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Utility of Plasma Fibrinogen Degradation Product Levels in the Assessment of Risk of Developing Oral Potentially Malignant Disorders in Subjects with Habit of Tobacco and Areca Nut Consumption

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Abstract: **Carcinogenic products like tobacco and areca nut which are widely consumed by the Indian population are major etiological factors which contribute to oral potentially malignant disorders which further lead to cancer. Some recent researches have led to detection of elevated fibrinogen degradation products in the plasma of patients with oral potentially malignant disorders and this has provided a new insight into their early diagnosis. Objectives: To detect the plasma FDP values in individuals with habit of tobacco and areca nut and in normal subjects without any habits, and to compare them so as to assess the risk at preliminary stage. Study Design: This study comprised of 30 subjects with habit of tobacco and areca nut and 30 normal subjects without any habits. The subjects were evaluated for plasma FDP levels using immunoturbidimetric assay. Results: The plasma FDP in subjects with habit of tobacco and areca nut when compared with normal subjects showed increased levels (p = 0.029). Conclusions: Since fibrinogen degradation product levels increase in subjects with chronic habit of carcinogenic product consumption, this test can be used to determine the risk of developing oral potentially malignant disorders in such patients.**

Keywords: **Arecanut, Fibrinogen Degradation products, Leukoplakia, Oral premalignant lesions, Oral submucous fibrosis, Tobacco.**

I. INTRODUCTION

Tobacco and areca nut pose a major threat to the Indian subcontinent. India is one of the leading global capital tobacco users in the world. As per the Global Adult Tobacco Survey (GATS-2010), more than one-third (35%) of adult Indian population use tobacco. Of them, 21% are addicted to smokeless tobacco whereas 9% are addicted to smoking. Rest 5% are addicted to both forms; i.e. smoking as well as smokeless tobacco.[1] Smoking plays a significant role in the development of leukoplakia. The fact was first recognized by Sir James Paget in 1837 and has been supported by many with experimental evidences.[2] This study was performed with an aim to determine whether plasma FDP levels can be used to predict the likelihood of developing oral premalignant lesions in future among subjects with a habit of carcinogenic product consumption using blood plasma in order to avoid invasive diagnostic procedures such as biopsy. Our objective was to correlate the plasma fibrinogen degradation product (FDP) levels, in subjects with carcinogenic

product habit; but without clinical findings with the control group. At the same time to assess 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.

II. BODY OF ARTICLE

Materials and Methods:

The study comprised of a total of 60 subjects divided into two groups: 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. After obtaining ethical clearance from the Institutional Ethical Committee, Sri Aurobindo Institute of Medical Sciences, Indore; written consent as approved by the committee, and necessary instructions, the subjects were incorporated in the study. The study was conducted in full accordance with the World Medical Association Declaration of Helsinki. A detailed case history was recorded and a thorough clinical examination was carried out for subjects in the study group. Under all aseptic precautions, 2 ml of venous blood was collected by venipuncture in a disposable vial containing 0.2 ml of sodium citrate. Blood samples were then allowed to stand for an hour at room temperature and then centrifuged at 3000 rpm for 3 minutes to separate the plasma. The plasma was stored at -70^{o} C and then FDP levels were quantified. 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 (Turbodyne D-Dimer-Tulip Diagnostics (P) Ltd; Goa, India). (Fig. 1 and 2)

Results:

The obtained D-Dimer values were analysed using statistical software, SPSS version 20.0. Chi square test ,unpaired -T test, correlation coefficient and one way ANOVA test were used in data analysis to compare the values of plasma fibrinogen degradation products between subjects with carcinogenic product consumption habit and normal subjects. The probability value from $p<0.05$ to $p<0.01$ was considered as statistically significant. The distribution of FDP levels amongst habit group showed 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. The results showed statistically significant difference with a p value of $p=0.029$. (Table 1 and Fig. 3)

Discussion:

This is the modern era of industrialization and urbanization that has seen a fast progress. This leads to physical and mental stress that cultivates in the lives of humans, which in turn ends up in alleviating measures like smoking, alcohol abuse, addiction to betel nuts and pan, and so. Not only are they obsessive, they wreak havoc on the body. Evidently, they have been observed to be abusing the oral cavity. Medical practitioners and dental surgeons encounter a wide spectrum of oral mucosal alterations in regular clinical practice.[3] Of these, oral carcinomas are the most prevalent. They are one of the leading cancers in India today, with an age standardized incidence rate of 12.6 per 100 000 population and account for around 30% of the cancer burden. A considerable number of such cases initially present with precursor lesions which further classify as precancerous lesions and precancerous conditions.[4]

In India, around 30 to 40% of the reported malignancies are oral in origin, which is attributable to the native habits of chewing tobacco, areca nut, betel leaf, lime and spices in a variety of combinations.[5] Different forms of tobacco and areca nut are in popular use amongst the general population such as khaini, gudakhu, snuff, bidi, paan and gutkha.

According to a general consensus, the stage of clinical advancement during the stage of diagnosis plays the most significant role in predicting the recurrence rate and mortality in patients with oral cancer. The time taken to diagnose has an influence of a wide number of clinical, demographic and social variables, which include reluctance on patient's side to consult a doctor, the reason being lack of easy availability of healthcare services, especially in patients from the lower strata of the society, as well as delay by the professionals in the diagnosis and treatment.[6] According to some studies, dental surgeons and other professionals in the field of healthcare are in requirement of educational updates on early diagnosing and preventing oral cancer. Health care professionals can improve mortality rates if cancerous lesions are detected at initial stages, or if precursor lesions are discovered and treated before they progress into malignancy.[7]

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A great challenge for early detection of the precursor lesions is attributed to our limited scope to differentiate the high risk lesions from lesions associated with low risk.[8] Thus, the preventing oral cancer and the related morbidity and mortality rates, depends upon the early detection of precursor lesions, leaving scope for histopathological evaluation and subsequent treatment plan according to the stage of diagnosis. Early detection of oral cancer has the ability to reduce the morbidity and mortality rates of the disease, but the screening measures have not been proven successful yet.

Recent advances in research on oral cancer have emerged to the development of sound diagnostic techniques at the clinical and molecular level for early screening of oral cancer. However, tissue biopsy procedures with histopathological assessment still remain the gold standard for the diagnosis of oral cancer, but this method requires an experienced professional, and is considered painful, invasive, costly and time consuming.[4] The recent clinical screening techniques for early diagnosis of oral cancer include tolonium chloride or toluidine blue dye, salivary diagnostics, oral CDx brush biopsy kits, and optical imaging systems.[9,10] Although these methods pose their own disadvantages and advantages, these non-invasive techniques have unfortunately crashed in being practically applicable in day-to-day setup, as patients are being still diagnosed at later stages of oral cancer.[11,12]

Fibrinogen is a liver-synthesized glycoprotein weighing 340 kDa with normal plasma levels around 1.5–3 g/L.[13,14]

Being an acute phase reactant, it increases throughout inflammatory process. In response to inflammation, the body ends up producing more fibrinogen and its degradation products. FDP has a gamut of functions. While fibrinopeptides aim at combating inflammation, FDP attempts at counteracting the fibrin-like behaviour of fibrin precipitating factor (FPF) and thrombin, which is produced in the autocatalytic process. So, with an increase in the severity of the disease, more FPF is produced, which in turn produces more FDP.[15]

D-dimers generated by plasmin digestion of fibrin are used as markers for fibrinolysis and disseminated intravascular coagulation in humans.[16]

In 1982, Chan *et al.* found in their study that the FDPs were increased in diabetic nephropathy leading to fibrin deposition in the endothelial and mesangial regions.[17]

Similarly, in 1993, Song *et al.* also reported in their study that FDPs were elevated in various liver diseases, especially cirrhosis of liver, leading to fibrin deposition and thus fibrosis.[18]

In 1894, a study conducted by Whitaker *et al.* showed that the mean concentration of FDPs was raised in plasma of patients with pulmonary embolism, deep venous thrombosis, arterial thromboembolism, and disseminated intravascular coagulation, as compared to the normal subjects.[19]

Similarly, in 1986, Elms *et al.* also discovered in their study that plasma levels in normal subjects were negative for FDPs. However, there were positive results in patients with pulmonary embolism, deep venous thrombosis or disseminated intravascular coagulation.[20]

The above studies support the hypothesis that FDP is an early diagnostic indicator of fibrin deposition. High levels of FDPs can be observed in certain systemic diseases. Some recent researches have shown that plasma FDP levels can also be used to diagnose oral premalignant and malignant lesions.

Out of the total 30 subjects with a habit of carcinogenic product consumption habit, 25 subjects showed raised FDP values. The mean FDP value in subjects with habit was 1517.86 mg/ml, which is significantly high as compared to the normal controls whose mean FDP value was 44.21 mg/ml.

Our findings are in accordance with a study conducted by Phatak AG(1984). He detected FPF in saliva of OSMF patients and showed that parotid saliva of three OSMF patients clotted both the oxalated plasma and fibrinogen, suggesting FPF has thrombin‐like behaviour. He hypothesized that when FPF detects fibrinous exudates in the mouth, it rapidly clots them and forms fibrin. The body, in response, produces more fibrinogen.[15]

Similarly, in 1990, Ghosh *et al* conducted a study and 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.[21]

Similar results were obtained by Koshti *et al*(2007), who performed a study on 35 patients of OSMF and found a significant increase in plasma FDP levels with an increase in histological grade of OSMF, but the increase was not statistically significant. Plasma FDP was found to be an early indicator of fibrin deposition. When the plasma FDP increased, the fibrin deposition also increased. The estimation was done using latex agglutination by XL-FDP kit.[22]

Wanjari *et al*(2011) also quantified the FPF in the saliva of OSMF patients. Subjects with positive FPF had increased FDP levels as compared to subjects with negative FPF. Subjects with increased FDP levels and positive FPF stand at a higher risk of developing OSMF as compared to those with normal FDP and negative FPF. These results provide ample evidence to prove the existence of a definite relationship between FPF and increased FDP in OSMF.[23]

Kiran *et al.*(2013) studied 35 cases of areca nut chewers with OSMF, 10 subjects with areca nut chewing habit but normal appearing oral mucosa, and 10 normal subjects without any habit. The patients were evaluated for plasma FDP. Plasma FDP was increased in areca nut chewers with OSMF. Although, FDP was not found in controls and subjects with habit but without OSMF. Also, no statistically significant association was noted between the levels of FDP in various clinical and histological grades of OSMF.[24]

Gharat *et al.*(2013) conducted a study including 25 cases each of leukoplakia, oral submucous fibrosis and oral squamous cell carcinoma, and normal cases. No significant association was observed between fibrinogen levels and premalignant lesions like OSMF. Although, raised serum FDP levels were observed corresponding to the stage of carcinoma, but no appreciable difference was noted amongst the histological grades.[25]

Kadani *et al.*(2014) in their study on FDPs, comprising a total of 30 subjects with OSMF and 30 control group subjects observed that out of total 30 cases of OSMF, 14 subjects showed FDP levels above 200ng/mg and 16 subjects showed negative response to the presence of FDP level. The method employed to detect FDP could be the probable reason for undetectable FDP levels in certain cases. Latex agglutination slide test method was used in their study, which according to previous studies were not sensitive enough to detect minimal increase in FDP levels, resulting in lower sensitivity. The qualitative method of estimation for FDP levels did not show an association between the FDP levels and OSMF. The semi-quantitative technique of detecting plasma FDP showed no association between increased level of FDP and the increase in severity of OSMF.[26]

Gupta *et al.*(2014) conducted a study on 35 patients with OSMF and with the habit of chewing areca nut, 30 subjects with areca nut chewing habit without OSMF, and 30 control group subjects. The study showed that plasma FDPs were detectable in all the subjects with areca nut chewing habit and OSMF, but could not be detected in the other two groups. Also, it was observed that with an increase in the clinical grades of OSMF, the plasma FDP levels also increased. The quantification was based on the principle of agglutination. The technique utilised by XL-FDP reagent kit was able to detect FDP above the level of 200ng/ml.[27]

According to our study, the increase in the FDP levels is a valuable early diagnostic indicator of increased rate of fibrin deposition. Hence, determination of FDP levels may further help in determining the risk of developing oral premalignant lesions in patients with chronic habit of carcinogenic product consumption.

Correlation of the FDP values among the different types of habits revealed high mean values in case of smoked tobacco(3005.60 mg/ml) and mixed habit(2788.32 mg/ml). The FDP values were lower in case of smokeless tobacco and areca nut habit(418.51mg/ml). This suggests that smoked tobacco causes higher systemic damage and is more harmful as compared to smokeless tobacco. So far duration is not a concern.

Various studies have been performed to detect FDPs in oral premalignant and malignant lesions using qualitative, semi quantitative and quantitative methods, but the latest automated latex-enhanced immunoturbidimetric method has not been used until now. Based on our knowledge of the current literature and studies, ours is the first study to detect the plasma FDPs in individuals with carcinogenic product consumption habit using the quantitative automated latex-enhanced immunoturbidometric method, which is the most reliable of all the other techniques.

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III. CONCLUSION

The plasma FDP values are an early diagnostic indicator of fibrin deposition and hence these values may be useful to determine the risk of development of oral potentially malignant disorders in individuals with a chronic habit of carcinogenic product consumption so as to warn them about the consequences even before the appearance of clinical manifestations. This would further help in the counselling of such individuals for quitting the habit and would definitely play a major role in reducing the disease burden that our country is facing at present. A larger sample size would be a better representation of the population and would also facilitate in obtaining more conclusive results.

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Conflicts of Interest: None Declared.

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REFERENCES

[1] Datta S, Chaturvedi P, Mishra A, Pawar P. A review of Indian literature for association of smokeless tobacco with malignant and premalignant diseases of head and neck region. Indian J Cancer 2014;51:200-208.

[2] Chitroda P, Shah J, Katti G, Ghali S. A Correlative Study of Smokeless Tobacco-induced Lesion and Smoke-induced Leukoplakia in Various Aspects. Journal Indian Aca Oral Med Radiol, April-June 2011;23(2):86-91.

[3] Ali FM, Aher V, Prasant MC, Bhushan P, Mudhol A, Suryavanshi H. Oral submucous fibrosis: Comparing clinical grading with duration and frequency of habit among areca nut and its products chewers. J Can Res Ther 2013;9:471-476.

[4] Nair DR, Pruthy R, Pawar U, Chaturvedi P. Oral cancer: Premalignant conditions and screening - an update. J Can Res Ther 2012;8:57-66.

[5] Khan MH, Mishu MP, Imam ST. Current Molecular Concept of Oral Carcinogenesis and Invasion. Medicine Today 2010;22:01.

[6] Ho MW, Risk JM, Woolger JA. The clinical determinants of malignant transformation in oral epithelial dysplasia. Oral Oncol 2012; 48(10): 969–976.

[7] Messadi DV, Wilder-smith P, Wolinsky L. Improving oral cancer survival: the role of dental providers. J Calif Dent Assoc 2009; 37(11): 789–798.

[8] Steele TO, Meyers A. Early detection of premalignant lesions and oral cancer. Otolaryngol Clin N Am 2011; 44(7): 221–229.

[9] Fedele S. Diagnostic aids in the screening of oral cancer. Head Neck Oncol 2009;1: 5–10.

[10] Epstein JB, Silverman JR, Epstein JD, Lonky SA, Bride MA. Analysis of oral lesion biopsies identified and evaluated by visual examination, chemiluminescence and toluidine blue. Oral Oncol 2008; 44(6): 538–544.

[11] Lingen MW, Kalmar JR, Karrison T, Speight PM. Critical evaluation of diagnostic aids for the detection of oral cancer. Oral Oncol 2008; 44(1): 10–22.

[12] Rosin MP, Poh CF, Guillard M, Williams PM, Zhang L, Macaulay C. Visualization and other emerging technologies as change makers for oral cancer prevention. Ann NY Acad Sci 2007; 1098: 167–183.

[13] Mosesson MW. Fibrinogen and fibrin structure and functions. J Thromb Haemost 2005; 3:1894–904.

[14] Lord ST. Fibrinogen and fibrin: scaffold proteins in hemostasis. Curr Opin Hematol 2007; 14:236–41.

[15] Phatak AG. Molecules Immunologically Similar to Fibrinogen (MISFI) in Oral Submucous Fibrosis (OSMF). Indian J Otolaryngol 1984;36:45‑47.

[16] Hamaguchi M, Morishita Y, Takahashi I, Ogura M, Takamatsu J, Saito H. FDP D-dimer induces the secretion of interleukin-1, urokinase-type plasminogen activator, and plasminogen activator inhibitor-2 in a human promonocytic leukemia cell line. Blood 1991;77:94–100.

[17] Chan V, Yeung CK, Chan TK. Antithrombin III and fibrinogen degradation product (fragment E) in diabetic neuropathy. J Clin Pathol 1982;35:661-666.

[18] Song KS, Kim HS, Park KE, Kwon OH. The fibrinogen degradation products (FgDP) levels in liver disease. Yonsei Med J 1993;34**:**234-238.

[19] Whitaker AN, Elms MJ, Masci PP, Bundesen PG, Rylatt DB, Webber AJ, Bunce IH. Measurement of cross linked fibrin derivatives in plasma: An immunoassay using monoclonal antibodies. J Clin Pathol 1984;37:882-887.

[20] Elms MJ, Bunce IH, Bundesen PG, Rylatt DB, Weber AJ, Masci PP, Whitaker AN. Rapid detection of cross-linked fibrin degradation products in plasma using monoclonal antibody-coated latex particles. Am J Clin Pathol 1986;85:360-364.

[21] Ghosh M, Aroor R, Raghavan MR. Clinical utility of serum fibrinogen degradation products in the diagnostic and prognostic evaluation of oral cancer. Ann Dent 1990;49:11-2.45.

[22] Koshti SS, Barpande S. Quantification of plasma fibrinogen degradation products in Oral Submucous Fibrosis: A Clinicopathologic Study. J Oral Maxil pathol 2007;11(2):48-50.

[23] Wanjari PV, Wanjari SP, Gharote HP, Warhekar AM. Correlation of Presence of Salivary Fibrin Producing Factor with Plasma Fibrinogen Level in Oral Submucous Fibrosis, Areca nut Chewers and Normal Subjects. J Indian Aca Oral Med Radiol 2011;23(4):559-562.

[24] Kiran G, Sekhar MS, Hunasgi S, Ahmed SA, Suri C, Krishna A. Plasma fibrinogen degradation products in betel nut chewers - with and without oral submucous fibrosis, J Oral Maxil Pathol 2013; 17(3):324.

[25] Gharat L, Rathod GP. Quantitative estimation of Serum Fibrinogen Degradation Product levels in Oral Premalignant and Malignant lesions. J Int Oral Health 2013; 5(5):65-72.

[26] Kadani M, Satish BN, Maharudrappa B, Prashant KM, Hugar D, Allad U, Prabhu PS. Evaluation of Plasma Fibrinogen Degradation Products and Total Serum Protein Concentration in Oral Submucous Fibrosis. J Clin Diagn Res 2014 ; 8(5): ZC54-ZC57.

[27] Gupta S, Manjunath SM, Jawanda MK, Bharti A. Quantification of plasma fibrinogen degradation products in areca nut chewers with and without oral submucous fibrosis. J Clin Diagn Res 2014;8:ZC27-ZC30.

APPENDIX: A

Table I: Distribution of FDP Level in Cases with Different Habits

Habit	N	Mean	Std. Deviation
Smoked Tobacco	10	3005.6060	6768.21251
Smokeless Tobacco + Areca nut	17	418.5112	484.53488
Mixed Habit	3	2788.3200	4729.70627
Normal	30	44.2067	41.50943
Total	60	781.0318	3027.89663

One way ANOVA value, F=3.244, p=0.029 (Significant)

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APPENDIX: B

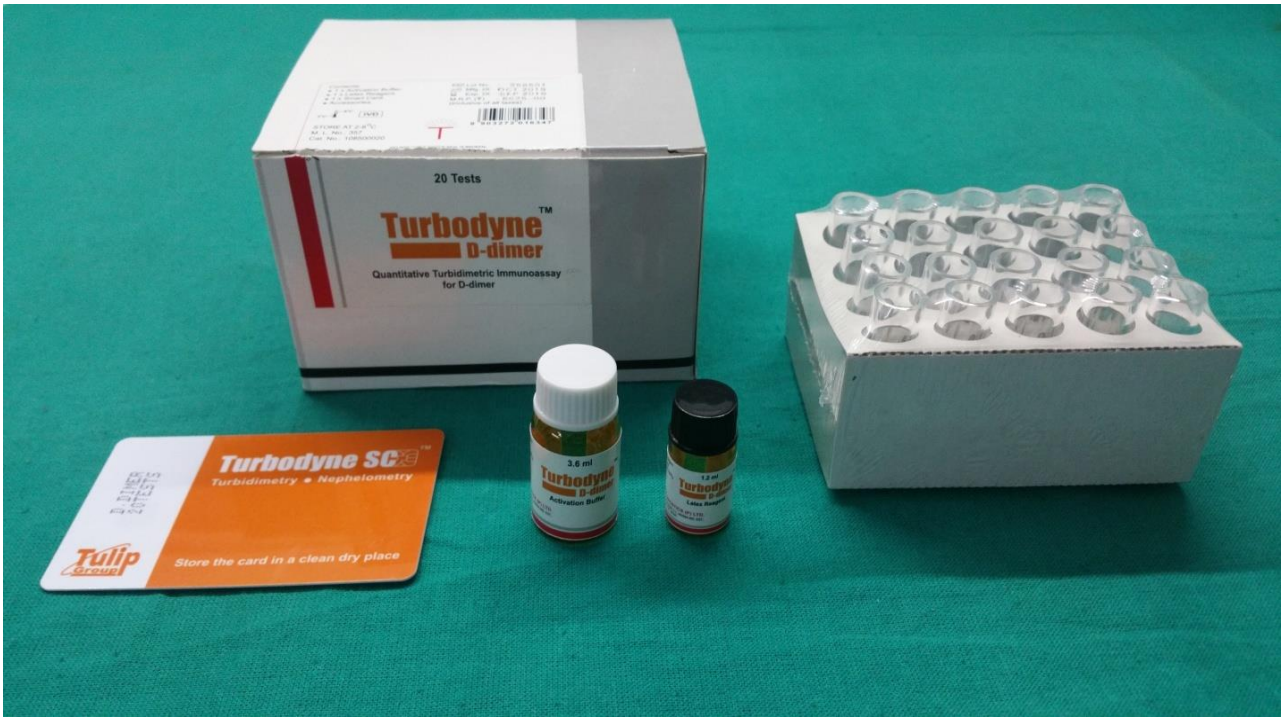

Figure.1: Photograph showing the Turbodyne D-Dimer Test kit with reagents and smart card.

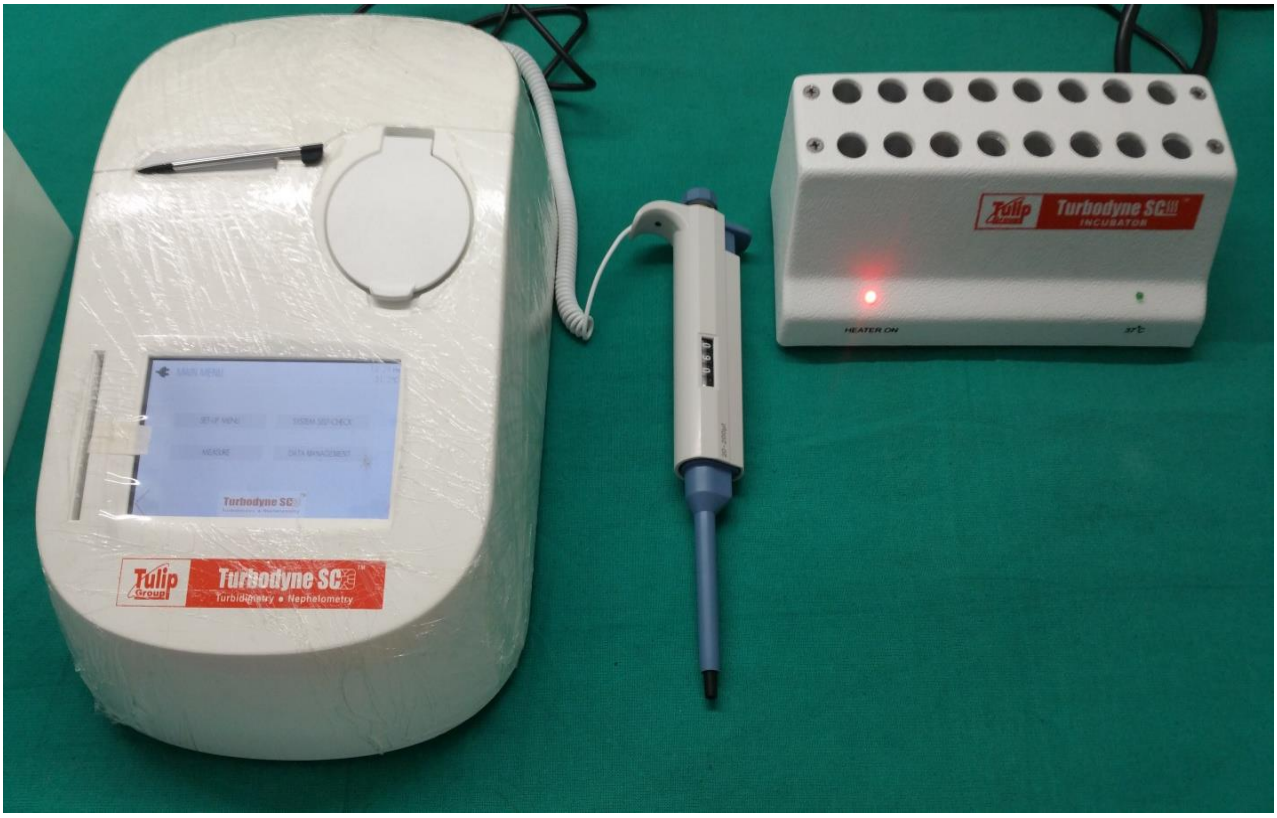

Figure.2: Photograph showing the Turbodyne SC Turbidometry-Nephelometry analyser unit with incubator.

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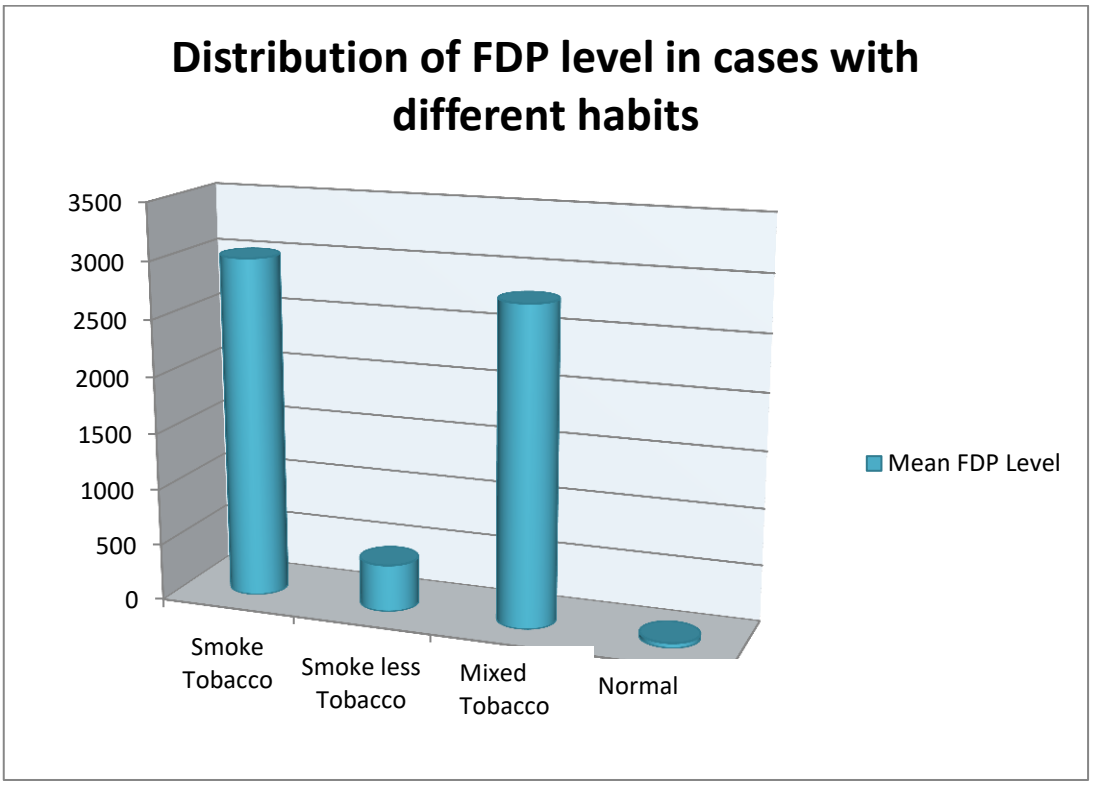

Figure.3: Bar diagram showing the distribution of FDP levels amongst cases with different types of habits.