QuitPuff: A Simple, Home-based, Salivary Diagnostic Test to assess Risk of Oral Pre-cancer and Cancer in Chronic Smokers

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Abstract - **High mortality rate in oral cancer is mainly due to late diagnosis. Current methods involve complex laboratory procedures and are unavailable in rural areas. In this study, a simple, home-based salivary diagnostic test for smokers is devised for early detection of oral pre-cancer and cancer.**

I. INTRODUCTION

Oral cancer is a cancerous growth in the oral cavity [1]. It most commonly involves the tongue, floor of the mouth, cheek lining, gums, lips or roof of the mouth. More than 90% of all oral cancers are squamous cell carcinoma [2].

In India, the incidence of oral cancer is the highest, accounting for almost one third cases found in the world [1]. The high prevalence is mainly due to influence of tobacco and betel quid chewing [4]. Over 5 people in India die every hour every day because of oral cancer [3]. The high mortality rate is attributed mainly to late diagnosis either due to ignorance or inaccessibility of medical care [1,2,7]. Most patients seek help only in the late stages when the symptoms are more prominent [1]. Detection of an oral cancer at stage I carries a prognosis of 80% survival, while the same lesion at stage III carries a 20% survival [8]. This difference could affect not only the quality of life for the patients but also the treatment cost. Thus, there is a need for improvement in early risk detection of oral carcinomas because in the initial stages treatment is more effective and morbidity is minimal.

Exposure to cigarette smoke/ tobacco is responsible for 90% cases of oral cancer [14]. When people smoke they generate Reactive Oxygen Species (ROS). ROS induced cell damage causes lipid peroxidation which is implicated in the pathogenesis of oral cancer [9,10,11,14,15]. It most commonly affects the polyunsaturated fatty acids, causing alteration in the structure and function of cell membranes. Cancer development is caused by cumulative action of multiple events i.e. initiation, promotion and progression, occurring in a single cell. ROS not only initiates but also promotes this multistep carcinogenesis [10]. Malondialdehyde (MDA) is the end product of lipid peroxidation and can be used as a marker for assessing the degree of lipid peroxidation [9,10,11,14,15]. MDA is mutagenic, genotoxic and a potential carcinogen and readily reacts with deoxy nucleosides to produce adducts causing DNA damage [11]. An increase in salivary MDA is widely reported in
various oral pre-cancers & cancers in the early stages [9,10,11,12, 13,14,15].

II. METHOD

One molecule of Malondialdehyde (MDA) reacts with two molecules of Thiobarbituric Acid (TBA) in an acidic medium at high temperature to produce a coloured adduct. A highly sensitive Thiobarbituric Acid (TBA) reagent was formulated by dissolving 0.375g of TBA in 85% Ortho-Phosphoric acid (1ml) and 1% Trichloro-Acetic Acid (1ml). MDA standards in saliva of 10 healthy people were prepared in the concentrations of 500, 250, 100, 50, 25 and 5ng/ml and TBA reaction was performed. The color change was noted. Samples were analysed by UV Spectroscopy, absorbance measured at 532nm, a standard curve (Figure 1) and colorimetric chart (Figure 2) were prepared.

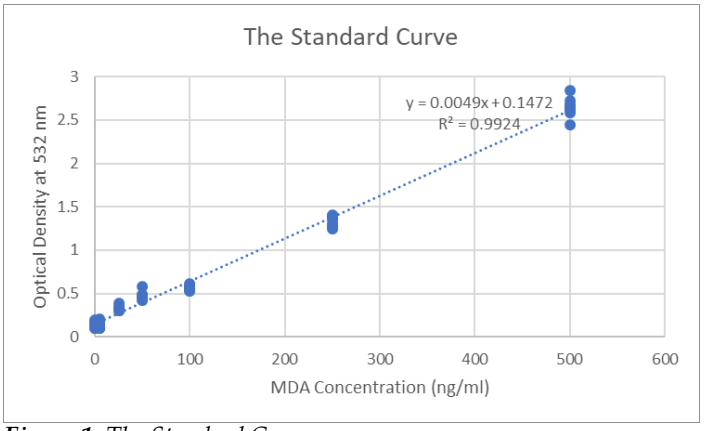

Figure 1: *The Standard Curve*

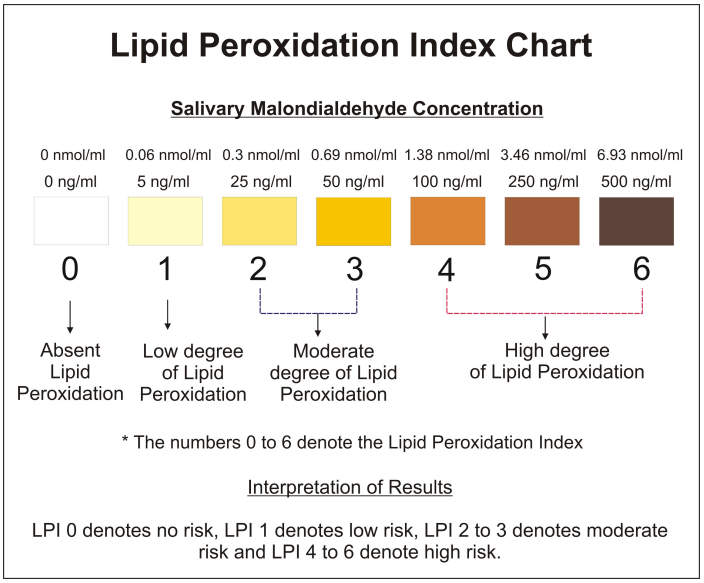

Figure 2: *The Colorimetric Lipid Peroxidation Index Chart*

The method was tested on 125 people (5 groups) from BMCRI Hospital. Ethics approval was granted by IEC. Informed consent was obtained. Group 1 had 25 non-smokers, Group 2, 3 and 4 consisted of 25 smokers who smoked less than 10, between 10-20 and more than 20 cigarettes per day respectively, none of them had any oral lesions, Group 5 consisted of 25 smokers who smoked 10-20 cigarettes a day, 20 with recently diagnosed pre-cancerous mouth lesions and 5 with Oral Squamous Cell Carcinoma stage 1-2, yet to start on treatment. Tests were done on fresh saliva samples. 1ml of saliva was taken in a test tube, 2ml of TBA Reagent was added and the mixture was heated in a boiling water bath for 15 minutes. The color change was matched with the colorimetric chart and the Lipid Peroxidation Index (LPI) was

noted. The test method was validated for its performance using the standard curve. All 125 samples were analyzed by UV Spectroscopy and the standard curve equation was used to determine the MDA levels. Based on the MDA values obtained by the validation method, the LPI (Lipid Peroxidation Index) was again derived and noted.

III. RESULTS AND CONCLUSION

The mean Lipid Peroxidation Index (LPI) obtained by the test method was compared with the mean LPI obtained by the validation method. The results agreed (Table 1). Two types of variations were found, small in 12% cases & large in 3.2% cases. Small variations were defined as those where a minor difference was found between the LPIs from the test and validation method. These minor variations did not warrant a zone change (low, moderate, high) in the LPI chart and hence considered as small variations. Large variations were defined as those where a large difference was found between the LPIs from the test and validation method, warranting a zone change. For purposes of calculation of accuracy only large variations were taken into consideration and thus it is derived that the diagnostic test was able to detect the degree of salivary lipid peroxidation with 96.8% accuracy.
Smokers exhibited a higher degree of salivary lipid peroxidation as compared to non-smokers, heavier the smoker, higher was the degree of lipid peroxidation (Table 2).

Table 1: *Comparison between the mean LPIs (Lipid Peroxidation Index) obtained by test and validation method.*

Group Name	Mean LPI obtained b test method	Mean LPI by validat method
Group 1	0.2	0.24
Group 2	3.64	3.56
Group 3	3.68	3.64
Group 4	4.52	4.48
Group 5	4.48	4.32

Table 2: *Degree of Lipid Peroxidation in Study Groups*

Group Name	Total No	Degree	of	Lipid	Peroxidation
		Zero	Low	Moderate	High
Group1	25	20	4	1	0
Group2	25	0	0	9	16
Group3	25	1	0	6	18
Group4	25	0	0	3	22
Group5	25	0	0	1	24

QuitPuff, a simple, quick, home-based, inexpensive method can serve as an early, non-invasive test for smokers to assess risk of oral pre-cancer & cancer.

IV. ACKNOWLEDGEMENT

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