predicting the levels of *Y*; against the alternative hypothesis that the two factors are not independent. That is, knowing the level of *X* can help in predicting levels of *Y*. To decide about the acceptance of hypothesis, the Chi-square test statistic is used which is defined as:

$$\chi^2 = \sum_{i=1}^{r}\sum_{j=1}^{s}\frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$

where O_{ij} is the observed frequency count for i^{th} level of variable *X* and j^{th} level of variable *Y*. E_{ij} is the expected frequency count for same cell. The expected count is given by

$$E_{ij} = \frac{n_i \times n_j}{n}$$

where n_i and n_j are the total counts for i^{th} level of variable *X* and j^{th} level of variable *Y*; and *n* is the total count. The calculated Chi-square value is compared with the tabulated one for $(r\text{-}1)\times(s\text{-}1)$ degrees of freedom. If the corresponding *p*-value is smaller than the pre-decided significance level, say 0.05, then we reject the null hypothesis and accept the alternative one. If the *p*-value is more than 0.05, then we accept null hypothesis.

D) Fisher's exact test

Fisher's exact test is a statistical test to determine if there are any non-random associations between two categorical variables.

If X and Y are the two categorical variables with *m* and *n* observed states respectively, then a *m* x *n* matrix can be generated with a_{ij} as the number of observations for i^{th} state of X and j^{th} state of Y. Accordingly, the row and the column sums R_i and C_j are

$$N = \sum_i Ri = \sum_j Cj$$

The conditional probability of getting the actual matrix given the particular row and column sums is given by

$$P_{cutoff} = \frac{R_1!R_2!...R_m!)(C_1!C_2!...C_n!)}{N!\prod a_{ij}!}$$

This is a multivariate generalization of hypergeometric distribution. All possible matrices of non-negative integers consistent with rows and column sums are determined, and for each matrix the conditional probability using above expression is determined, such that the sum of probabilities is 1.

To determine the P-value of the test, the tables needs to be ordered by some criterion that measures dependence, and those tables that represent equal or greater deviation from independence than the observed table are the ones whose probabilities are added together. In a typical 2 × 2 case, the P-value of the test is simply the sum of P-values of matrices that are less than P_{cutoff}. In R package, the test can even be applied for $r \times s$ tables where r and $s > 2$.

PHOTO PLATE I

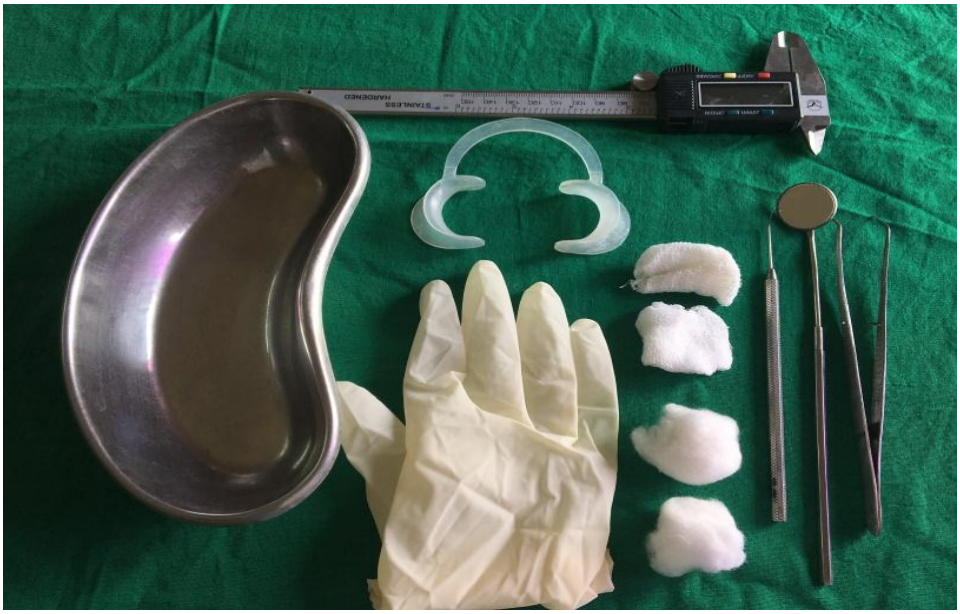


Fig 1. Armamentarium for clinical examination.

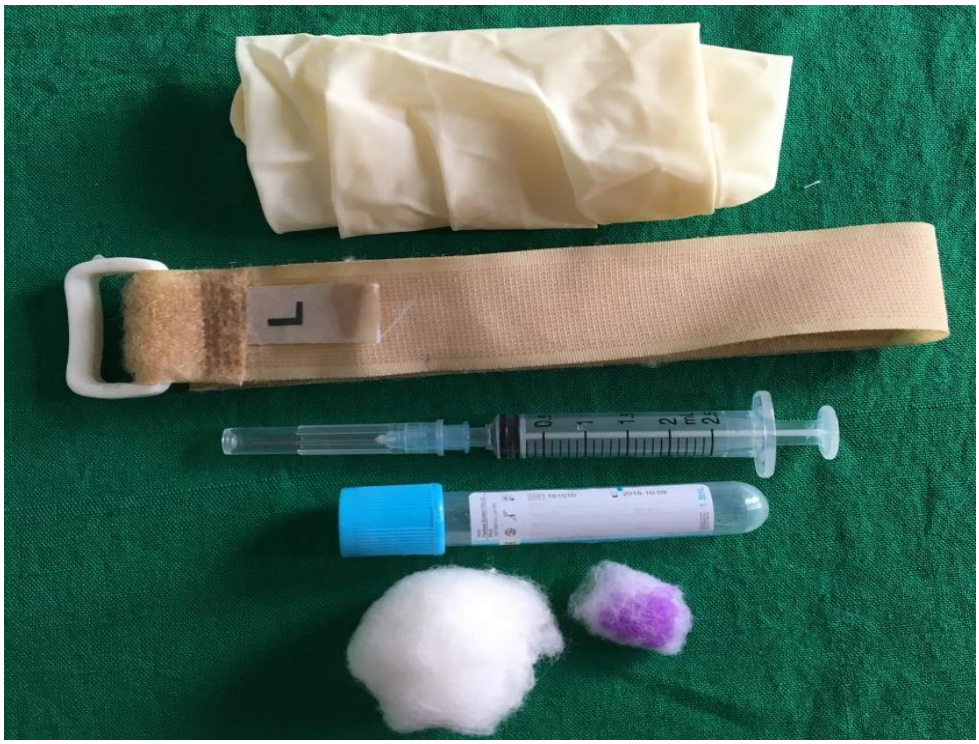


Fig 2. Armamentarium for blood sample collection.

PHOTO PLATE II

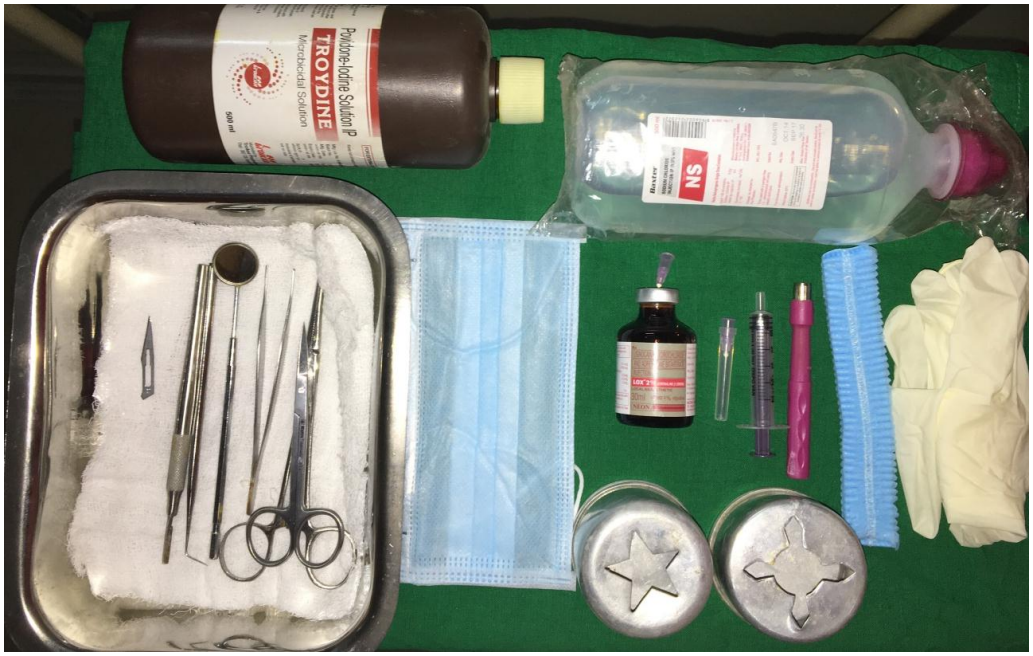


Fig.3 Armamentarium for biopsy procedure.

Fig.4 Centrifuge machine

PHOTO PLATE III

Fig. 5 : Plasma FDP diagnostic kit (Tulip XL FDP diagnostic kit)

Positive control and Negative control

XL FDP latex reagent

Phosphate buffer solution

Plastic slide with six reaction circles, disposable sample dispensing dropper, mixing sticks.

PHOTO PLATE IV

CLINICAL PICTURES OF DIFFERENT STUDY GROUP

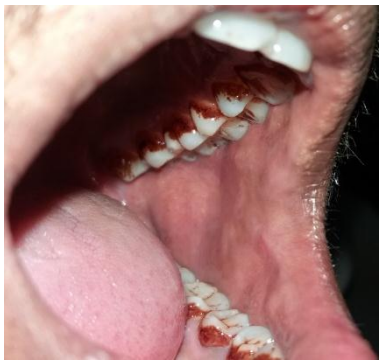


Fig 6: Group II: Normal mucosa with habit

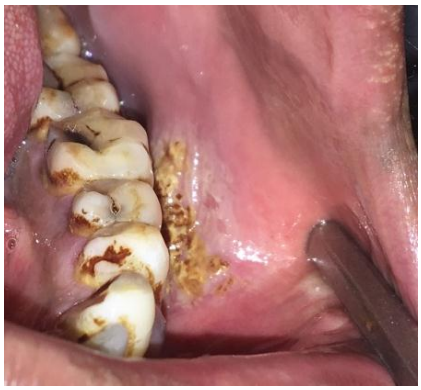


Fig 7: Group III: tobacco pouch keratosis

Fig 8: Group IV: OSMF

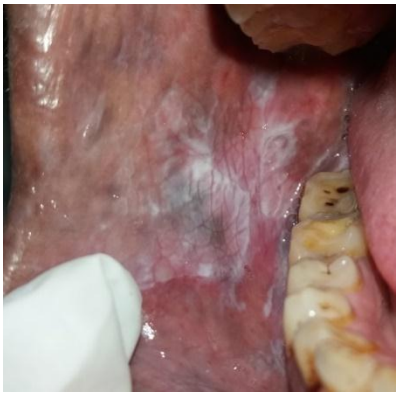


Fig 9: Group V: Oral Leukoplakia

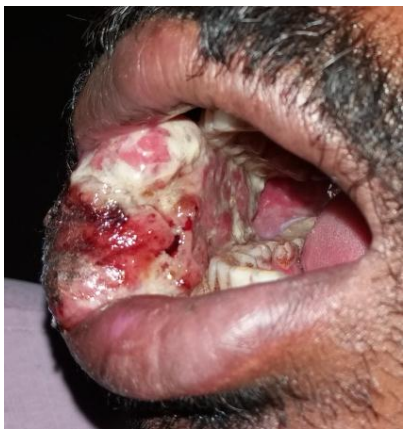


Fig 10: Group VI: OSCC

PHOTO PLATE V

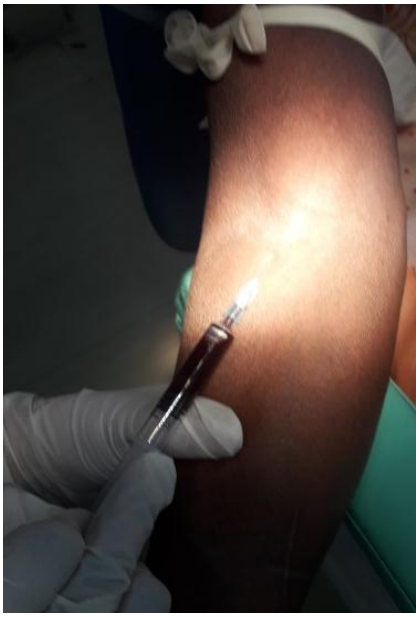


Fig. 11. Venipuncture under aseptic precaution

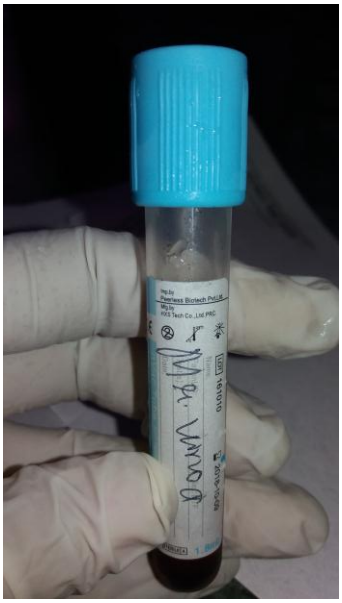


Fig 12: Transfer of blood into citrate bulb

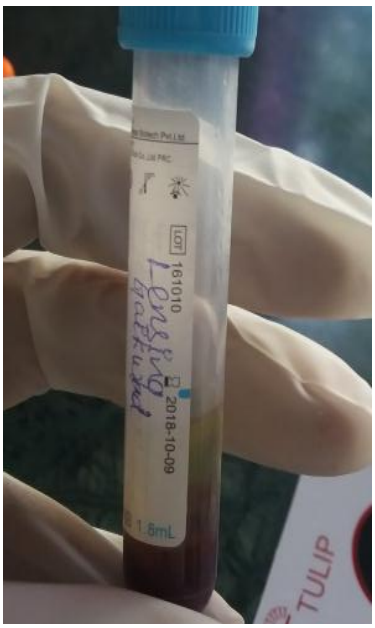


Fig 13 Plasma obtained after centrifugation

PHOTO PLATE VI

Qualitative method for FDP estimation.

Fig 14: Sample placed over the slide

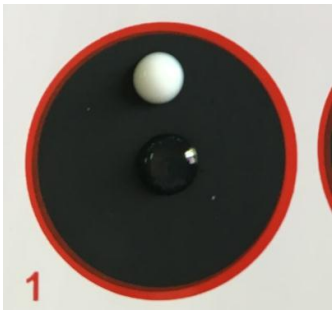


Fig 15: XL FDP latex reagent added adjacent to sample.

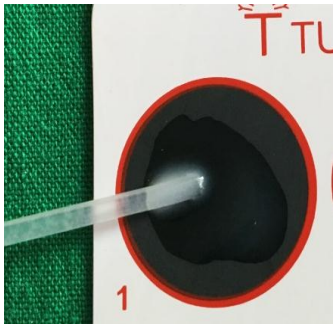


Fig 16: Mixing done with mixing stick

Fig 17 a: Positive i.e. D dimer level above 200 ng/ml.

Fig 17 b: Negative i.e. absence of FDP

PHOTO PLATE VII

Semi quantitative test after positive qualitative test

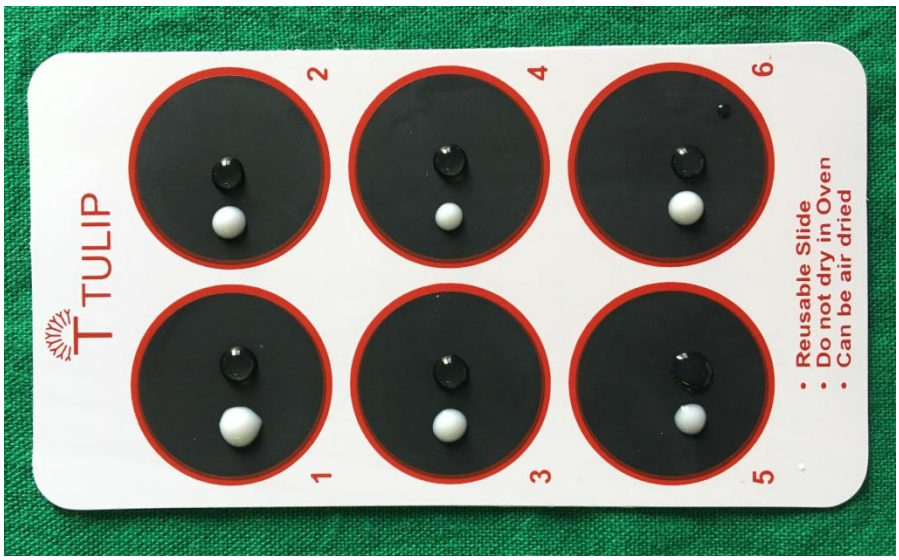


Fig.18: Serial dilutions of the plasma sample by using PBS buffer solution (1:2,1:4,1:8,1:16,1:32 and so on)

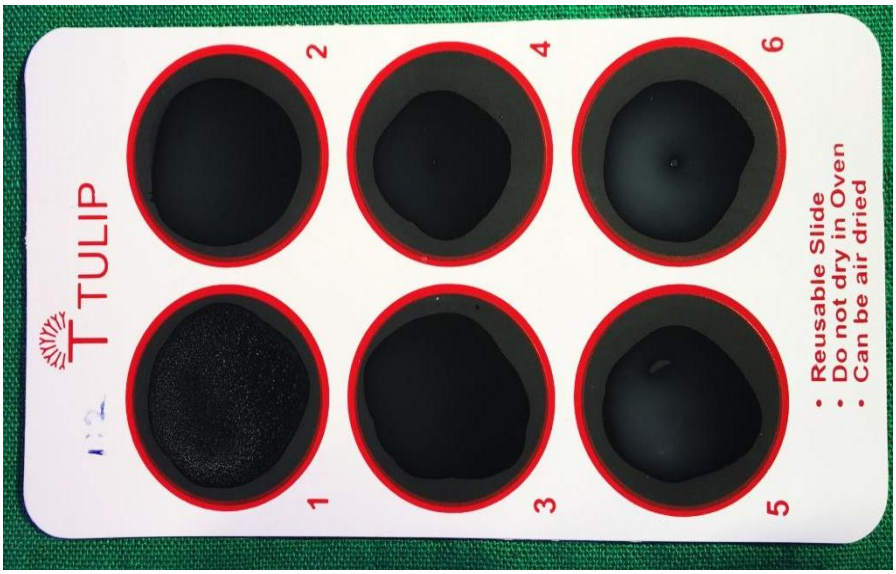


Fig 19. Positive agglutination in circle 1 for 1:2 dilution which is indicative 400 ng/ml of FDP levels

RESULTS

A hospital based cross sectional study was undertaken to evaluate the levels of plasma FDP levels in habit induced oral lesions. Total 150 subjects were selected randomly from the departmental OPD and categorized in **SIX** groups of 25 subjects each and are as follow:

- Group I : Normal healthy individual with habit and normal oral mucosa.
- Group II : Individuals with habits and normal mucosa.
- Group III : Individuals with habit and having oral lesion those are not premalignant in nature.
- Group IV : Individuals with habit and having oral submucous fibrosis.

- Group V : Individuals with habit and having oral leukoplakia.
- Group VI : Individuals with habit and having oral squamous cell carcinoma.

The results and observation of the study were evaluated and interpreted according to following parameters:

DISTRIBUTION OF AGE

Table 1 provides the descriptive statistics for age of patient in six groups. The mean age for all the individuals in the study was 40.17 ± 15.73 with the age range of 10-80 years. The mean age in group I was 31.24 ± 12.57 years with a range of 12-67 years. For group II, the mean age was 36.80 ± 13.82 years with range of 19-69 years. In group III, the mean age was 42.20 ± 19.88 years with range of 16-80 years. In group IV, the mean age was minimum 30.44 ± 9.82 years with age range of 10-48 years. Group V reported mean age of 47.20 ± 11.03 years with range of 24-68 years. Group VI showed maximum mean age of 53.16 ± 12.17 years with range of 31-72 years. A graphical representation of the mean age of patient in six groups is given in Graph 1.

DISTRIBUTION OF GENDER

Table 2 provides the distribution of patient as per gender amongst groups. Out of total 150 individuals, 106 (70.67 %) were males and 44 (29.33%) were females. Amongst the males the maximum number i.e 20 (80%) were in group II while minimum i.e 14 (56%) in group I. Males in group III, IV, V and VI were 18 (72 %), 19 (76 %), 18 (72 %) and 17 (68 %) respectively. Amongst the female the

maximum number i.e 11 (44 %) were in group I and minimum i.e 5 (20 %) in group II. Number of females in group III, IV, V and VI were 7 (28%), 6 (24%), 7 (28%) and 8 (32%).A graphical representation of the gender distribution in six groups is given in Graph 2.

DESCRIPTIVE BEHAVIOURAL CHARACTERISTICS IN DIFFERENT GROUPS

Table 3 provides the distribution of habits in individuals according to age. The total number of patients consuming areca nut was 22 (17%), and the age of individuals with this habit ranged from 18 - 76 years with the mean age being 40.59 years. There were 5 (4%) patients consuming Gutkha. The age of individuals with this habit ranged from 32 - 41 years with the mean age of 38.2 years.

The maximum number i.e. 71 (56.8%) of individual had habit of consuming kharra. The age of individuals with this habit ranged from 10 to 80 years with the mean age being 39.52 years. The total number of patients consuming pan masala was 5 (4%). The age of individuals with this habit ranged from 23 to 51 years with the mean age being 39.8 years. The total number of patients consuming tobacco was 46 (36.8%). The age of individuals with this habit ranged from 16 to 80 years with the mean age of 48.41 years.

The total number of patients consuming alcohol was 17 (13.6%). The age of individuals with this habit ranged from 21 to 69 years with the mean age of 43.24 years. The total number of patients smoking was 32 (25.6). The age of individuals with this habit ranged from 19 to 66 years with the mean age being 39.44 years.

Graphical presentation of distribution of patient according to the mean age of individuals is given in graph 3.

Table 4 shows distribution and association of type of habit with gender. In the individual consuming areca, 14 (63%) were males and 8 (36.3%) were females. In gutkha chewers, 4 (80%) were males and 1 (20%) was female, while for kharra 50 (70.4%) were males and 21 (29.5%) were females. In individuals consuming pan masala, 3 (60%) were males and 2 (40%) were females and in individual consuming tobacco, 33 (71.7%) were males and 13 (28.2%) were females. Individual with alcohol consumption included 13 (76.4%) males and 4 (23.52%) females. Among the smokers, 26 (81.5%) were males and 6 (18.7%) were females. The association between the type of habit and gender was statistically insignificant with a p-value of 0.8207 as obtained through chi-square test. Graphical presentation of distribution of individuals according to gender and habit is given in graph 4.

Table 5 Shows distribution of number of individual according to habits in each group. Group 1 shows no habits which is a control group. Group II shows 3 (12%), 1 (4%), 13 (52%), 3 (13%), 4 (16%), 1 (16%) and 10(40%) individual had habit of areca nut, ghutkha, kharra, pan masala, tobacco, alcohol and smoking respectively. In group III and IV individuals with habit of areca nut, ghutka, kharra, pan masala, tobacco, alcohol and smoking were 8(32%), 3 (12%), 12 (48%), 0, 9 (36%), 6 (24%), 5(20%) and 7 (28%), 1(4%), 22 (88%), 1 (4%), 6 (24%), 1(4%), 2 (8%) respectively. In group V and VI individuals with habit of areca nut, ghutkha, kharra, pan masala, tobacco, alcohol and smoking were 2 (8%), 0, 12 (48%), 0, 11 (44%), 4 (16%), 13 (52%) and 2 (8%), 0, 12 (48%),1 (4%), 16 (64%), 5 (20%),

4 (16%) respectively. Graphical presentation of distribution of number of individuals according to habit is given in graph 5.

DISTRIBUTION OF INDIVIDUALS ACCORDING TO DIFFERENT STUDY GROUP

Table 6 shows distribution of individuals in group III (habit induced lesion which are not potentially malignant lesion) where 6 (24%) had tobacco pouch keratosis, 16 (64%) had habit induced lichen planus and 3 (12%) had smokers palate. Graphical representation is given in graph 6.

Table 7 provides the distribution of patients in group IV (OSMF patient) according to clinical diagnosis. On the basis of clinical diagnosis, there were 3 (12%) patient in stage I, 11 (14%) in stage II, 8 (32%) in stage III and 3 (12%) in stage IV. Graphical representation is given in graph 7.

Table 8 provides the distribution of patient in group V (leukoplakia patient). Out of the 25 cases, 20 (80%) were in homogeneous leukoplakia type followed by 4 (16%) in erythematous leukoplakia and 1 (4%) in verrucous leukoplakia. Graphical representation is given in graph 8.

Table 9 provides the distribution of OSCC patients according to TNM staging. Of the 25 patients of OSCC, there was 1 (4%) patient in stage I, 5 (20%) in stage II, 6 (24%) in stage III and 13 (52%) in stage IV. Graphical representation is given in graph 9.

Table 10 provides the distribution of OSCC patients according to histological grades. Of the 25 patients in the OSCC group, 13 (52%) were from well

differentiated squamous cell carcinoma (WDSCC), 8 (32%) were from moderately differentiated squamous cell carcinoma (MDSCC) and 4(16%) were from poorly differentiated squamous cell carcinoma (PDSCC).Graphical representation is given in graph 10.

QUALITATIVE AND QUANTITATIVE ASSESSMENT OF FDP IN DIFFERENT GROUPS

Table 11 provides the number of individuals in different groups with positive qualitative test for FDP and their mean quantitative values. In group IV, FDP level were present in 3 (12%) individuals with a mean quantitative level of 200 ng/ml, while in group V it was present in 1 (4%) individual with a mean levels of 200 ng/ml. FDP was present in 5 (20%) individuals in group VI with a mean levels of 280 ± 109.55. There was statistically significant association between the FDP outcome and the groups as indicated by p-value of 0.0005. The proportion of cases with positive FDP outcome was significantly higher in OSCC, OSMF groups as compared to other groups as obtained by using Fisher's exact test. Graphical presentation for positive qualitative test for FDP in different group is given in graph 11.

Table 12 provides the qualitative and quantitative distribution of FDP levels according to the stages of OSMF. None of the individual in stage I showed positive for FDP. Out of the 11 cases of stage II, 8 of stage III and 3 of stage IV showed 1 case each positive for qualitative test for FDP each with mean level of 200 ng/ml. The association of OSMF staging and FDP outcome was statistically insignificant as indicated by p-value of 0.6748 using Fisher's exact test. Graphical presentation for

positive qualitative test for FDP in OSMF patients according to clinical stage is given in graph 12.

Table 13 provides the distribution of FDP according to type of leukoplakia. Out of the 20 cases of homogeneous leukoplakia 1 case showed positive test with mean levels of 200 ng/ml, while none of non-homogeneous group showed positive test for FDP. The association of type of leukoplakia and FDP outcome was statistically insignificant as indicated by p-value of 0.9999 using Fisher's exact test. Graphical presentation for positive qualitative test for FDP in Leukoplakia patients according to type is given in graph 13.

Table 14 provides the distribution of FDP levels in OSCC group according to TNM staging. In TNM stage IV, there were 10 (76.92%) individuals with negative FDP test and 3 (23.08%) individuals with positive FDP test with a mean quantitative FDP value of 333.33 ± 115.47 ng/ml for the positive FDP test. In TNM stage III, there were 4 (66.67%) individuals with negative FDP test and 2 (33.33%) individuals with positive FDP test with a mean FDP value of 200 ng/ml for the positive FDP levels. The association of TNM staging and FDP outcome was statistically insignificant as indicated by p-value of 0.6403 using Fisher's exact test. Graphical presentation for positive qualitative test for FDP in OSCC patients according to TNM staging is given in graph 14.

Table 15 provides distribution of FDP levels in OSCC group according to histological grading. Of the 25 patients in the OSCC group, 13 were from WDSCC, 8 were from MDSCC and 4 were from PDSCC. In WDSCC group, FDP level was present in 1 (7.69%). In the MDSCC group, it was present in 3 (37.5%); and in the

PDSCC group, it was present in only 1 (25%) patient. The mean FDP level for WDSCC and PDSCC groups was 200, while for MDSCC it was 333.33 ± 115.47. The association of histological grades with FDP outcome was statistically insignificant as indicated by p-value of 0.2522 using Fisher's exact test. Graphical presentation for positive qualitative test for FDP in OSCC patients according to histological grading is given in graph 15.

DISCUSSION

Oral cancer is amongst the most alarming health problem facing mankind. Potentially malignant disorder like oral submucous fibrosis, leukoplakia, erythroplakia, and lichen planus carry an increased risk for malignant transformation[52]. The prevalence of potentially malignant disorders and OSCC isincreased by prevalent habit of tobacco, betel quid, areca nut and their products. These products are available in various forms like kharra, gutkha, panmasala etc. Gutkha is the combination of crushed areca nut, tobacco, catechu, paraffin wax, slaked lime and sweet or savory flavorings. Kharra is similar to gutkha, the major difference between them is that the former is packed and marketed, while the latter is prepared locally. Pan masala is the combination of sugar coated seeds of sesame,

fennel, and coriander, mint leaves, cardamom, powdered lime, pure menthol, catechu and areca nuts.

Early diagnosis of oral cancer is important as it reduces the morbidity and mortality associated with it drastically. As oral cancer is one of the leading cause of death, introduction of newer diagnostic approach for detection of dysplastic changes at early stage is important. Although variety of diagnostic aids and adjunctive techniques are available, they vary in their sensitivity and specificity which makes the early diagnosis difficult worsening the prognosis of patients. Thus, histopathological examination remains the gold standard for evaluating the dysplastic changes in these patients. However it is an invasive procedure and may show false negative results if biopsy site is not representative. In such situations a tumour marker can be useful as a diagnostic as well as prognostic indicator.

Tumor markers are some inconspicuous agents produced by tumor cells or other cell within the body in response to cancer or certain benign conditions. They can be detected as circulating agents within the peripheral blood or plasma and help in distinguishing different pathologies especially at early stage[9]. FDP are the degradation product of fibrinogen and fibrin which are produced under the influence of thrombin and plasmin in coagulation and fibrinolytic pathway respectively. Fragment D and E, D-dimer, X and Y are its principle products. The physiological range of FDP levels is less than 10 mg/L while an FDP level of more than 40 mg/mL is considered pathologic[53].

Elevated levels of FDP are seen in normal physiological conditions like menstruation (9.8µg/ml) and pregnancy (2.5 mg/l) and also in systemic conditions

like hypertension (395.59 mg/dl), patients with ischemia diseases (656 µg/L)[54,55,34,30]. However the range of degradations products varies depending upon the disorders. The degradation product of fibrinogen in cancer also tends to elevate due to activation of coagulation and fibrinolytic pathways and is one of the factors necessary for growth and metastasis of tumour[10].

Elevated FDP levels were reported by **Astedt et al[21]** and **Svanberg et al[22]** in ovarian malignant tumours, **Kosugi et al[24]**in head and neckcancer**, Song et al[27]** in liver diseases including hepatocellular carcinoma, **Sato et al[28]** in leukemias, **Okholm et al[29]** and **Oya et al[31]** in colorectal cancer, **Khan et al[32]** in breast carcinoma, **Ay et al[33]** in large group of various cancers, **So et al[36]** in lung cancer, **Dastjerdi et al[37]** in malignant tumors of uterus, cervix and ovary.

Various studies have shown association of oral premalignant and malignant lesions with plasma, serum and saliva FDP levels, however, some showed no association with it. Thus to investigate whether FDP can be used in early detection or staging of pre-cancer and cancer we conducted this study to evaluate levels of plasma FDP in patients with oral premalignant and malignant lesions as well as in other habit induced lesions.

In the present study, patient included were of wide age range i.e. 10-80 years. 25 individuals in group I had no habit with apparently normal mucosa (control group). Individual with any physiological or systemic illness which could cause elevation in FDP levels were excluded. On estimation of FDP levels none of them were positive.

FDP levels were estimated in 25 individuals of group II who did not develop any lesion even after variable duration and frequency of habit were in age range of 19-69 years with mean age of 36years and male predominance. Majority of the individuals had kharra chewing habit. On estimation of plasma FDP levels in them, none of them showed positive qualitative test which was in accordance with **Wanjari et al[44], Kiran et al[45] and Gupta et al[47]**. However **Sharma et al in 2017[48]** found elevated FDP level in subjects with tobacco smoking habit (3005.60 mg/ml) as compared to smokeless tobacco form (418.51 mg/ml).

The present study (Group III) included individuals who had habit and lesion which were not pre malignant or malignant in nature. No previous studies had mentioned about the FDP levels in such kind of population. It consisted of lesion like habit induced lichenoid reaction, tobacco pouch keratosis and smoker's palate etc. 64% individuals in this group were having habit induced lichenoid reaction. Age range for this group was 16-80 years with the mean age 42.20 years and had male predominance. Maximum individual (48%) had habit of kharra chewing. None of the individual in this group had positive qualitative test for plasma FDP.

In the present study, OSMF patients (Group IV) were in age range of 10-48 years with mean age of 30 years which was in accordance with **Gupta et al[47], Pandya et al in[57], Koshti and Barpande[43], Wanjari et al[44], Kiran et al[45] Gharat et al [17],** and **Kadani et al[46].** Male predominance was seen in this group which was in accordance with studies by **Reddy et al[58]** and **Chaturvedi et al[59]** and unlike other studies by **Sinor et al[60]** and **Murti et al[61]** where female predominance was seen. Male predominance in our study was may be due to easy accessibility for males to

use kharra, areca nut and ghutka in our society. Maximum individual had habit of chewing kharra followed by areca nut and tobacco.12% patients of OSMF showed increased plasma FDP levels and were statistically significant when compared with controls. However, there was no association of clinical OSMF staging and FDP levels. It was in accordance with **Kadani et al**[46] while **Gharat et al**[17] did not show association between the presence of FDP levels with clinical staging in OSMF patients. In contrast to these studies, **Koshti and Barpande**[43]**, Kiran et al**[45] and **Gupta et al**[47] reported statistically significant increase in the mean FDP levels with increase in clinical grades of OSMF.

In the present study individuals who had leukoplakia (Group V) were in the age range of 24-68 years with mean age of 47.20 years with male predominance. Similar distribution of age and gender were reported by **Bánóczy J**[62] and **Espinoza et al** [63]. However, **Liu et al**[64] found that peak incidence was the fifth decade of life and the gender ratio was equal. **Brzak et al**[65] and **Brouns et al**[66] found women predominance. In the present study buccal mucosa was found to be the most common site involved which was in accordance with **Axéll et al**[67] whereas **Liu et al**[64] and **Brouns et al**[66] reported that tongue was most commonly involved site. Present study showed 80% of individuals with homogeneous leukoplakia and 52% had smoking habit which was in accordance with **Brouns et al**[66] and **Schepman et al**[68]. On estimation of FDP levels in this group, only one individual was positive for FDP and was not significant when compared to control group. Only one study by **Gharat et al**[17] had mentioned about the levels of FDP in oral leukoplakia patients who found no significant rise in FDP as compared to normal individuals.

In the present study group VI consisted of 25 OSCC patients with mean age of 53.16 years; male predominance and most prevalent was tobacco chewing habit. Maximum patient belonged to TNM stage IV (52%) and histologically well differentiated SCC (52%). This group had Only 5 individuals showed positive FDP level among which 3 were of TNM stage IV and 2 of stage III. FDP levels were statistically increased in OSCC group when compared to control group but was not significantly associated in different TNM stages and histological grading.

Ghosh et al[42] observed significant increase in the mean values of plasma FDP levels with the advancement of stages in oral cancer patients as compared to normal individuals. Similar results were obtained by **Gharat et al**[17] who reported similar results however the study used serum samples instead of plasma. Although the present study showed significant difference in plasma FDP levels when compared to control group but did not show association with TNM staging and histological grades.

The increased FDP levels in OSCC is due to the tumour itself as it has proteolytic enzymes which are capable of causing degradation of fibrin or fibrinogen.[21]but the absence of the FDP levels in present study could be due to the lack of the sensitivity of the kit to detect minimal rise of FDP levels or smaller sample size. In OSMF patients, the probable reason for non-detection of FDP levels could be because of the absence of proteolytic enzyme due to absence of tumour while in leukoplakia it could be due to lack of inflammatory component.

FDP were assessed in plasma, serum and saliva by variety of kits available in market with varying sensitivity and specificity. All the types of assays use

monoclonal antibodies against the epitomes on the D-dimer fragment that are absent on fibrin, fibrinogen, and non-cross linked fragments of fibrin. Depending on the extent or degree of lysis of cross linked fibrin different molecular weight D-Dimer moiety may be formed. In the present study FDP were detected in plasma by Tulip XL FDP-kit which uses a monoclonal antibodies and is sensitive to D dimer. It is readily available, easy to use, cost effective, and produced rapid results.

Gupta et al[69] evaluate the diagnostic ability of whole blood D-dimer and Plasma D-dimer in patients with disseminated intravascular coagulation and found the sensitivity for plasma FDP latex method was 66.7% while **Nisio et al in 2007**[70] found sensitivity of 69% for qualitative and 85% for semiquantitative assessment to detect FDP levels in deep vein thrombosis.

However, studies done by **Rathi et al**[71] indicate 100% sensitivity of the assay in suspected pulmonary thromboembolism. **Koshti** and **Barpande**[43] used the same kit and reported statistically significant increase in the mean FDP levels with increase in clinical grades of OSMF.

Thus, different assays have different results in variable patients. This is due to variation in reactivity of different D-dimer monoclonal antibodies to different D-dimer moiety. In addition to this some monoclonal antibodies may sometimes react with non-cross linked degradation products of fibrin or fibrinogen. Sometimes alpha 2 antiplasmin neutralize plasmin thereby restricting fibrinogenolytic activity and localizing the fibrinolysis on the fibrin clot which also influences the presence of FDP levels in plasma[46,72]. These could also be the probable reason for undetectable FDP levels in the present study.

The present study included 150 patients out of which only 9 individuals were positive for FDP (5 OSCC, 3 OSMF and 1 leukoplakia). There was no significant difference in levels of FDP in stages of OSMF as well as with TNM stages and histological grades of OSCC. Also none of the individuals in habit induced lesion which are not premalignant as well as in individual with habit showed positive FDP. This suggests that the estimation of plasma FDP levels in habit induced oral lesions is may not be a reliable method in the early diagnosis, staging or prognosis of the disease process but more studies are required with larger sample size and a kit with maximum sensitivity for detection of FDP in oral potentially malignant disorder and malignancy.

SUMMARY

The present study was a hospital based cross sectional analytical study carried out in the department of Oral Medicine and Radiology. A total of 150 individuals were selected and divided into six groups i.e. healthy individuals with no habits (Group I), individuals with habits and normal mucosa (Group II), with habits and having oral lesion those are not premalignant in nature (Group III), individuals with habit having oral submucous fibrosis (Group IV), individual with habit and having oral leukoplakia (Group V) and individuals with habit having oral squamous cell carcinoma (Group VI).

Considering the need for early detection of changes in potentially malignant disorder and early malignancy so as to reduce the mortality rate, the present study

was conducted to evaluate the levels of plasma FDP in patients with habit induced oral lesion.

The present study results are summarized as,

- The mean age for all the individuals in study groups was 40.17 years.
- The mean age for control group and individuals with habit and no lesion, habit with lesion (not potentially malignant), OSMF, leukoplakia and OSCC was 31.24, 36.80, 42.20, 30.44, 47.20 and 53.16 years respectively.
- All the groups showed male predominance.
- Kharra chewing was the most common habit in all the study groups, except the OSCC Group where tobacco chewing and smoking was more prevalent .
- Maximum number of the OSMF patients belonged to stage III while in leukoplakia maximum cases were of homogeneous type of leukoplakia. In OSCC group maximum cases belonged to TNM stage IV and well differentiated squamous cell carcinoma.
- In qualitative estimation of plasma FDP, only 12 % of OSMF, 4% of leukoplakia and 20 % of the OSCC cases were positive.
- The quantitative range of plasma FDP for OSMF and leukoplakia was 200ng/ml while the range for OSCC group was 200-400 ng/ml.
- On comparison with the control group, individuals with habit but no lesion and individual with habit induced lesion which are not pre malignant in nature showed no significant difference in FDP levels.

- On comparison with control group, patients with OSMF, leukoplakia and OSCC showed significantly high levels of plasma FDP.
- On comparison of FDP levels with the stage of OSMF, there was no significant association between them.
- Similarly no significant association was found in FDP levels according to TNM staging as well as histological grades of OSCC with FDP.

Out of 150 individuals only 9 were positive for FDP which suggest that use of plasma FDP levels for diagnosis, staging and prognosis would be inadequate and cannot be used as reliable tumour marker.

Limitation and future scope

Results can alter on incorporating larger sample size of each group. For the estimation of the plasma FDP levels more sensitive kits are available which on use may detect even minimal rise in their levels.

In conclusion, it can be said that qualitative method of plasma FDP level estimation did not show any association between the presence of FDP levels in habit induced oral lesions especially potentially malignant disorders and malignancies. Semi-quantitative method for estimation of plasma FDP do not show any association between the increase of FDP levels with the increasing in severity of OSMF, OSCC or type of leukoplakia. Thus the present study results conclude that estimation of plasma FDP levels for diagnosis, staging and prognosis of potentially malignant and malignant diseases is not reliable.

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TABLE

Table 1: Descriptive statistics for age of patients in six groups

GROUP	MEAN (Age of the Pt. in years)	SD	RANGE
I	31.24	12.57	12-67
II	36.80	13.82	19-69
III	42.20	19.88	16-80
IV	30.44	9.82	10-48
V	47.20	11.03	24-68
VI	53.16	12.17	31-72
Total	40.17	15.73	10-80

Table 2: Frequency distribution of patient as per gender in six groups.

	No. (Percentage)	
GROUP	MALE	FEMALE
I	14 (56%)	11 (44%)
II	20 (80%)	5 (20%)
III	18 (72%)	7 (28%)
IV	19 (76%)	6 (24%)
V	18 (72%)	7 (28%)
VI	17 (68%)	8 (32%)
Total	106 (70.67%)	44 (29.33%)

Table 3: Distribution of habits in individuals according to the age

Habits	Number of patients (%)	Range of age (yr)	Mean age (yr)
Areca nut	22 (17%)	18 – 76	40.59
Gutkha	5 (3.33%)	32 – 41	38.2
Kharra	71 (56.8%)	10 – 80	39.52
Pan Masala	5 (4%)	23 – 51	39.8
Tobacco	46 (36.8%)	16 – 80	48.41
Alcohol	17 (13.6%)	21 – 69	43.24
Smoking	32 (25.6%)	19 – 66	39.44

Table 4. Distribution and association of type of habit with gender

Type of habit	Gender		Chi - square value	P-value
	Male	Female		
Areca nut	14 (63%)	8 (36.3%)	2.9044	0.8207 (NS)
Gutkha	4 (80%)	1 (20%)		
Kharra	50 (70.4%)	21 (29.5%)		
Pan Masala	3 (60%)	2 (40%)		
Tobacco	33 (81.5%)	13 (18.5%)		
Alcohol	13 (76.4%)	4 (23.5%)		
Smoking	26 (81.5%)	618.7%)		

Table: 5 Distribution of habits according to the Groups (n=25)

Habits	Groups [no. of subjects (%)]					
	I	II	III	IV	V	VI
Areca nut	0	3 (12%)	8 (32%)	7 (28%)	2 (8%)	2 (8%)
Gutkha	0	1 (4%)	3 (12%)	1 (4%)	0 (0%)	0 (0%)
Kharra	0	13 (52%)	12 (48%)	22 (88%)	12 (48%)	12 (48%)
Pan Masala	0	3 (13%)	0 (0%)	1 (4%)	0 (0%)	1 (4%)
Tobacco	0	4 (16%)	9 (36%)	6 (24%)	11 (44%)	16 (64%)
Alcohol	0	1(4%)	6 (24%)	1 (4%)	4 (16%)	5 (20%)
Smoking	0	10 (40)	5 (20%)	2 (8%)	13 (52%)	4 (16%)

Table 6: Distribution of individuals in Group III (habit induced lesion which are not potentially malignant lesion (n=25).

GROUP III	[Number (%)]
Tobacco pouch keratosis	6 (24%)
Habit induced lichen planus	16 (64%)
Smokers palate	3 (12%)

Table 7: Distribution of OSMF patients according to clinical staging (n=25)

Clinical diagnosis	[Number (%)]
Stage I	3 (12%)
Stage II	11 (44%)
Stage III	8 (32%)
Stage IV	3 (12%)

Table 8: Distribution of type of leukoplakia patients (n = 25)

Type Leukoplakia	[Number (%)]
Homogeneous leukoplakia	20 (80%)
Erythro-leukoplakia	4 (16%)
Verrucous leukoplakia	1 (4%)

Table 9: Distribution of OSCC according to TNM staging (n=25)

TNM stage	OSCC [Number (%)]
Stage I	1 (4%)
Stage II	5 (20%)
Stage III	6 (24%)
Stage IV	13 (52%)

Table 10: Distribution of OSCC according to histological grades (n==25)

Histological grades	OSCC [Number (%)]
WDSCC	13 (52%)
MDSCC	8 (32%)
PDSCC	4 (16%)

Table 11: Distribution and association of individuals in different groups with positive qualitative test for FDP and their mean quantitative values.

FDP	Group [No. (%)]						P-value *
	I	II	III	IV (n=25)	V (n=25)	VI (n=25)	
Negative	25	25	25	22 (88)	24 (96)	20 (80)	0.0005 (S)
Positive	0	0	0	3 (12)	1 (4)	5 (20)	
Min. quantitative Levels	-	-	-	-	-	200	
Max. quantitative Levels	-	-	-	-	-	400	
Mean ± SD quantitative Levels	-	-	-	200	200	280 ± 109.55	

***Using Fisher's exact test; S: Significant*

Table 12: Association of levels of FDP according to staging of

Staging	FDP			P-value*
	Negative Qualitative test	Positive qualitative test	Quantitative test (ng/ml) Mean ± SD	
	Number (%)	Number (%)		
I	3 (100 %)	0	-	0.6748 (NS)
II	10 (91%)	1 (9%)	200	
III	7 (87.5%)	1(12.5%)	200	
IV	2 (66.6%)	1(33.4%)	200	

***Using Fisher's exact test; NS: Not Significant*

Table 13: Association of levels of FDP according to type of Leukoplakia (n = 25)

Type	FDP			P-value*
	Negative Qualitative test	Positive Qualitative test	Quantitative test Mean ± SD	
	Number (%)	Number (%)	(ng/ml)	
Homogeneous Leukoplakia	19 (95%)	1 (5%)	200	0.9999 (NS)
Erythroleukoplakia	4 (100%)	0	-	
Verrucousleukoplakia	1 (100%)	0	-	

***Using Fisher's exact test; NS: Not Significant*

Table 14: Association of levels of FDP in OSCC patients according to TNM staging (n = 25)

TNM	FDP			P-value*
	Negative Qualitative test	Positive Qualitative test	Quantitative test Mean ± SD	
	Number (%)	Number (%)	(ng/ml)	
I	1 (100%)	0	-	0.6403(NS)
II	5 (100%)	0	-	
III	4 (66.6%)	2 (33.4%)	200 ± 0	
IV	10 (77%)	3 (23%)	333.33 ± 115.47	

***Using Fisher's exact test; NS: Not Significant*

Table 15: Qualitative distribution and Quantitative levels of FDP in OSCC patients according to shistological grades (n = 25)

HG	Total	FDP			P-value*
		Negative Qualitative test	Positive Qualitative test	Quantitative test Mean ± SD	
		[Number (%)]	[Number (%)]	(ng/ml)	
WDSCC	13 (52%)	12 (92.31%)	1 (7.69%)	200	0.2522 (NS)
MDSCC	8 (32%)	5 (62.5%)	3 (37.5%)	333.33 ± 115.47	
PDSCC	4 (16%)	3 (75%)	1 (25%)	200	

***Using Fisher's exact test; NS: Not Significant ;HG: histological grade*

GRAPHS

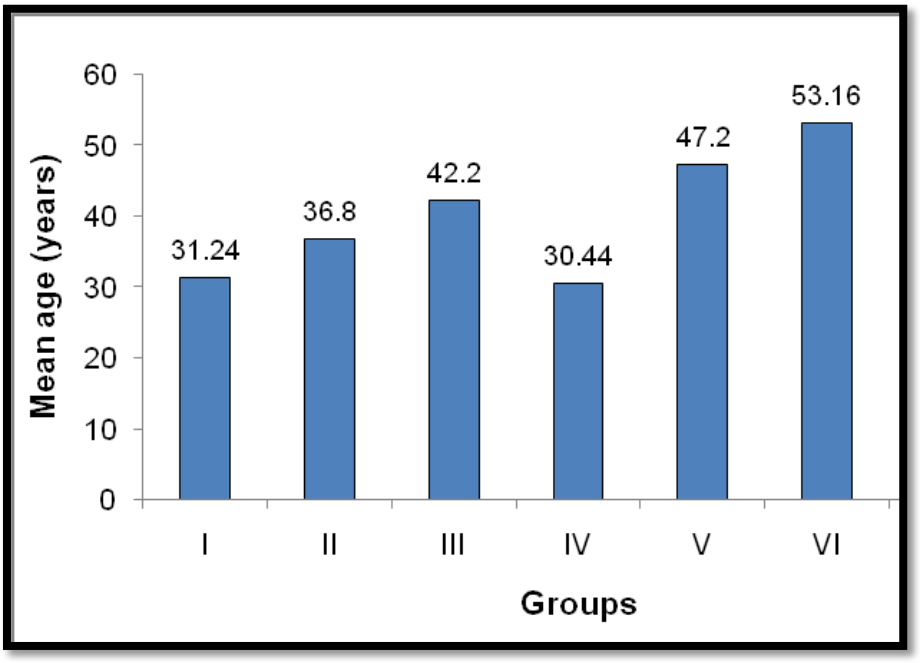

Figure 1: Column chart showing mean age of individuals in each group

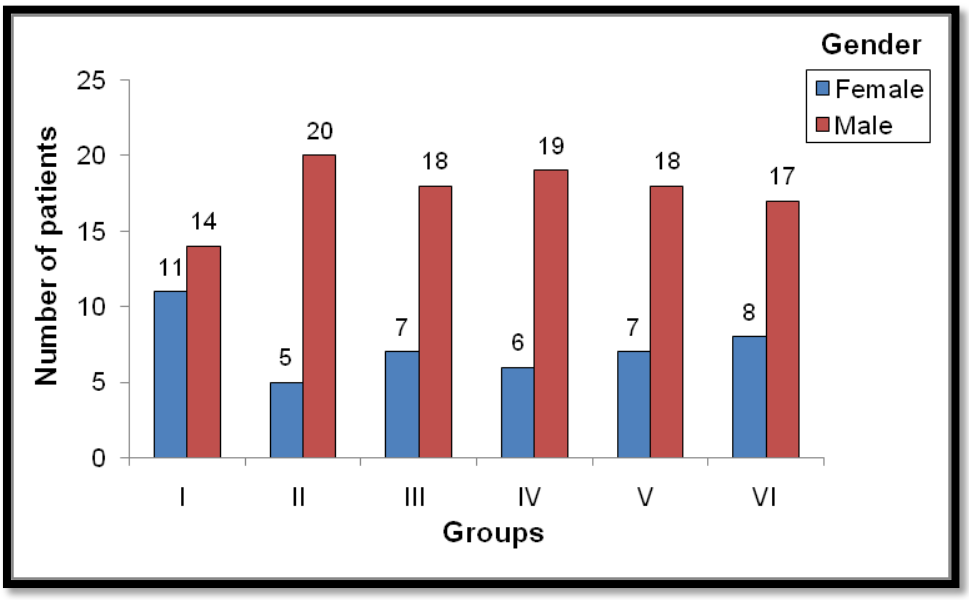

Figure 2: Column chart showing the number of individuals in each group as per gender

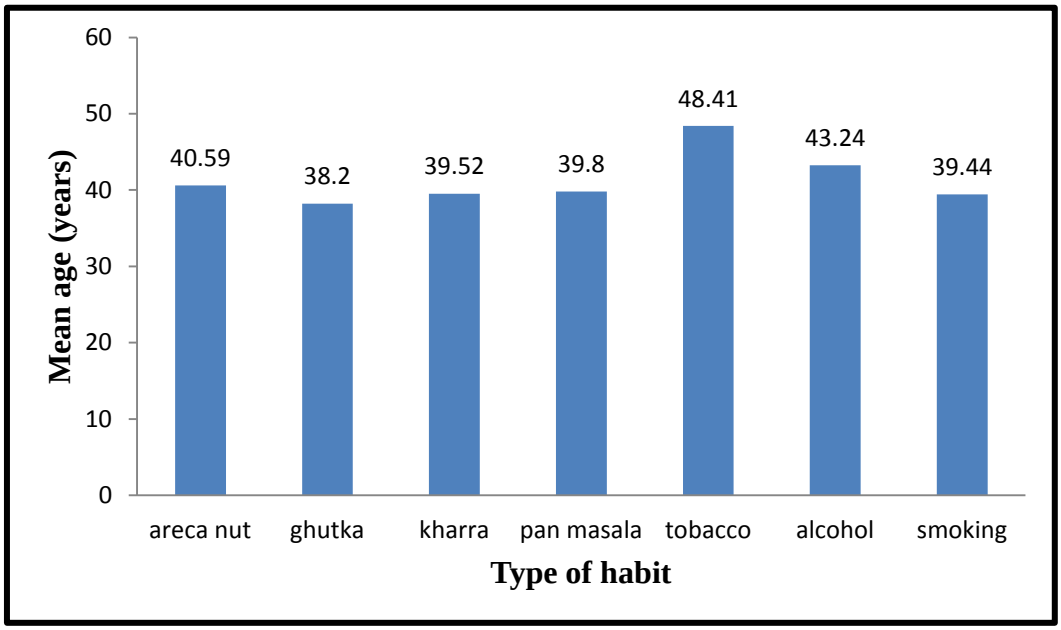

Figure 3: Column chart showing mean age of individuals with different types of habit

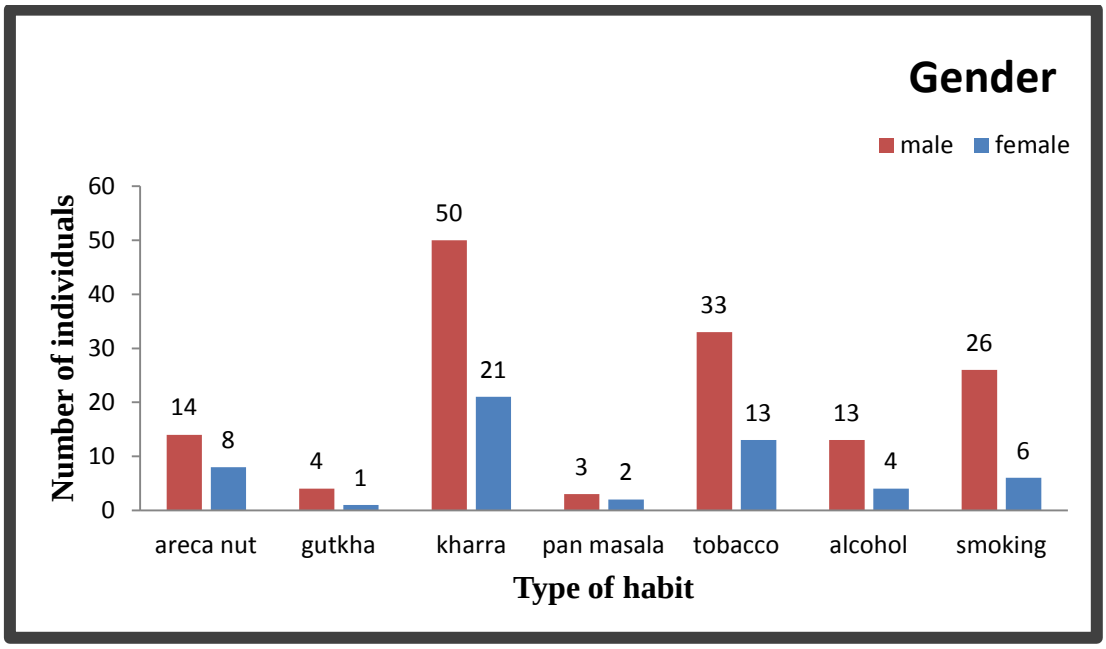

Figure 4: Column chart showing number of individuals according to gender and habits

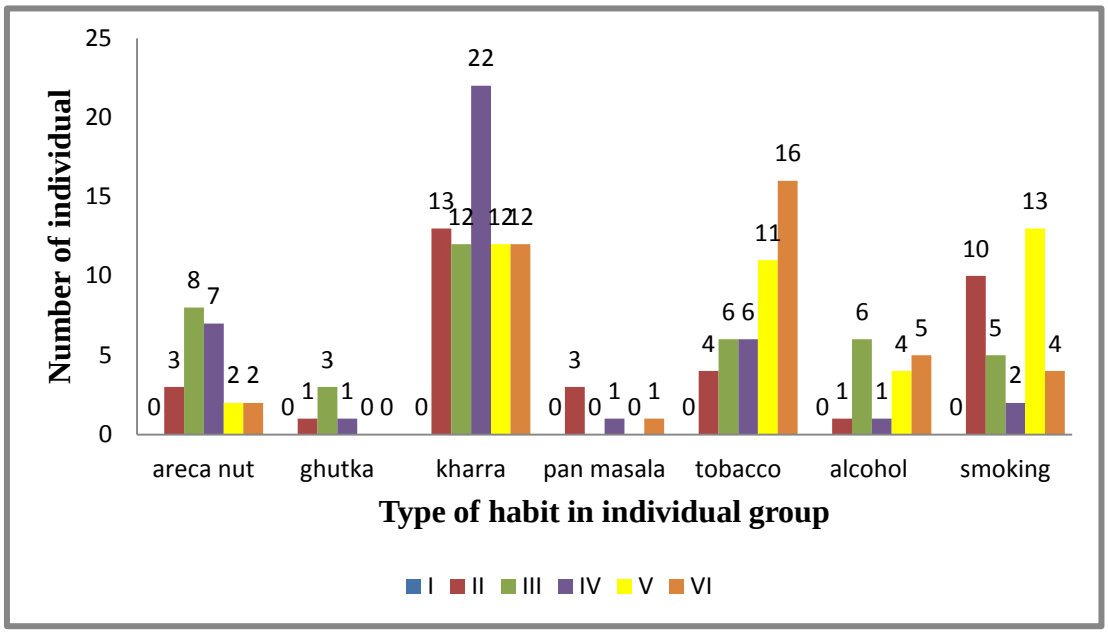

Figure 5: Column chart showing number of individual according to habit in each group

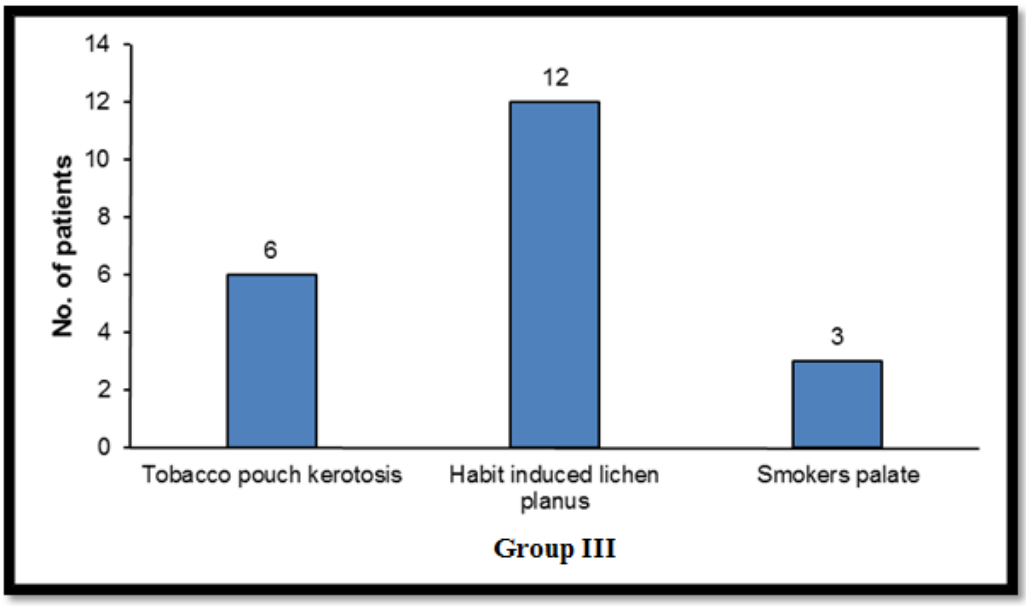

Figure 6: Column chart showing the distribution of individuals in group III

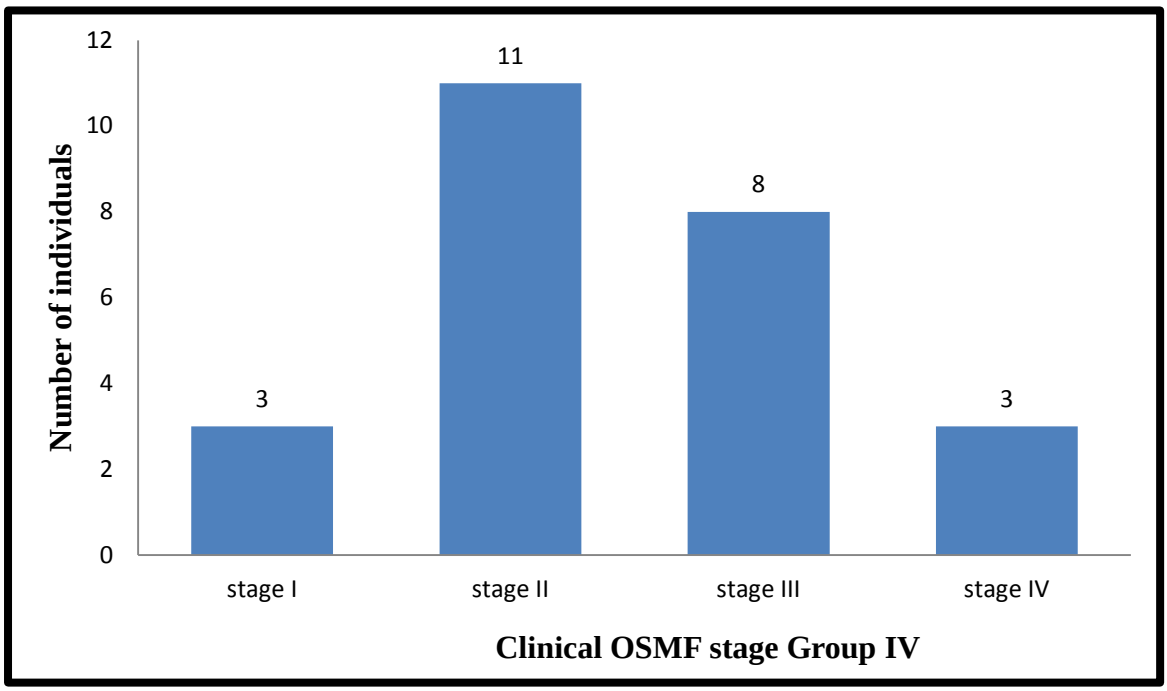

Figure 7: Column chart showing number of patients as per clinical OSMF stage.

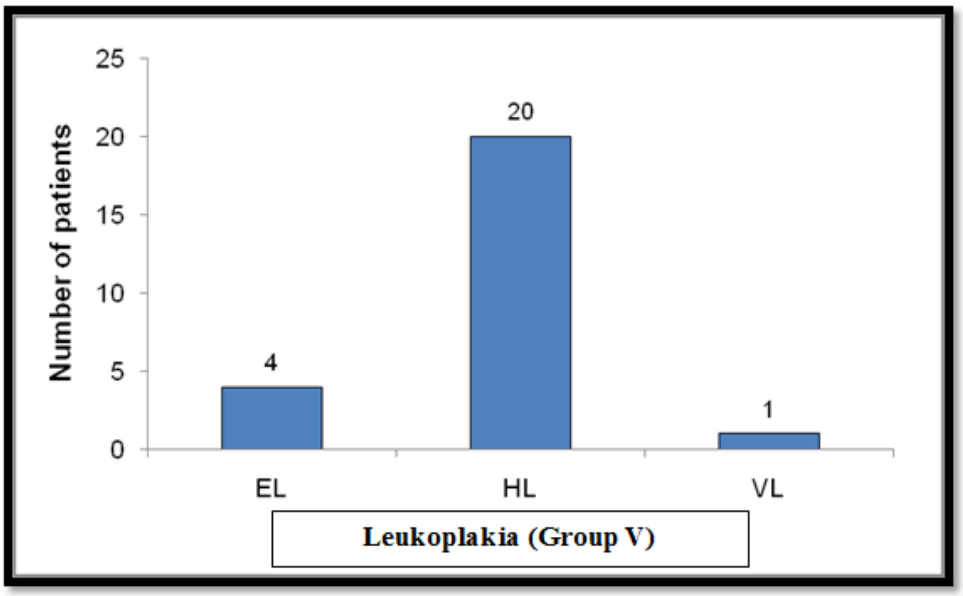

Figure 8: Column chart showing number of patients according to type leukoplakia

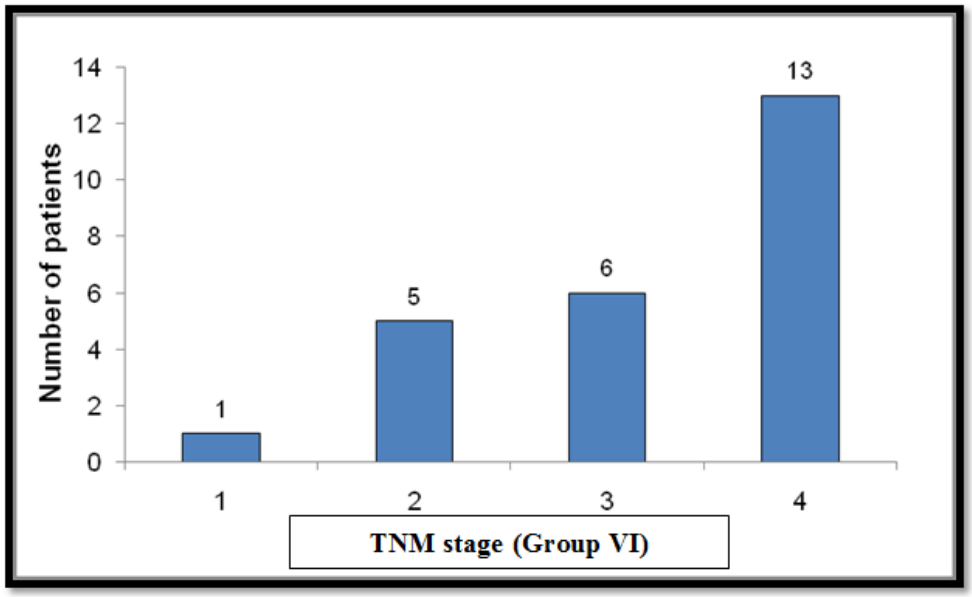

Figure 9: Column chart showing number of patients as per TNM stage in OSCC group

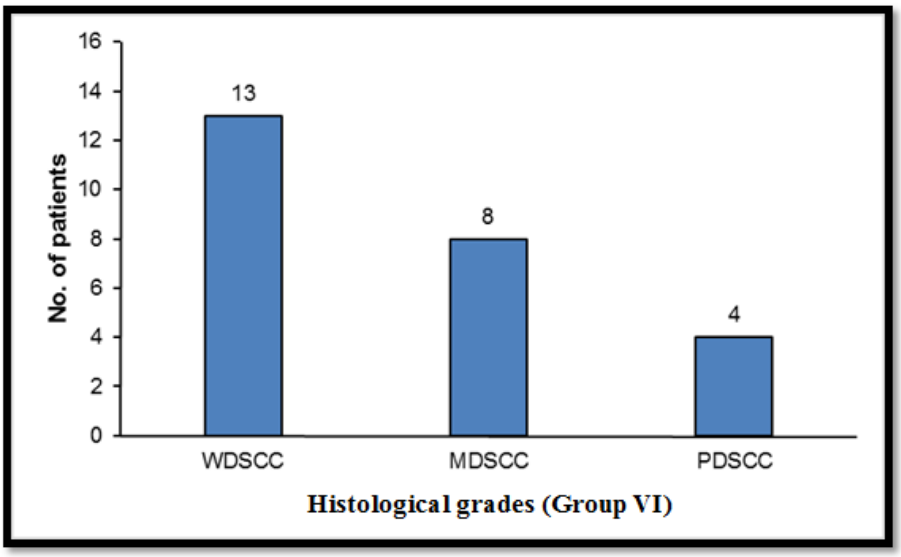

Figure 10: Column chart showing number of patients as per histological grades in OSCC group

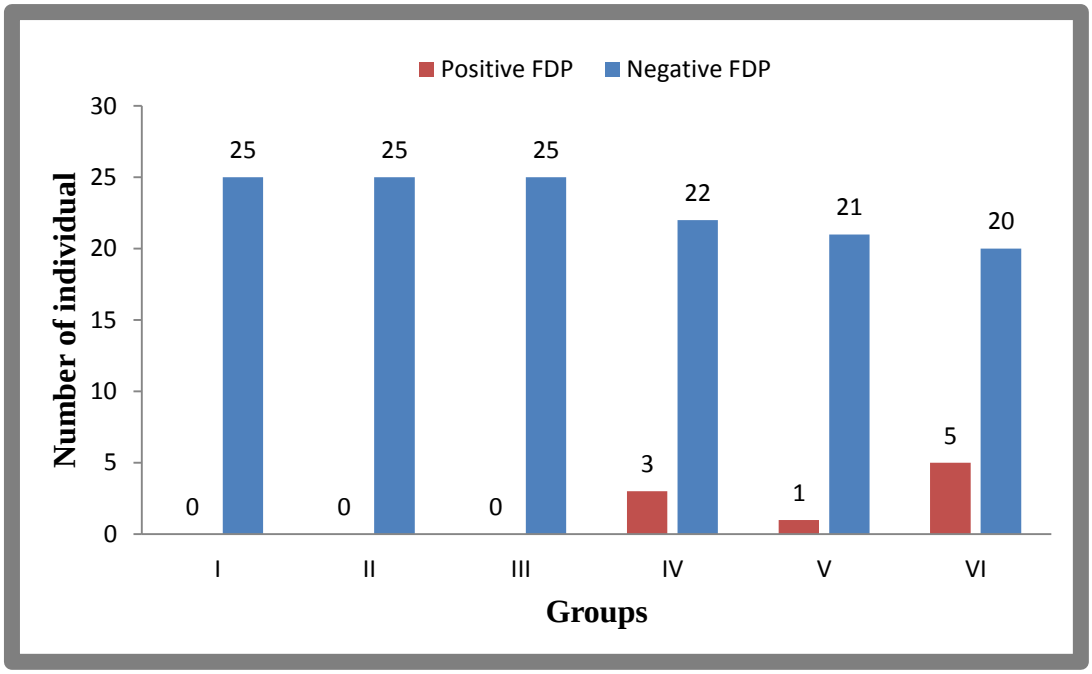

Figure 11: Column chart showing number of patients with positive and negative qualitative FDP test in each group

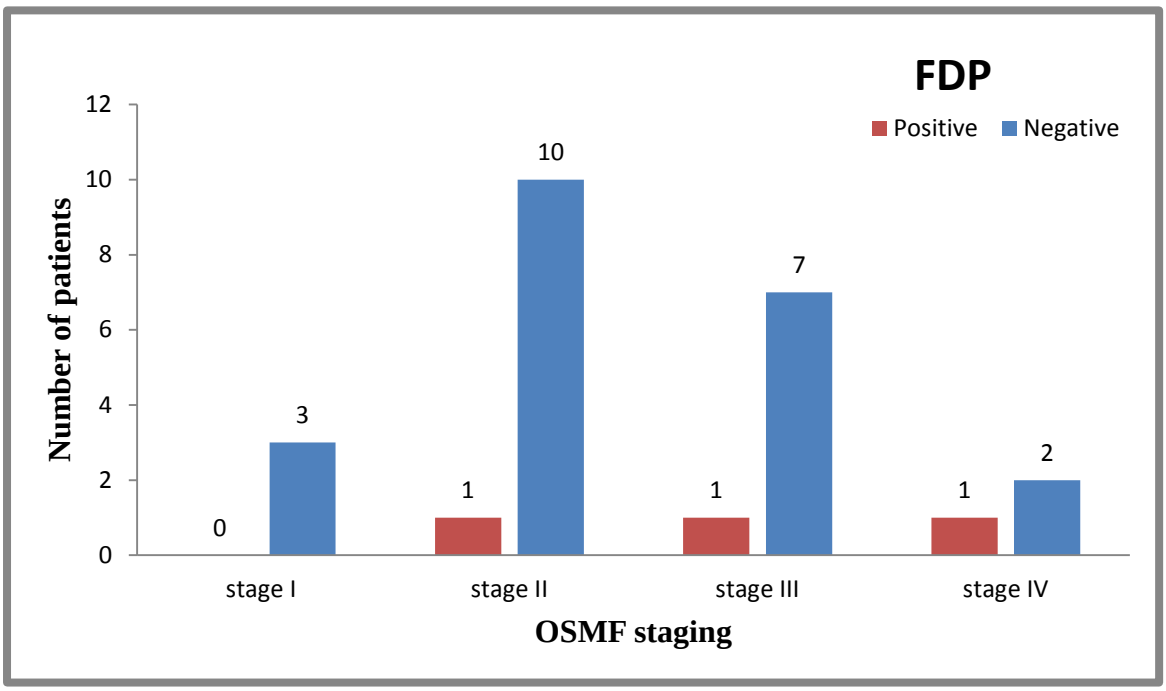

Figure 12: Column chart showing distribution of FDP in different OSMF stages

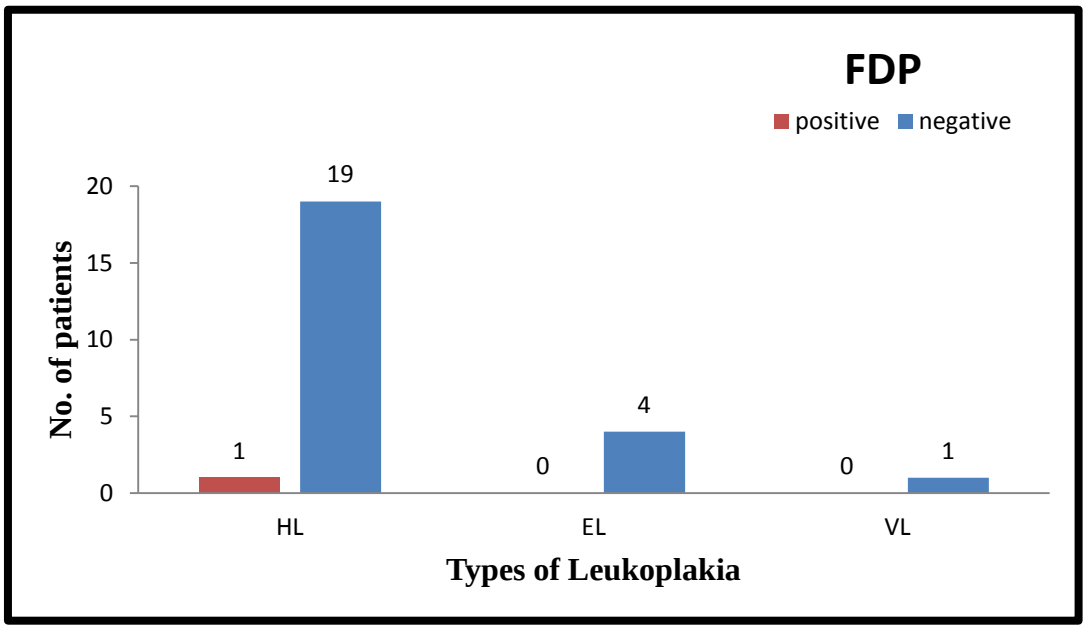

Figure 13: Column chart showing distribution of FDP in different type of leukoplakia patients.

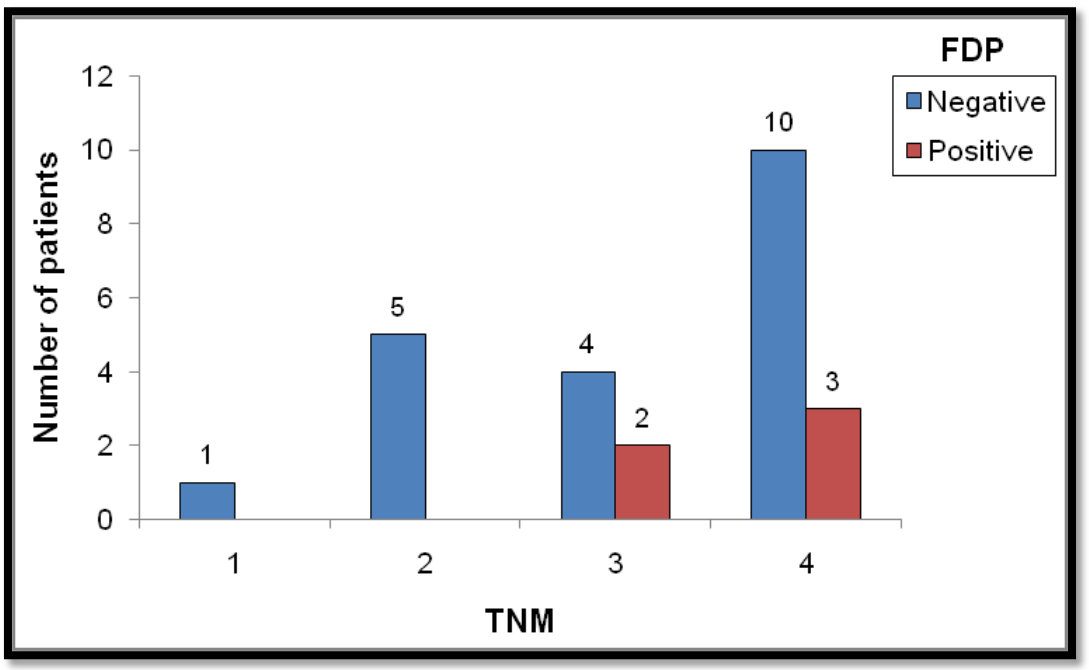

Figure 14: Column chart showing FDP in number of patients as per TNM stage

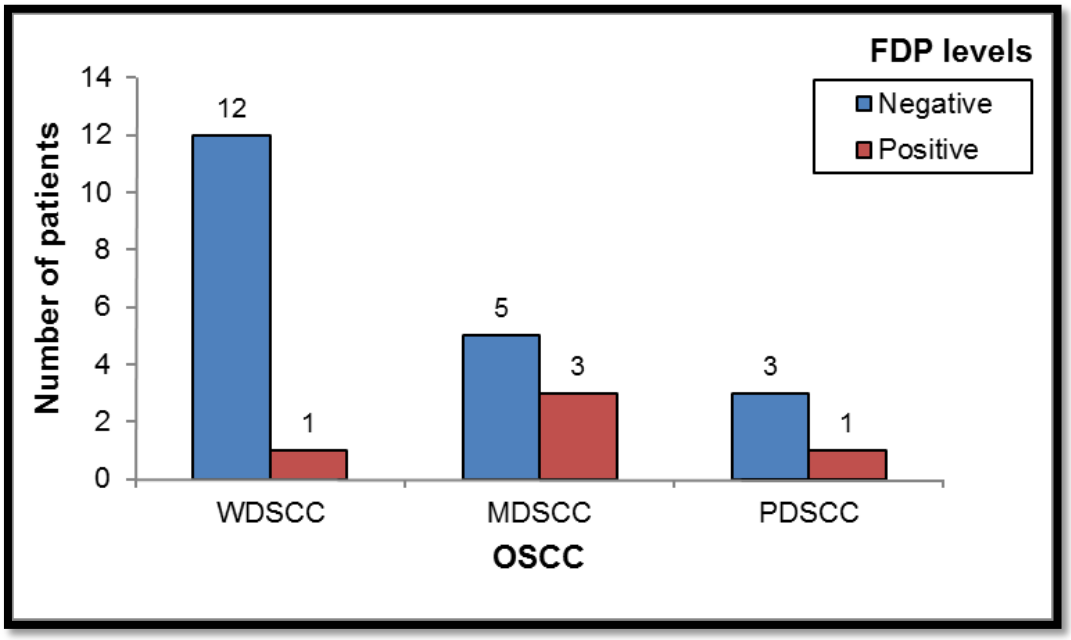

Figure 15: Column chart showing FDP in OSCC group according to histological grades.

ANNEXURE I

CASE HISTORY PROFORMA

Serial No:

Registration No:

Name :

Age/Sex :

Occupation :

Address :

Date:

Chief Complaint :

History of present illness :

Past treatment for premalignant and malignant condition:

Past Medical History :

Drug History :

Personal History : Socioeconomic status : Low / Middle / High

Educational Status : Literate / Illiterate

Habit :

Type of food :

Spicy/ Normal/ Nonspicy

Hot / Normal

Veg/ Non-veg

Chewing :

	Duration	Frequency	Quantity
Betal nut			
Pan masala			
Gutkha			
Pan with betel nut			
Pan with betel nut + zarda			
Tobacco + lime			

Smoking

	Duration	frequency	Quantity
Bidi			
cigarette			
chillum			
Any other			

General Examination

Built/Thin/Average/Heavy

Vital Sign - Temp.: Pulse : RR: BP:

Regional Lymphadenoapthy- Present Absent

On Examination

Soft tissue examination

Buccal Mucosa

Labial mucosa

Tongue

Uvula

Hard and Soft Palate

Retromolar region

Gingiva

Interincisal distance

Area of chief complaint

Hard Tissue Examination

Teeth Present

Caries

Wasting Disease

Restoration

Prosthesis

Stain

Calculus

Others

Provisional diagnosis:

Clinical Diagnosis

Clinically healthy with habits:

Habit induced oral lesion which are not malignant:

Premalignant lesion and condition:

ORAL LEUKOPLAKIA	
Homogenous leukoplakia	Non homogenous leukoplakia
Plaque type	Verrucous leukoplakia
	Papillary leukoplakia
	Exophytic leukoplakia
	Erythroleukoplakia

OSMF	
OSMF stage I	
OSMF stage II	
OSMF stage III	
OSMF stage IV	

ORAL MALIGNANCY

Stage 0 - T_{is} N0 M0

Stage 1- T1 N0 M0

Stage 2- T2 N0 M0

Stage 3- T3 N0 M0

T1 N1 M0

T2 N1 M0

T3 N1 M0

Stage 4A – T4 N0 M0

T4 N1 M0

ANY T N2 M0

Stage 4B- any T N3 M0

Stage 4C- Any T, Any N, M1

FDP levels estimation:

PATIENT WITH HABIT	FDP LEVEL	
	Qualitative Test	Semi Quantitative Test
Healthy Individuals		
Individuals with Premalignant lesions and conditions		
Individuals having OSCC		

Semi quantitative assessment:

Plasma FDP levels:

ANNEXURE II

INFORMED CONSENT FORM

Evaluation of levels of plasma fibrinogen degradation products in patients with habit induced oral lesions.

Patients I.D.:

I, Mr./Master/Mrs./Miss._____

Resident of: _____

_____

_____ aged _____ years, exercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent/consent on behalf of patient named
Mr./Master/Mrs./Miss._____
Resident of: _____aged _____ years,

as his/her _____.

I acknowledge that doctor has informed me about this research project suitably and sufficiently to my satisfaction. I agree to let my X-rays, photographs, impressions and other investigations to be taken as required. I agree to take part in this project and will not mix any other projects during the period of this trial. I shall report to the dental hospital or other place where called on given appointment dates and time. I shall inform the doctor on any adverse effects or unusual symptoms noticed by me. I shall co-operate with the doctors and paramedical staff, in all respects. I permit to publishing the results of my participation in this study. I shall not be given any reimbursement or compensation. I have been informed of my right to opt out of this research project at any time without giving any reason for doing so.

I hereby record my consent for participation in the said trial.

1.	_____	_____	_____	_____	
	Patient's name	Signature/thumbprint	Date	Time	
Or	_____	_____	_____	_____	
	Person providing consent	Signature/thumbprint	Date	Time	
2.	_____	_____	_____	_____	
	Witness name	Signature	Date	Time	
3.	_____	_____	_____	_____	
	Investigator's name	Signature	Date	Time	

(Confidential)

Informed Consent Form

Evaluation of levels of plasma fibrinogen degradation products in patients with habit induced oral lesions

वैयक्तीक जानकारी

मरीज का नाम : तारीख :

उम्र/लिंग :

पत्ता :

मोबाईल नंबर :

मैं मानता हूं कि चिकित्सक ने मुझे इस शोध परियोजना के बारे में उपयुक्त और पर्याप्त रूप से मेरी संतुष्टि के बारे में बताया है। मैं अपने एक्स-रे, फोटो, इंप्रेशन और अन्य जांचों को जरूरी के रूप में लेने के लिए सहमत हूं। मैं इस परियोजना में भाग लेने के लिए सहमत हूं और इस परीक्षण की अवधिके दौरान किसी भी अन्य परियोजनाओंको मिला नहीं करेगा। मैं सभी मामलों में डॉक्टरों और पैरामेडिकल स्टाफ के साथ मिलकर काम करूंगा। मैं इस अध्ययन में अपनी भागीदारी के परिणामोंको प्रकाशित करने की अनुमति देता हूं। मुझे कोई प्रतिपूर्ति या क्षतिपूर्ति नहीं दी जाएगी। मुझे ऐसा करने के लिए किसी भी कारण के बिना किसी भी समय इस शोधपरियोजनासे ऑप्टआउट करने का मेरे अधिकार के बारे में सूचित किया गया है। मैं एतद्द्वारा परीक्षण में भाग लेने के लिए मेरी सहमति रिकॉर्ड करता हूं।

१) मरीज का नाम	सही	तारीख	समय
२) साक्षीदार का नाम	सही	तारीख	समय
३) डॉक्टर का नाम	सही	तारीख	समय

(Confidential)

Informed Consent Form

Evaluation of levels of plasma fibrinogen degradation products in patients with habit induced oral lesions

वैयक्तीक माहिती

रूग्णाचे नाव : दिनांक :

वय/लिंग :

पत्ता :

मोबाईल नंबर :

मी कबूल करतो की डॉक्टरांनी मला या संशोधन प्रकल्पाबद्दल समाधानकारक माहिती दिली आहे. मी माझ्या एक्स-रे, छायाचित्रे, इंप्रेशन आणि आवश्यकतेनुसार अन्य तपासण्या करण्यास सहमत आहे. मी या प्रकल्पात भाग घेण्यास सहमती देतो आणि या चाचणीच्या कालावधीत कोणतेही अन्य प्रकल्प एकत्रित करणार नाही. मला डेन्टल हॉस्पिटल किंवा इतर ठिकाणी दिलेल्या भेटीचो तारीख आणि वेळ सांगितली आहे. मी डॉक्टर आणि पॅरामेडिकल कर्मचा-यांना सर्व बाबतीत सहकार्य करेल. या अभ्यासात मी माझ्या सहभागाचे निकाल प्रकाशित करण्यास परवानगी देतो. मला कोणतीही नुकसान भरपाई दिली जाणार नाही. असे करण्यासाठी कोणतेही कारण न देता मला कोणत्याही वेळी या संशोधन प्रकल्पातून बाहेर पडण्याचा अधिकार मिळालेला आहे. मी या अन्वये केलेल्या चाचणीत सहभागासाठी माझी संमती नोंदवित आहे.

१) रूग्णाचे नाव	स्वाक्षरी	तारीख	वेळ
२) साक्षीदाराचे नाव	स्वाक्षरी	तारीख	वेळ
३) डॉक्टरचे नाव	स्वाक्षरी	तारीख	वेळ

MASTER CHART

GROUP I

SR.NO	**SEX**	**AGE**	**HABIT**	**Lymphade-nopathy**	**Clinical diagnosis**	**Histopathological diagnosos**	**TNM stage**	**Qualitative FDP test**	**Quantitaive FDP test (ng/dl)**
1	female	35	absent	absent	normal	not applicable	not applicable	negative	not applicable
2	female	47	absent	absent	normal	not applicable	not applicable	negative	not applicable
3	male	29	absent	absent	normal	not applicable	not applicable	negative	not applicable
4	female	67	absent	absent	normal	not applicable	not applicable	negative	not applicable
5	male	36	absent	absent	normal	not applicable	not applicable	negative	not applicable
6	male	17	absent	absent	normal	not applicable	not applicable	negative	not applicable
7	female	21	absent	absent	normal	not applicable	not applicable	negative	not applicable
8	male	26	absent	absent	normal	not applicable	not applicable	negative	not applicable
9	male	26	absent	absent	normal	not applicable	not applicable	negative	not applicable
10	female	23	absent	absent	normal	not applicable	not applicable	negative	not applicable
11	female	27	absent	absent	normal	not applicable	not applicable	negative	not applicable
12	male	22	absent	absent	normal	not applicable	not applicable	negative	not applicable
13	male	27	absent	absent	normal	not applicable	not applicable	negative	not applicable
14	male	12	absent	absent	normal	not applicable	not applicable	negative	not applicable
15	male	30	absent	absent	normal	not applicable	not applicable	negative	not applicable
16	male	26	absent	absent	normal	not applicable	not applicable	negative	not applicable
17	female	30	absent	absent	normal	not applicable	not applicable	negative	not applicable
18	male	47	absent	absent	normal	not applicable	not applicable	negative	not applicable
19	male	60	absent	absent	normal	not applicable	not applicable	negative	not applicable
20	male	24	absent	absent	normal	not applicable	not applicable	negative	not applicable
21	female	35	absent	absent	normal	not applicable	not applicable	negative	not applicable
22	female	23	absent	absent	normal	not applicable	not applicable	negative	not applicable
23	female	25	absent	absent	normal	not applicable	not applicable	negative	not applicable
24	male	30	absent	absent	normal	not applicable	not applicable	negative	not applicable
25	female	36	absent	absent	normal	not applicable	not applicable	negative	not applicable

GROUP II

SR.NO	**SEX**	**AGE**	**HABIT**	**Lymphade-nopathy**	**Clinical diagnosis**	**Histopathological diagnosos**	**TNM stage**	**Qualitative FDP test**	**Quantitaive FDP test (ng/dl)**
26	female	35	kharra	absent	normal	not applicable	not applicable	negative	not applicable
27	male	19	kharra	absent	normal	not applicable	not applicable	negative	not applicable
28	male	41	tobacco	absent	normal	not applicable	not applicable	negative	not applicable
29	male	46	tobacco,smoking	absent	normal	not applicable	not applicable	negative	not applicable
30	male	55	smoking	absent	normal	not applicable	not applicable	negative	not applicable
31	male	69	tobacco	absent	normal	not applicable	not applicable	negative	not applicable
32	male	23	panmasala,smoking	absent	normal	not applicable	not applicable	negative	not applicable
33	male	24	kharra	absent	normal	not applicable	not applicable	negative	not applicable
34	male	20	kharra	absent	normal	not applicable	not applicable	negative	not applicable
35	male	41	gutkha	absent	normal	not applicable	not applicable	negative	not applicable
36	male	21	areca nut, kharra	absent	normal	not applicable	not applicable	negative	not applicable
37	male	24	kharra	absent	normal	not applicable	not applicable	negative	not applicable
38	male	30	kharra, smoking	absent	normal	not applicable	not applicable	negative	not applicable
39	male	21	smoking	absent	normal	not applicable	not applicable	negative	not applicable
40	male	23	smoking	absent	normal	not applicable	not applicable	negative	not applicable
41	female	45	panmasala, kharra, tobacco	absent	normal	not applicable	not applicable	negative	not applicable
42	male	50	smoking	absent	normal	not applicable	not applicable	negative	not applicable
43	female	45	kharra,smoking	absent	normal	not applicable	not applicable	negative	not applicable
44	male	59	areca nut, kharra	absent	normal	not applicable	not applicable	negative	not applicable
45	male	48	panmasala,smoking, alcohol	absent	normal	not applicable	not applicable	negative	not applicable
46	female	35	smoking	absent	normal	not applicable	not applicable	negative	not applicable
47	male	31	kharra	absent	normal	not applicable	not applicable	negative	not applicable
48	male	25	kharra	absent	normal	not applicable	not applicable	negative	not applicable
49	female	45	kharra	absent	normal	not applicable	not applicable	negative	not applicable
50	male	45	kharra	absent	normal	not applicable	not applicable	negative	not applicable

GROUP III

SR.NO	SEX	AGE	HABIT	Lymphade-nopathy	Clinical diagnosis	SITE	Histopathological diagnosos	TNM stage	Qualitative FDP test	Quantitaive FDP test (ng/dl)
51	female	38	areca nut, kharra	absent	tobacco pouch keratosis	buccal mucosa	not applicable	not applicable	negative	not applicable
52	female	61	kharra	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
53	male	38	gitkha	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
54	female	34	tobacco	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
55	male	55	tobacco	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
56	male	30	kharra, areca nut, alcohol	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
57	male	23	kharra	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
58	male	65	alcohol	absent	tobacco pouch keratosis	buccal vestible, buccal mucosa	not applicable	not applicable	negative	not applicable
59	female	41	areca nut, gutkha	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
60	male	32	alcohol	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
61	male	27	smoking	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
62	male	38	areca nut, smoking	absent	tobacco pouch keratosis	buccal vestible	not applicable	not applicable	negative	not applicable
63	male	21	smoking, alcohol	absent	tobacco pouch keratosis	buccal vestible	not applicable	not applicable	negative	not applicable
64	female	16	kharra	absent	tobacco pouch keratosis	buccal vestible	not applicable	not applicable	negative	not applicable
65	male	33	kharra	absent	tobacco pouch keratosis	buccal vestible	not applicable	not applicable	negative	not applicable
66	male	39	gutkha	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
67	female	24	kharra	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
68	female	32	alcohl, kharra	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
69	male	19	smoking, kharra	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
70	male	24	alcohol	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
71	male	66	kharra	absent	smokers palate	palate	not applicable	not applicable	negative	not applicable
72	male	78	kharra, smoking	absent	smokers palate	palate	not applicable	not applicable	negative	not applicable
73	male	65	areca nut	absent	smokers palate	palate	not applicable	not applicable	negative	not applicable
74	male	76	areca nut	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable
75	male	80	kharra	absent	habit induced lichen planus	buccal mucosa	not applicable	not applicable	negative	not applicable

GROUP IV

SR.NO	SEX	AGE	HABIT	Lymphade-nopathy	Clinical diagnosis	Histopathological diagnosos	TNM stage	Qualitative FDP test	Quantitaive FDP test (ng/dl)
76	male	24	smoking, kharra	absent	III	OSMF	not applicable	present	200
77	male	44	tobacco, kharra	absent	I	OSMF	not applicable	negative	not applicable
78	male	18	areca nut, kharra, smoking	absent	II	OSMF	not applicable	negative	not applicable
79	female	35	kharra	absent	III	OSMF	not applicable	negative	not applicable
80	male	48	areca nut, smoking, alcohol	absent	I	OSMF	not applicable	negative	not applicable
81	female	31	kharra	absent	III	OSMF	not applicable	negative	not applicable
82	female	10	areca nut, kharra	absent	IV	OSMF	not applicable	negative	not applicable
83	male	34	kharra	prsent	IV	OSMF	not applicable	negative	not applicable
84	female	13	kharra	absent	II	OSMF	not applicable	present	200
85	male	34	kharra	absent	III	OSMF	not applicable	negative	not applicable
86	male	40	kharra, tobacco	present	IV	OSMF	not applicable	present	200
87	male	32	kharra	absent	III	OSMF	not applicable	negative	not applicable
88	female	31	kharra	absent	I	OSMF	not applicable	negative	not applicable
89	male	28	kharra, tobacco	absent	II	OSMF	not applicable	negative	not applicable
90	male	16	kharra, tobacco	present	III	OSMF	not applicable	negative	not applicable
91	male	40	kharra	absent	II	OSMF	not applicable	negative	not applicable
92	male	38	kharra, tobacco	absent	II	OSMF	not applicable	negative	not applicable
93	male	40	kharra	absent	II	OSMF	not applicable	negative	not applicable
94	male	21	kharra	present	III	OSMF	not applicable	negative	not applicable
95	male	22	guthka,smoking	present	III	OSMF	not applicable	negative	not applicable
96	male	32	tobacco, kharra	absent	II	OSMF	not applicable	negative	not applicable
97	male	25	kharra, panmasala,smoking	absent	II	OSMF	not applicable	negative	not applicable
98	male	32	tobacco, kharra	absent	II	OSMF	not applicable	negative	not applicable
99	male	41	kharra	absent	II	OSMF	not applicable	negative	not applicable
100	female	32	kharra	absent	II	OSMF	not applicable	negative	not applicable

GROUP V

Sr no.	GEN	AGE	HABIT	LYMPHADENOPATHY	Location of lymph node	Clinical diagnosis	SITE	Histological diagnosis	TNM	Qualitative FDP test	Quantitaive FDP test (ng/dl)
101	male	50	kharra	absent	not applicable	homogeneous, leukoplakia	labial mucosa	leukoplakia	not applicable	negative	not applicable
102	male	50	tobacco, kharra	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
103	male	43	kharra	absent	not applicable	erythroplakia	buccal mucosa	erythrolakai	not applicable	negative	not applicable
104	female	51	tobacco, kharra	absent	not applicable	homogeneous, leukoplakia	labial mucosa	leukoplakia	not applicable	negative	not applicable
105	male	50	kharra, tobacco,smoking	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
106	female	45	smoking, kharra	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
107	male	43	areca nut, alcohol	absent	not applicable	erythroplakia	buccal mucosa	erythroplakia	not applicable	negative	not applicable
108	male	24	kharra, tobacco,smoking	absent	not applicable	homogeneous, leukoplakia	gingiva	leukoplakia	not applicable	negative	not applicable
109	female	54	smoking, tobacco	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
110	male	34	smoking	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
111	male	41	alcohol, smoking	absent	not applicable	homogeneous, leukoplakia	B,L	leukoplakia	not applicable	negative	not applicable
112	male	65	tobacco, kharra	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	positive	200
113	male	65	smoking	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
114	male	43	tobacco,kharra	absent	not applicable	errucous leukoplakia	buccal mucosa	verrucous leukoplakia	not applicable	negative	not applicable
115	male	38	smoking	absent	not applicable	homogeneous, leukoplakia	retromolar, buccal mucosa	leukoplakia	not applicable	negative	not applicable
116	male	56	smoking	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
117	female	68	kharra, tobacco,smoking	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
118	male	41	smoking	absent	not applicable	homogeneous, leukoplakia	labial mucosa	leukoplakia	not applicable	negative	not applicable
119	male	32	smoking	absent	not applicable	erythroplakia	buccal mucosa	erythroplakia	not applicable	negative	not applicable
120	female	51	tobacco, kharra	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
121	male	60	kharra	present	submandibular bilateral	erythroplakia	buccal and labial mucosa	erythroplakia	not applicable	negative	not applicable
122	male	46	alcohol, kharra	present	submandibular bilateral	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
123	male	57	smoking	present	submandibular bilateral	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
124	female	40	tobacco	absent	not applicable	homogeneous, leukoplakia	buccal mucosa	leukoplakia	not applicable	negative	not applicable
125	female	33	smoking, acrca nut	present	submandibular bilateral	homogeneous, leukoplakia	gingiva	leukoplakia	not applicable	negative	not applicable

GROUP VI

Sr no.	GEN	AGE	HABIT	LYMPHAD - ENOPATHY	Clinical diagnosis	SITE	Histological diagnosis	TNM	Qualitaive FDP test	Quantitaive FDP test (ng/dl)
126	female	66	alcohol, kharra	present	malignacy	buccal mucosa	well differentiated	iv	negative	not applicable
127	male	42	tobacco	present	malignacy	buccal mucosa	well differentiated	iii	negative	not applicable
128	male	57	kharra, smoking	present	malignacy	buccal and labial mucosa	well differentiated	iv	negative	not applicable
129	male	62	tobacco	present	malignacy	buccal mucosa, alveolus	well differentiated	iv	negative	not applicable
130	female	49	tobacco	present	malignacy	buccal mucsa, alveolus	moderately differentiated	iv	positive	400
131	male	50	tobacco, alcohol	present	malignacy	alveolus	well differentiated	iii	negative	not applicable
132	female	69	kharra, alcohol	present	malignacy	buccal mucosa, alveolus	moderately differentiated	iv	negative	not applicable
133	male	72	kharra	present	malignacy	buccal mucosa	well differentiated	ii	negative	not applicable
134	male	51	smoking, panmasala,tobacco	present	malignacy	buccal mucosa	well differentiated	ii	negative	not applicable
135	male	62	tobacco	present	malignacy	retromolar area	well differentiated	iv	negative	not applicable
136	male	58	tobacco,alcohol	present	malignacy	tongue	well differentiated	iii	negative	not applicable
137	male	36	kharra	present	malignacy	buccal mucosa,palate,alveolus,gingiva	moderately differentiated	iv	positive	400
138	male	32	kharra,smoking, alcohol	present	malignacy	alveolus, gingiva, buccal mucosa	moderately differentiated	iv	negative	not applicable
139	female	31	kharra	absent	malignacy	labial mucosa	moderately differentiated	ii	negative	not applicable
140	female	50	tobacco	present	malignacy	alveolus	well differentiated	ii	negative	not applicable
141	male	54	tobacco, kharra	present	malignacy	ailveolus	poorly differentiate	ii	negative	not applicable
142	female	72	tobacco	present	malignacy	alveolus,floor of mouth	moderately differentiated	iii	positive	200
143	male	38	kharra, tobacco, areca nut, smoking	present	malignacy	tongue	well differentiated	iv	negative	not applicable
144	male	64	kharra	present	malignacy	alveolus	moderately differentiated	iv	negative	not applicable
145	male	50	kharra	present	malignacy	buccal mucosa, alveolus	well differentiated	iii	positive	200
146	male	45	tobacco, kharra	present	malignacy	buccal mucosa	poorly differentiate	iv	negative	not applicable
147	female	60	areca nut, tobacco	present	malignacy	tongue	poorly differentiate	iii	negative	not applicable
148	female	38	tobacco	absent	malignacy	gingiva	well differentiated	i	negative	not applicable
149	male	57	tobacco, kharra, smoking	present	malignacy	buccal mucosa	poorly differentiate	iv	positive	200
150	male	64	tobacco	present	malignacy	soft palate	moderately differentiated	iv	negative	not applicable