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Urinary 8-Ohdg an Oxidative Stress Marker to DNA and Total Antioxidant Status levels in Oral Cancer Patients

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Abstracts
Recently there has been a significant shift in mortality rate from infectious diseases to non-communicable diseases. Oral cancer is one of the commonest malignancies in India. Approximately 60,000 newly diagnosed cases of oral cancer are reported to occur every year. Oxidative stress is known to be a major cause of development of many diseases attributed free radical injury, which is often neutralized by various endogenous and exogenous antioxidant systems. 8-hydroxy-2-deoxyguanosine (8-OHdG) is a sensitive marker oxidative stress in DNA that could be detected in urine, plasma and leukocytes. Very few studies from this region on oxidative DNA stress as assessed by urinary 8-OHdG, malondialdehyde (MDA), protein thiols and ferric reducing ability plasma (FRAP) in oral cancer subjects have been undertaken and hence the present study.
Objectives: *To compare the serum levels of lipid peroxidation, total antioxidant status and protein thiols in patients with oral cancer and healthy controls. To evaluate whether the estimation of urinary 8-OHdG could serve as a marker to predict the extent of cellular oxidative stress in oral cancer patients.*
Methods: *The study included 60 volunteers, who were divided into 30 controls, and 30 early stage oral cancer patients who were further classified into newly diagnosed (n=15) and one year treatment follow-up oral cancer subjects (n=15). Random urine samples were collected and analyzed for the concentration of 8-OHdG. Serum lipid profile and lipid peroxidation, protein thiols and FRAP assay were performed by photometric assay methods. The urinary 8-OHdG and serum MDA levels were significantly higher in newly diagnosed oral cancer subjects than one year treatment follow up subjects. Atherogenic markers were significantly altered in oral cancer subjects. A significant correlation exists between urinary 8-OHdG and FRAP in both the groups of oral cancer patients.*
Conclusions: *Significant increase in the urinary 8-OHdG and other oxidative stress markers firmly suggest increased rate of oxidative DNA modifications in oral cancer subjects. The association between urinary 8-OHdG and FRAP in oral cancer patients suggest the preventive role of antioxidant on DNA modifications as induced by oxidative stress. In future, more research has to be focused to study gender variation, histological types, and different grades of tumour as well as survival. This would be taken up in our laboratory, with near future in a phased manner.*
Key Words: *Oral cancer, lipid profile, oxidative stress, antioxidant, 8-OHdG 8Hydroxy de oxygunosine, MDA, ferric reducing ability plasma (FRAP).*

INTRODUCTION

Cancer is a disease caused by an uncontrolled division of abnormal cells in a part of the body. Most cancers are named for the organ or type of cell in which they start – for example, cancer that begins in the colon is called colon or colorectal cancer, cancer that begins in basal cells of the skin is called basal cell carcinoma [1] Oxidative stress is a condition where there is an imbalance between pro-oxidant and antioxidants in aerobic organ-isms. In recent years, increasing experimental and clinical data have provided compelling evidences to implicate oxidative stress in a large number of pathological states including carcinogenesis. Oxidative stress can induce deleterious effect on lipids, proteins, carbohydrates and nucleic acids. As a result the product of lipid peroxidation namely Malondialdehyde (MDA), protein thiols, sialic acid and 8-hydroxy-2'-deoxyguanosine (8-OHdG) are formed respectively. These modifications due to oxidative stress might alter signal transduction, membrane transport, and other cellular metabolic functions [2] 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most sensitive and biomarker for oxidative stress in DNA that can be detected in urine, plasma and leukocytes. In conditions of oxidative stress, DNA gets damaged by reactive oxygen species (ROS) to produce modified base in 8-hydroxy-2'-deoxyguanosine (8-OHdG).Studies have documented individual variations in the concentrations of 8-OHdG in random urine, exhibited due to time, exercise, drinking water and other miscellaneous factors. Studies have also documented higher serum 8-OHdG levels in smokers than non-smokers as well as a marker of cancer incidence [3] Each year about 5, 75,000 new cases and 3, 35,000 deaths occur worldwide due to oral cancer. In India, it is estimated that there is an incidence of 12.48 male cases per 100,000 population and 5.52 females per 100,000 populations in all cancers [4] Squamous cell type of the oral cancer is responsible for considerable morbidity and mortality. Tobacco chewing with betel, gutkha, smoking and alcohol consumption are the most important etiological factors associated with the high incidence of oral cancer in India [5] Experiments showed that the mutagenic potential of 8- oxodG is supported by a loss of base-pairing specificity, misreading of adjacent pyrimidines, or insertion of adenine opposite the lesions [6] Squamous cell type of the oral cancer is responsible for considerable morbidity and mortality. Tobacco chewing with betel, gutkha, smoking and alcohol consumption are the most important etiological factors associated with the high incidence of oral cancer in India [7]

Till date, very few studies from the region of South India are available on the extent of oxidative stress, as assessed by 8-OHdG, MDA, protein thiols and (Ferric reducing ability plasma) FRAP assay levels in oral cancer subjects of south Indian Pondicherry population. Moreover, not many studies are available in the literature on oxidative DNA damage in newly diagnosed oral cancer and treatment follow up subjects. Hence, the present study was planned.

MATERIAL AND METHODS

All volunteers enrolled in this study were aged between 20 – 65 years. Healthy controls constituted Group I. Newly diagnosed Oral cancer patients without any previous history of anticancer medication constituted Group II. Oral Cancer Patients who were on treatment constituted Group III. Subjects with hypertension, past history of coronary artery disease, myocardial Infarction, peripheral vascular disease, renal disease, diabetes mellitus and those on vitamin supplements were excluded from the study.All values are entered in micro soft excel 2010 and data were expressed in mean ± SD. Student t test and one way ANOVA and post hoc analysis was performed for group comparison. Pearson's correlations analysis was done using commercial statistical software graph pad InStat. A p value less than 0.05 consider as significant.

RESULT

In the present study, oral cancer subjects were between the age group of 25 to 65 years. The mean age of affected patient was 36.96± 7.25 standard deviation. Glycaemic status in the study subjects showed no significant difference (p value = 0.7) as depicted in Table 1.When compared to healthy controls, MDA level significantly higher in oral cancer subjects ($p<0.0001$) but, between subgroups the levels of MDA low in subjects on treatment follow up (Table 2). Antioxidant capacity in plasma as assessed by FRAP assay significantly low in newly diagnosed subjects when compared to control ($p<0.01$) in table 1.There is significant increase in the levels of FRAP assay in subjects with one year of treatment follow up in comparison to the newly diagnosed subjects ($p<0.001$) table 2. There is no significant alteration noted in the levels of serum Protein thiols. When compared to the control, urinary 8-OHdG level in oral cancer subjects is significantly higher (Table 1.) But the levels of urinary 8-OHdG in newly diagnosed subjects of oral cancer is significantly high ($p<0.001$) as depicted in Table 2. But when compared to controls there is no significant differences in the patients of one year of treatment follow up ($p>0.05$). When compared between the subs groups of oral cancer subjects, there is a significantly lower level of 8-OHdG in the patient on treatment of one year follow up. When compared to control, serum total cholesterol, TAG and LDL levels are significantly higher and HDL level is lower in oral cancer subjects as depicted in table 1. Similarly both the newly diagnosed and subjects who were on treatment follow up showed significantly higher level of total cholesterol levels, TAG and LDLc ($p<0.001$).HDLc level was significantly lower in the treatment follow up subjects when compared to control ($p<0.01$.) There is a significant correlation was noted between urinary 8 OHdG and FRAP assay in both newly diagnosed($r = 0.7729$); and treatment follow-up subjects ($r = 0.5946$) both ($p<0.05$) as shown in figures.

Table: 1 Characteristic of study parameters between control and oral cancer patients.

PARAMETERS	CONTROL [N=30]	PATIENTS[N=30]	P-Value
AGE [years]	33.3±8.49	36.96±7.25	0.07
GLUCOSE	92.9±17.0	96.83±13.08	0.319
TC	150.4±34	213.9±40.310*	0.001
TAGs	86.6±27.10	170.3±49.26*	0.001
HDL	47.81±11.7	39.6±11.47*	0.008
LDL	85.5±18.5	126.533±28.40*	0.000
MDA	8.03±1.45	13.42±1.90*	0.000
FRAP	465.4±60.13	446.033±114.85	0.416
PROTEIN THIOLS	7.09±.6.3	7.923±1.11	0.478
8-Ohdg	1.030±0.73	2.32±2.54*	0.004

Data presented are mean ± SD. Analysis of data was done by student t test. A p value < 0.05 was considered significant.

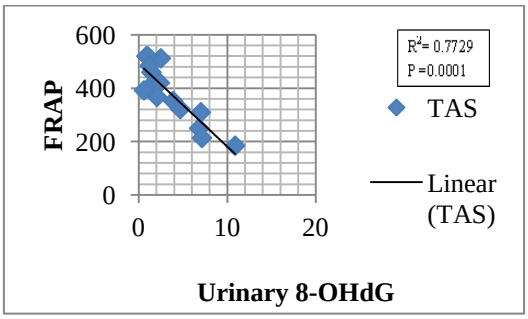
Table: 2 Comparison of oxidant, antioxidant status and 8-OHdG levels in the different study groups

Parameters	Control [n=30]	Newly diagnosed [n=15]	One year follow up [n=15]	p-Value
Age	33.3±8.49	36.8±5.18	37.13±9.062	0.21
Glucose	92.9±17.0	91.13±11.9	102.53±11.92	0.07
MDA	8.03±1.45	14.06±1.86*	12.78±1.77*	0.000
FRAP assay	465.4±60.13	380.6±109.43 *	511.6±78.56#	0.000
Protein thiols	7.09±.6.3	8.48±1.15	7.36±0.74	0.622
8-ohdg	1.030±0.73	3.58±3.093*	1.06±0.64#	0.044

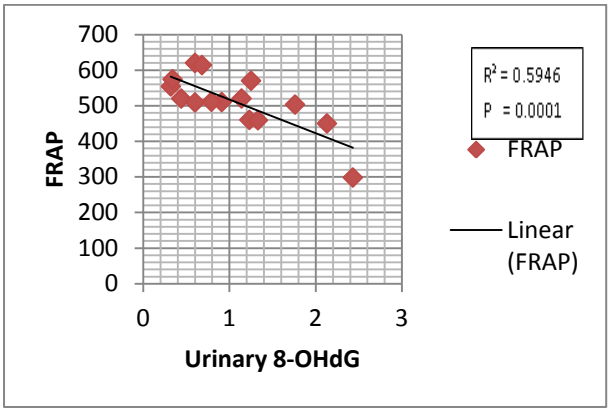
Data presented represent mean ± SD. Analysis of data was done by one-way ANOVA and post-hoc Tukey-Krammer test.

depicts comparison with Control group and the # depicts comparison with newly diagnosed group.$p < 0.05$; # $p<0.05$.

Graph- 1: Association of FRAP and 8-OHdG in new patients.



Graph 2 Association of FRAP and 8-OHdg in patients on one year treatment follow up subjects.



DISCUSSION

OXIDATIVE STRESS & ORAL CANCER

Oral cancer accounts for one third of all malignancies in India and remains to be one of the major public health problems; the important cause for high incidence of oral cancer is tobacco chewing and poor oral hygiene [8]. In our study all the oral cancer subjects had history of tobacco or betel nut chewing, irrespective of sex, several studies had documented tobacco induced oxidative damage *in vivo and in vitro*[9]. Cancer is essentially an event occurring at the level of gene where there is DNA damage that occurs due to various reasons including virus, chemicals, and irradiation. Sometimes familial preponderance can also play a role in carcinogenesis. Earlier, studies have documented oxidative stress induced by ROS, as a modifier of *nucleic acid, proteins and lipids*, and thereby alter the cell signals in triggering the biochemical event of pre-cancerous and malignant transformations [10]. In our study, there is a high level of oxidative stress as assessed by MDA levels in oral cancer subjects and the MDA levels is much higher in newly diagnosed than subjects on treatment follow up (Table 1& 2).In concordance with our study, several studies have documented the increased level of oxidative stress (lipid peroxide levels) in oral cancer subjects [11][12][13]. In our study, the antioxidant status, as assessed by FRAP is comparatively low along with dyslipidaemia, in oral cancer emphasising a cumulative evidence to support the role of oxidative stress in the pathogenesis oral cancer. Though in our study, there is no significant change in protein thiols, a decrease in FRAP level is relatively pronounced in newly diagnosed untreated cases. [14]Studies also have documented the involvement of free radicals in both the initiation and the promotion of multistage carcinogenesis. These free radicals have been shown to cause DNA damage, activating the pro-carcinogens and alter the cellular anti-oxidant defense mechanisms [15]. Antioxidant defenses of the body have been proposed to be the inhibitors of initiation, promotion and transformational stages of carcinogenesis and to protect the cells against oxidative DNA damage.

URINARY 8-OHDG A MARKER OF ORAL CANCER

Several proposed markers of oxidative stress and antioxidant enzymes could aid in the early identification of cancer; in our study, urinary 8-OHdG an endogenous noninvasive marker of oxidative DNA damage is significantly higher. Also in addition there is an association between urinary 8-OHdG level and antioxidant status as assessed by FRAP in both the sub groups of oral cancer subjects. [16] Similarly, a study by *Han et al* showed oxidative DNA damage in smokers of who were exposed more to the environmental pollutants. Studies have also shown 8-OHdG as a stress marker in cardiovascular diseases. Furthermore, it is suggested that the urinary 8-OHdG a modified nucleobase can serve as a sensitive biomarker of oxidative DNA damage. [17] *Boonla C et al* showed an increased excretion of urinary 8-OHdG in patients with renal calculi,

indicating that oxidative damage to DNA as increased in these patients. Oxidative stress also mediates the development of several pathological conditions and the urinary excretion of oxidatively modified products of lipids (MDA and isoprostanes) and DNA 8-OHdG is currently used to assess the overall oxidative stress in diseases [18] Although, the metabolic and chemical stability of 8-OHdG exhibits favorable properties for biomarker purposes, the levels of urinary 8-OHdG exhibit marked inter-individual variations [19].

DYSLIPIDAEMIA IN ORAL CANCER

In our study, subjects of newly diagnosed oral cancer and subjects who were on treatment showed altered lipid profile with significant enhancements in LDL level and decreased HDL level in both newly diagnosed cases who were on treatment ,when compared to controls. This makes us to conclude that patient with oral cancer are susceptible to cardiovascular risk. In addition oxidative stress and decreased antioxidant status can thus play synergistic role in increasing mortality of oral cancer patients.

CONCLUSION

Significant increase in the urinary 8-OHdG and other oxidative stress markers firmly suggest increased rate of oxidative DNA modifications in oral cancer subjects. The association between urinary 8-OHdG and FRAP in oral cancer patients suggest the preventive role of antioxidant on DNA modifications as induced by oxidative stress. In future, more research has to be focused to study gender variation, histological types, and different grades of tumour as well as survival. This would be taken up in our laboratory, with near future in a phased manner.

LIMITATION OF OUR STUDY

Small sample size, assessment of other antioxidant enzymes, classification based on histological type or precancerous lesions continue to act as a major drawback of our study due to paucity of time.

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