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To Estimate the level of Oxidative stress parameter among Diabetic persons with Squamous cell carcinoma of oral cavity: A Case Control Study

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ABSTRACT

Background: Diabetes mellitus and cancer share common modifiable and non- modifiable risk factors. Both conditions have a great impact on social and economic status of an individual and their families. Diabetes is rapidly becoming a common metabolic problem in rural populations. At the same time, the incidence of oral cancer has not decreased over the years despite exhaustive research and has become the most common cause of death. Oral cancer accounts for approximately 4-5% of all cancers in the world. Recent epidemiological studies have shown a strong link between diabetes and cancer. Objective: To Estimate the concentration of Oxidative stress markers among Diabetic persons with Squamous cell carcinoma of oral cavity .Materials and Methods: A Case Control study, conducted at Sri Devaraj Urs Medical College attached to Sri R.L. Jalappa Hospital a constituent of Sri Devaraj Urs Academy of Higher Education and Research, Kolar. A total of 60 subjects were included in the study and categorised into equal number of cases and controls. Diabetic and lipid profiles were estimated by enzymatic methods in Dry chemistry Autoanalyser, Vitros 5.1FS, HbA1c analysed by HPLC (BioRad D10 USA), Nitric Oxide, Malondialdehyde, and Vitamin C by spectrophotometer Perkin Elmer UV/ VIS Lambda-35 methods respectively. Results: Our patients were in the age group of 30- 80 years with a mean age of 52.62± 12.67. Diabetic profile and oxidant markers were significantly elevated in cases compared to controls. However, mean values of lipid profile and antioxidants were significantly reduced in cases with an exception of LDL cholesterol, when comparing with controls Conclusion: Observations that has been made with lipid profile and antioxidant variables in diabetes with SCC was reduced might be due to their increased utilization by neoplastic cells for new membrane biogenesis.

These findings strongly suggest, evaluation of these variables may help better patient care and build nation as well in addition to health education.

Keywords: Diabetes Mellitus, Lipid Profile, Oral Cancer, Squamous cell Carcinoma

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Introduction:

Cancer is a significant global health care-problem, with a worldwide estimation of approximately 14 million new cases and 8.2 million cancer related deaths in 2012. It is expected that annual cancer cases will raise from 14 million in 2012 to 22 million within next 20 years [1]. Worldwide, the incidence of cancer of oral cavity varies greatly. France, India, Brazil, Central and Eastern Europe have the highest rates of Cancer of oral cavity around world [2].Studies conducted by Malawall AM et al in 1976, documented that India had highest rate of cancer of oral cavity compared to any other Asian countries [3].

Type 2 diabetes mellitus (T2DM) consists of an array of dysfunctions characterized by hyperglycemia and results from the combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion. Diabetes of long duration leads to various multi-organ dysfunctions such as atherosclerosis, peripheral neuropathy, retinopathy, nephropathy and heart disease [4].

Both diabetes mellitus and cancer share common modifiable and non- modifiable risk factors. These conditions together individually has a great impact on socio- economic status of an individual and their families [5].

Even though it is a well known fact that end product of Glycolysis, lactic acid can get converted into pyruvate and further to Acetyl CoA, and/ or in case where there is no demand for pyruvate synthesis or deficiency of LDL or inactivity of LDH, lactic acid may get elevated in the cytosolic component of cell there by affecting the cell cycle; termed as Warburg's hypothesis. This hypothesis is less studied with respect to Oral SCC.

In normal population, oral cancer mainly involves the tongue, oropharynx, floor of the mouth and buccal mucosa. Lips, gingiva, dorsum of the tongue and palate are usually not affected[6].

But in people with diabetes, tumors most commonly involve the gums and labial mucosa. Also in contrast to the normal population, in which males are most commonly affected, with an increased incidence among alcoholics and smokers of sixth and eighth decades of life. Most common risk factor for oral cancer is tobacco. Other risk factors include alcohol, immune defects, genetic factors and viruses such as human papilloma virus, Epstein–Barr virus, hepatitis virus, etc [7].

In diabetic patients, increased oxidative stress may lead to lipid, protein, and DNA modifications. Hyperglycemia causes glycation of proteins, auto-oxidation of glucose, and activation of polyol metabolism that contributes to the formation of reactive oxygen species. This reactive oxygen species causes damage to cellular macromolecules that may lead to protein and DNA modification. The elevated reactive oxygen species in diabetes leads to DNA strand breaks and base modifications

Epidemiological studies have shown strong association between type- 2 diabetes and cancer. But, whether diabetes is cause or consequence of cancer is not clear. HbA1c an indicator of chronic hyperglycemia as it is not altered due to daily variation of glucose levels. Studies have shown that there is an increase cancer risk with HbA1c of 6% to 6.9% [8].

Most of the studies have shown that oxidative stress is an important event in development of tobacco related disease as well as diabetes. Antioxidant enzymes Superoxide dismutase, catalase and glutathione peroxidase serves as the backbone of cellular antioxidant mechanism and these enzymes are reported to get altered in both oral carcinogenesis and diabetes [8, 9].

Biochemical alterations in SCC are improperly researched. Though few studies are done in this regard, lacunae still exists regarding other biochemical alterations such as glucose metabolism and oxidative stress parameters in oral SCC [9, 10].

Most of the studies doesn't mention definitive

inclusion criteria for oral leukoplakia, which itself is a controversial issue and none has excluded other possible causes for white lesions in these patients, such as lichenoid lesions due to oral hypoglycemic drugs and studies also lack information about the onset of diabetes. Did the premalignant lesions develop before or after diagnosis of diabetes mellitus? or how many years after diagnosis of diabetes did the patients develop premalignant lesions? Needs to be addressed. Although it is observed that both leukoplakia and lichen planus occur most often in the second year of established diabetes, these questions needs to be investigated in greater detail before we draw any conclusions.

These controversies created an interest in us to study oral cancer, squamous cell carcinoma with minimal investment and maximal outcome with an objective! to estimate the level of oxidants and antioxidants in both clinically proven healthy controls and diabetes with oral SCC .

Materials and Methods:

A case Control study, conducted at RL Jalappa Hospital attached to Sri Devaraj Urs Medical College a Constituent of Sri Devaraj Urs Academy of Higher Education and Research, Kolar. Controls was selected from patients attending the ENT outpatient department of the Hospital and Cases were included with all clinically and Histopathologically proven oral squamous cell malignancy cases admitted in the Head and Neck oncology ward in RL Jalappa hospital. A total of 60 Subjects (cases = 30 and controls = 30) were included in the study which was done from July 2016 to September 2016.

Study included diabetic subjects with clinically and histopathologically proven oral squamous cell malignancy cases admitted in the Head and Neck oncology ward in RL Jalappa hospital. Subjects with age and gender matched healthy volunteers with no history of diabetes and oral precancerous/cancerous lesions were included as controls. Present study excluded patients on hypolipidemic drugs and antioxidants known to alter the SCC condition and histological variants other than SCC.

Method of collection

Institutional Ethical clearance (IEC) was obtained to start the study. Written informed consent was taken from both cases and control groups. An overnight of minimum 8hrs fasting, 5ml venous blood was collected. All possible standard precautions were taken while collecting the blood samples. Sterile disposable needle and vacutainer was used for sample collection. Correct procedure was followed at every each step of blood collection, transportation and analysis. Overall preanalytical, analytical and transcriptional errors were taken care.

Fasting Blood Glucose, Postprandial Blood Glucose and lipid profile were estimated in vitros 5.1 FS Dry Chemistry, auto analyzer from Johnson and Johnson works on the principle of “reflectance photometry” and HbA1c percentage was analysed in HPLC (Bio Rad D10 USA).

Concentration of Malondialdehyde (MDA), Vitamin C (Vit C), Nitric Oxide (NO), Glutathione peroxidase (GpX) was estimated by Thiobarbibituric acid reactive substances (TBARS), 2,4- Dinitro phenyl hydrazine, modified Griess and UV Colorimetric (Sigma Aldrich) respectively. All these variables were done manually by Spectrophotometer Perkin Elmer UV/Vis Lambda-35.

The collected data were tabulated in MS excel and the analysis was performed using the statistical package SPSS version 21.0. All quantitative variables were analyzed using student’s t test. $p < 0.05$ was taken as statistically significant.

Results:

In our study, patients were in the age group of 30- 80 years and the mean age of study subjects was 52.62± 12.67 years. Nearly 67% of the study subjects were Females and 33% were Males.

Among the Cases group the site of Squamous Cell Cancer in the oral cavity was more common in Buccal Mucosa tumor (n=15) 50%, tumor of base of tongue was seen in (n=5) 17%, hard palate malignancy in n=3 (10%), labial mucosa in n=3 (10%), Pyriform fossa n=2 (5%) and Gums n=2 (6%).

Tobacco Consumption for more than 10 years was one of the common factor in all the 30 cases of cancer in our study with few cases reporting of both Alcohol and Tobacco consumption for years was seen. Chewing of tobacco in 60%, smoking in 27% and in 13% the cases both smoking and tobacco chewing was present.

Table 1: Site of Squamous cell carcinoma in Oral Cavity in Case Group

Site of Carcinoma	Number (n=30)	Percentage
Buccal Mucosa	15	50
Base of tongue	5	17
Hard palate	3	10
Labial mucosa	3	10
Pyriform fossa	2	7
Gums	2	6

Table 2: Comparison of Diabetic Profile between controls and cases

Parameters	Controls Mean ± SD	Cases Mean ± SD	'p' value with significance
FBS (mg/dL)	96.53±29.58	115.90±75.53	0.001*
PPBS(mg/dL)	127.57±41.97	167.62±98.29	0.05*
HbA1c %	5.95±1.22	6.63±2.54	0.006*

*statistically significant

Table 2 shows the mean values of diabetic profile such as FBS, PPBS and HbA1c were found to be significantly elevated in cases compared to controls. Mean values of serum lipid profile for controls and cases at the time of diagnosis are represented in table 3. A significant decrease of TC, TG, HDLC and VLDLC was observed in cases as compared to controls. However, LDLC was reduced in cases but did not reveal any significant difference among the two groups. Further, when we compared NO, MDA, Vitamin C and GPx between cases and controls, concentration of NO (p=0.033) and MDA (p=0.007) was significantly elevated, whereas antioxidant concentration was significantly reduced in cases on comparing with controls. Same is depicted in table 4.

Discussion:

Various factors are known to play in etiopathogenesis and progression of oral squamous cell carcinoma. Many times it is the interplay of socioeconomic factors, etiological factors such as exposure to tobacco, nutritional status of patient that leads to development of squamous cell carcinomas of oral cavity. Compared to the studies conducted by Kamath A et al and Patel et al, [11,12] we have observed a median age of 51.53 and 52.62

years with range of 30- 80 years which is similar to finding of our study. A female preponderance was seen in our study, this may be due to the habit of keeping the tobacco mixed with betel quid in the buccal cavity for a prolonged contact period by females when working in the fields. This may also be a reason for finding more malignant lesions in buccal mucosa.

Table 3: Comparison of Lipid Profile between controls and cases

Parameters	Controls Mean ± SD	Cases Mean ± SD	'p' Value with Significance
TC (mg/dL)	164.89±33.73	146.55±37.21	0.043*
TG (mg/dL)	195.18±110.7	150.52±78.97	0.038*
HDL (mg/dL)	40.46±8.03	35.93±6.26	0.029*
LDL(mg/dL)	84..96±28.98	82.86±25.22	0.356
VLDL (mg/dL)	39.03±22.15	30.10±15.79	0.038*

*statistically significant

Table 4: Comparison of Oxidative and Anti oxidative parameters between controls and cases

Parameters	Controls Mean ± SD	Case Mean ± SD	'p' value with significance
NO (nmol/mL)	0.031±0.0027	0.36±0.0035	0.031*
MDA (ng/mL)	0.426±0.030	0.59±0.024	0.007*
Vit C(mg/dL)	0.1857±0.0082	0.1548±0.028	0.001*
Gpx(mU/mL)	1.91±0.90	0.41±0.12	0.02*

In our study all cases were associated with tobacco exposure in some form or other form. It is believed that tobacco carcinogens induce generation of free radicals and reactive oxygen species, which are responsible for high rate of oxidation/ peroxidation of polyunsaturated fatty acids. Lipid peroxidation further release peroxide free radicals. There is a substantial evidence that the hydroxyl radical generated, can destruct tissue by initiation and propagation of lipid peroxidation by abstracting hydrogen from unsaturated fatty acids. This affects essential constituents of the cell membrane and may be involved in carcinogenesis [9,13].

Recent studies have shown that diabetes is one of the major contributing factors in the initiation and progression of certain cancers [14]. Epidemiological studies have shown that diabetic patients have higher risk of developing common cancers such as oral, pancreas, liver, breast, colorectal cancers etc [15]. Our study explored the relationship between diabetes and oral cancer and the impact of oxidative stress parameters and lipids in SCC.

Our findings correlated with above study. However post prandial levels was almost near statistical significant which may be due to the food patient had consumed and also they were on Total Parental Nutrition (TPN). Recent evidences suggest that diabetic patients show more pre-cancerous lesions such as leukoplakia and erythroplakia leading to oral

cancer [16]. The coronal part of gingival connective tissue underneath the junctional epithelium shows decreased collagen density. Reduction in collagen synthesis and replication of DNA in dermal fibroblast are seen more in diabetic patients as compared to non -diabetic subjects. Increased collagenase activity and abnormalities in neutrophil degranulation due to gingival crevicular fluid collagenase or other metabolic abnormalities in periodontal ligament fibroblast was observed in diabetics. The histological sections in diabetic patients also showed thickened basement membrane, swollen and proliferated endothelial cells and obliteration of capillaries with narrow capillary lumen. These findings were consistent with our study [17]. These changes may occur initially before progressing to invasive lesions. In animal models diabetes seems to promote the activation of the Ras/ Raf/ MAPK signal transduction pathway mainly by induction of erbB2 and erbB3 receptors [18].

In the present study TC, TG, HDL values were significantly decreased in oral cancer groups compared to controls. This is correlated well with our previous institutional study [11].

Lipids are the important constituents of cell which are required to carry out several vital biological functions. Lipids are essential for the maintenance of the structural and functional integrity of all biological membranes and are also involved in the activity of the membrane bound enzymes and are important for stabilization of DNA helix [11].

Lipoproteins are responsible for transportation of lipids and cellular uptake and regulation of cholesterol is mediated by lipoproteins situated on the surface of cell. These lipoproteins are broken down by cells to meet the demands for cellular functions. The blood cholesterol undergoes early and significant changes in some malignant diseases [11].

Lohe et al. have observed an inverse relationship between serum lipid profile, oral Cancer and oral precancerous lesions. But in our study we have considered clinically proven and histopathlogically confirmed cases [19]. Thus, whatever the alteration in the lipid varies such as TC, TG, and HDLC, we have observed lower in SCC cases compared to controls except LDLC which is more in controls compared to cases with non-significant p-value.

HDLC levels may be a useful indicator, reflecting the initial changes occurring in neoplastic conditions [20]. A drastic reduction in levels of HDLC was observed in our study, clearly indicating that HDLC may have role in SCC. This is in accordance with the previous reports [19, 20] stating that low HDLC is an additional predictor of cancer and it might be a consequence of disease that is mediated by utilization of cholesterol for membrane biogenesis of the proliferating malignant cells [20].

According to the studies conducted by Alexopoulos et al. and Chawda et al, increased concentration of glucose leads to increased triglycerides by Glycolytic pathway and Total Cholesterol by the end product of glycolysis converted to Acetyl CoA and fatty acids [20,21].

In our study NO and MDA parameters were elevated in diabetes groups compared to controls. Our results agree partly with the study done by Chavez J et al. Formation of oxidative stress parameters might be the result of damage to critical cellular macromolecules including DNA, lipids, and proteins. The oral peroxidase activity loss was accompanied by increased carbonylation of salivary proteins, an indicator of oxidative damage to proteins[22].

In our study antioxidant variables such as enzymatic Glutathione Peroxidase (GPx) and non- enzymatic parameter Vitamin C concentrations was decreased in diabetes group compared with controls, this may be due to recession caused by the diabetes or its sequelae. Our results were consistent with the study conducted by Mulholland CW et al. Studies have reported lowered antioxidant or antioxidant capacity in blood and tissues of oral precancerous lesions and malignancies [23]. This is observed even in our study.

The findings observed with respect to antioxidants and SCC could be due to

1. Increased utilization of antioxidants to scavenge Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS).

2. Poor antioxidant defence systems in cancerous environment.

3. Inadequate production of antioxidant enzymes and increased destruction of antioxidants by reactive oxygen metabolites.

Lowered capacity to defence ROS/ RNS might be one of the possible mechanisms operating in the progression of oral cancer.

However, this needs to be further evaluated with large sample size, multicentric centric study considering the population lifestyle, socioeconomic conditions, demography, diet, environment etc.

We propose clinicians to consider examination of oral cavity in all diabetic patients for lesions, diabetic status in all SCC patients, get lipid profile in all patients with oral SCC, Oxidative stress evaluation in all cases of SCC and start antioxidants at initial evaluation of the case for better patient care.

Evaluation of these parameters may help in early diagnosis and management which may help

better patient care and build nation as well in addition to health education.

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