A Comparative Study of Lipid Profile in Oral Squamous Cell Carcinoma (OSCC) Cases and Controls.

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Abstract

The purpose of this study is intended to detect and evaluate serum lipid concentrations and establish the interrelationship between the lipid profile variables for both oral and non oral cancer. This case control study included 100 clinically diagnosed and histopathologically confirmed OSCC cases and 100 age and gender matched healthy control subjects who had neither any history of cancer nor suffered from any major illness. Serum lipid concentrations such as total cholesterol, HDL cholesterol and triglycerides were determined by enzymatic colorimetric assays. LDL and VLDL cholesterol concentrations were calculated from the above findings. ANOVA was performed to compare mean, standard deviation and P- values of the parameters using SPSS 15.0 version for windows. The results have shown significantly decreased levels of serum lipid concentrations in OSCC cases when compared to normal control subjects, showing an inverse relation between serum lipid levels and Oral Squamous cell carcinomas. The detected lower concentrations of serum lipid components in the head and neck cancer cases might be due to the utilization of lipids by the cancer cells to maintain cell integrity. Plasma lipid status may be a useful bio-marker indicator for initial changes occurring in neoplastic cells.

Lipids are chief cell membrane components crucial for various biological functions including cell growth and division of normal and malignant tissues, maintenance of the structural and functional integrity of all biological membranes, activity of membrane-bound enzymes and stabilization of the DNA helix. Total cholesterol (TC), triglycerides (TG), high density lipoproteins cholesterol (HDLC), low density lipoproteins cholesterol (LDLC), and very low density lipoproteins cholesterol (VLDLC) constitute the lipid profile. Lipoproteins transport free cholesterol in the circulation, LDLC help in the transportation of cholesterol from liver to the other cells, HDLC in the transportation of cholesterol from other cells to the liver, VLDLC packs and transports the triglycerides, which are taken up and degraded by cells to perform cellular functions. Cholesterol, an amphipathic lipid is vital for cellular uptake and is distributed systematically in the domains of membranes and exists either as free cholesterol or connected with a long chain fatty acid, as cholesteryl ester. Cholesterol is synthesized in many tissues from acetyl-Co-A,

and is eliminated from the body in the bile as cholesterol or bile salts. Specialized membranes called 'lipid rafts' are maintained enriched with sphingolipids and cholesterol, which act as the platform for signal transduction. Head and Neck Squamous Cell Carcinoma (HNSCC) advances from the mucosal epithelial cells that underlines the oral cavity, larynx, pharynx, sinonasal tract and the use of tobacco products is associated with inflammation in the exposed tissues (1). Tobacco smoke has been reported to kindle H_2O_2 and hydroxyl radicals in addition to direct carcinogenic effects on the epithelial cells of the oral mucous membrane. Extensive use of areca nut and tobacco carcinogens induce the production of free radicals and reactive oxygen species (ROS), which has been implicated in pathogenesis of several diseases including cancer. Oxidative stress is the chief leading causes of toxicity attributed to the interactions of ROS and reactive nitrogen species (RNS) with cellular macromolecules like DNA, lipid, and proteins, which hamper the signal transduction pathways such as protein kinases, phosphatases, and transduction mechanisms (2). ROS enhances the rate of oxidation and per-oxidation of polyunsaturated fatty acids, thus disturbing essential constituents of cell membrane and apparently involving carcinogenesis. Animal studies have shown that nicotine, a tobacco carcinogen, affects the activity of enzymes responsible for lipid metabolism (3). Lipid metabolism in rapidly proliferating cancerous cells is based on redirecting the carbon from energy production into membrane biosynthesis. During signal transduction cascades, lipids are broken down into bioactive lipid mediators, which regulate an array of carcinogenic processes like cell growth, cell migration and metastasis formation (4).

Cancer is a leading cause of morbidity globally and a growing public health concern, with the increasing annual number of new cancer cases it is projected to amplify to 13.1 million deaths in 2030 (5). In 2015 caused over 8.7 million deaths globally, being the second leading cause of death behind cardiovascular diseases (6). The most common histological type of oral cavity cancer is Squamous cell carcinoma, which accounts for more than 90% of cases (7), with more than 450,000 new cases and 350,000 deaths annually (8-11). The distressing rates of mortality reported in HNSCC are usually owing to high incidence of loco-regional recurrence and metastatic disease (12). The incidence of HNSCC continues to mount and is estimated to enhance by 30% (1.08 million new cases annually) by 2030 (13-14), and critical needs for novel therapies to improve overall survival is indispensable (15) .Despite some modest improvements in the treatment ,the overall 5-year survival rate remained just about 40–50% chiefly due to poor accessibility of effective therapeutic options for HNSCC cases with recurrent disease (16) whereas the recurrence rates remain high (17). Cancers of the lip and oral cavity are vastly common in South Central Asia (India, Sri Lanka, and Pakistan) (18) as well as Melanesia (Papua New Guinea, with the highest incidence rate worldwide in both sexes), reflecting the popularity of betel nut chewing (19). Incidence rates are also high in Eastern and Western Europe and in Australia/ New Zealand and have been linked to alcohol consumption, tobacco smoking, HPV infection for cancers of the oropharyngeal region, and to ultraviolet radiation from sunlight exposure for lip cancer (19-22). OSCC develops in the oral cavity and oropharynx and can occur due to many etiological factors, but smoking and alcohol remain the most common risk factors especially in the Western world (23). Though treatment is complex for cases with head and neck cancers[24], its early detection can improve the prognosis significantly (25). Oral cancer is a preventable disease, where smoking and alcohol, considered major risk factors are present in 90% of cases (26), and having them both derives a synergic effect (27). There is

now sufficient understanding of the causes, and adequate information to facilitate early detection and well-timed treatment of another third of cases (28) which remains to be the key to improving survival (29).

Materials and Methods

A total of 200 individuals were included in our case control study. Out of them 100 were histopathologically confirmed and untreated OSCC cases from MNJ cancer hospital, Hyderabad and the remaining 100 were normal healthy subjects who had never suffered any major illness that alters serum lipid concentrations. Fasting blood samples were collected from the cases and controls by obtaining a written informed consent from each of them. Serum was separated and stored in small aliquots at -20º C. Total cholesterol, HDL cholesterol and triglyceride concentrations in serum were determined in mg/dl by using standard commercially available kits manufactured by Transasia Bio-Medicals Ltd, Daman (India) in collaboration with ERBA Diagnostics Mannheim GmbH, Germany.

Serum Total cholesterol concentrations in mg/dl were determined by CHOD-PAP enzymatic method, a modified Roeschlau's method in which 20 µl of serum was mixed with 1 ml of working enzyme reagent containing cholesterol esterase, cholesterol oxidase, peroxidase, sodium phenolate, 4-aminoantipyrine, phosphate buffer (pH: 6.5) and lipid clearing agent. Then the mixture was incubated at 37º C for 10 minutes and the absorbance was measured at 505 nm.

Serum HDL cholesterol concentrations in mg/dl were determined by phosphotungstic acid method in which 250 µl of serum was mixed with 500 µl of precipitating reagent that precipitates LDL and VLDL fractions of lipid cholesterol. The mixture was then allowed to incubate at room temperature for 10 minutes and then centrifuged at 4000 rpm for 10 minutes. The clear supernatant obtained was then assayed as per the serum total cholesterol estimation.

Serum triglyceride concentrations in mg/dl were determined by GPO-Trinder Method, an enzymatic method in which 10 µl of serum was mixed with 1 ml of working enzyme reagent containing lipoprotein lipase, glycerol kinase, glycerol phosphate oxidase, di-hydroxy-acetone phosphate, adenosine triphosphate, 4-aminoantipyrine, 3,5-dichloro-2-hydroxybenzene sulfonate, peroxidase and buffer (pH:7.0). The mixture was incubated at 37º C for 10 minutes and then the absorbance was measured at 505 nm. The serum VLDL and LDL cholesterol concentrations in mg/dl were calculated as per the following formula:

Serum VLDL cholesterol = Serum Triglycerides ÷ 5

Serum LDL cholesterol = Serum Total cholesterol – (HDL cholesterol + VLDL cholesterol)

Statistical analysis: Results of the study were tabulated for OSCC and control groups. All the parameters in the study were statistically analyzed for mean values, standard deviation, range and *P*- values. ANOVA was performed to compare mean, standard deviation and *P*-values of the parameters using SPSS version 15 (SPSS, Chicago, IL) for windows.

Results and Discussion:

Age range for OSCC cases was 9-87 years in males and 27-75 years in females, and in controls 21-80 years in males and 22-87 years in females. However, many of the ages mentioned in case sheets are given by cases were subjective, while exact age of 100 OSCC cases (Males 45 and Females 55) and 100 controls (Males 55 and Females 45) was available, hence analysis was carried out with those, mean age at which OSCC identified was 9-87/ 49.30±15.55 in male and 27-75/84.20±11.26 in female years .To comprehend the role of

gene mutations/ polymorphisms in onset of the disease, the cases and controls were divided into 4 categories, <25 years (1% cases and 3% controls), 26 to 45 years (27% cases and 19% controls), 46 to 65 years (60% cases and 70% controls), and above 66years (12% cases and 8% controls). Highest percentage of OSCC cases was identified between 46-65 years in both case and control group. Regarding the primary tumor site, there was a neat predominance on the BM adding up 35 cases (35.0%), followed by tongue adding up 23 cases (23.0%), then Mandible, oral cavity and RMT adding up 12, 10, & 8 cases (12%, 10% and 8%) (Table 1). In the present study Stage III showed the highest frequency (39%) when compared to Stage IV (34 %) and Stage II (21%), and further types of tumor grade like Stage I (6%) showed very low frequency when compared to other stages (table 1). Histopathologically OSCC were graded as: well differentiated Squamous cell carcinoma (WD SCC), moderately differentiated, (MD SCC) and poorly differentiated (PD SCC) based on the degree of differentiation. In the present study, the serum lipid levels in OSCC cases were found to be slightly elevated in WD SCC and MD SCC compared to PD SCC. The levels showed significant correlation with serum TC, LDL, VLDL, HDL and triglycerides among the degrees of differentiation (Table2). The gender wise levels of the various parameters of lipid profiles were found significantly lower in OSCC cases as compared with controls (table 3). ANOVA and P-values were calculated (Table 3). The P-values were found significant for serum total cholesterol, VLDL, LDL, HDL and for serum triglycerides, revealing an inverse relationship between serum lipid profile in OSCC and controls. The mean serum levels of total cholesterol was significantly (p= 0.0001) lowered in OSCC cases (148.1±22.8) compared to controls (181.45±23.67). In OSCC cases both the HDL (45.52±11.1) and LDL (84.69±17.4) were also lowered when compared to levels of HDL (47.92±10.60) and LDL (98.86±17.58) in

controls. However the decreased HDL levels were not found significant (p=0.13) when compared to controls. There was significant (0.0001) decrease in the levels of VLDL (18.0±6.0) when compared to controls (29.05±8.19) and triglycerides were found to be little higher in controls (145.53±40.73) than compared to cases (89.7±30.4) respectively.

Among one hundred OSCC cases, the number of cases who had only Chewing habit showed a high frequency of 31%, than only smokers 6% while we did not find, only alcohol habituates in cases. Analysis pattern of the cases with two habits revealed that chewing and alcohol habituate cases were more prevailing (26%) than Alcohol and Smoking (14%) and Smoking and chewing (2%), and cases who were having all the three risk factors: alcohol, smoking and chewing habits were found to be 10%. In the present study 11% were found without any habitual risk factors.

95% C.I and t stat values were observed and p values were calculated correlating the risk factors with the lipid components in OSCC cases and control subjects and marked for significance (table 5).

- We observed that only alcohol risk factor habituates were not present in our study.

- The smoking risk factor was significant in TC, VLDL and TG values (<0.0001), and insignificant in HDL (>=0.014) and LDL values (=0.001).

- Chewing risk factor was significant in all the cholesterol components TC, HDL, LDL, VLDL and TG (<0.0001).

- Smoking and alcohol combination risk factor also was relatively significant in this case study (<0.0001).

- In alcohol and chewing combination risk factor, only TG was insignificant (<0.0003).

- With the other risk factor combinations:

Samata *et al*

chewing and smoking (<0.0001), chewing, smoking and alcohol (<0.0001), all showed a statistical significance in TC, HDL, LDL, VLDL and TG values.

- No habits group also showed a statistical significance (<0.0001), in our case control study.

Majority of the cases in our study were in the age group of 46-65 years (60%), which is in accordance, and observed a similar range of 41-60 years (40%) (30). Interesting finding observed was that low serum cholesterol concentration (i.e. <5.16 mmol/L) magnifies the overall mortality from cancer and enhances prostate, colon and lung cancer among men over 60 years of age (31).

Male predominance was observed in studies (32-33) which is similar with our findings. While numerous studies suggest that low cholesterol levels are linked with various cancers, it was recorded that high total cholesterol level (≥240 mg/dL) and was positively associated with breast cancer in women and prostate, colon cancers in men, but negatively associated with risk of liver, stomach cancer in both men and women, and lung cancer in men (34).

Cases with tumors in advanced stages might have lower lipid levels due to malnutrition caused by an inadequate food intake. In accordance to our study well and moderate groups were higher than poorly differentiated (35-36), and no correlation between the T stage and lipid levels was also observed excluding the possible link between tumor stages, malnutrition and low lipid levels in cases (37). While the correlation between histological grading and serum lipid profile in Oral Cancer and pre-cancer was not noted (38), studies have established the linkage with tobacco abuse and histological grading (39).

Carcinogenesis leads to reduced level of cholesterol in proliferating tissues and blood (40), probably due to the augmented tumor cells. The new membrane biogenesis occurs due to degradations of lipoprotein fractions (41), like HDL, VLDL and LDL of cholesterol in the blood compartments (42). A significant decrease was noticed in mean serum cholesterol levels of lipoproteins in oral carcinoma and leukoplakia groups of cases (43) while both total cholesterol (TC) levels and triglyceride levels were considerably reduced in oral Squamous cell carcinoma cases as compared to the healthy controls (44). TC, LDL and TG were lowered and a significant decrease in all the lipid fractions correlates to oral malignancy (45-46) is in accordance with our study. The lower serum lipid level status may be an important indicator for initial changes occurring in neoplastic cells which might be due to utilization of cholesterol for membrane biogenesis. Hypolipidemia may occur due to the direct lipid lowering effect of tumor cells or the malfunction of the lipid metabolism (47), while cancer mortality risk with low plasma cholesterol was attributed to the effect of existing cancer disease (31). However, earlier studies observed that higher mortality due to cancer was observed in cases with high total cholesterol levels (38) although total cholesterol (mg/dL) was significantly lower in cases who were deceased (48). The crucial role of lipid classes and molecular species in supporting tumor growth and metastatic dissemination (49), and the dependence of cancer cells on aberrant lipid and cholesterol metabolism might point to these pathways as prominent targets to treat cancer as well as to sensitize them to anticancer therapies(50). Total lipids, cholesterol and HDL cholesterol levels are inversely associated with incidence of cancer whereas triglycerides levels were significantly elevated in cancer cases (51).

Lipids are major cell membrane components which are vital for various biological functions involving cell division and growth of normal and malignant cells (52) and cholesterol,

an integral part of lipid rafts plays a vital role in pathways governing carcinogenesis, drug resistance and metastasis (53). Oxidative stress increases with the increase of lipid peroxidation, causing inflammation and tissue damage in OSCC cases with compromised antioxidant defence (54). Tumor promotion is associated with perioxidant status as ROS participates in carcinogenesis (55). Carcinogens from tobacco cause liberation of free radicals and reactive oxygen radicals which cause high rate of peroxidation of unsaturated fatty acids causing increased utilization of lipids like TG, TC, lipoproteins for membrane biogenesis of cancer cells. The present study could be a preliminary study where such a correlation of lipid profile with risk factors was done.

Smoking alone:

Approximately 90% of people with oral cancer are tobacco users. A reduction in the total cholesterol, TG, LDL and HDL in all forms of tobacco users with OPC and OSCC cases was observed[56] in accordance with our study, but the HDL values were slightly increased in our study. A decreasing serum lipid status in the chronic smokers, especially the LDL can be used as an early indicator for changes in the neoplastic cells. Using tobacco is an important etiologic factor which aids in the development of oral precancerous lesions /conditions and head and neck cancer (30). HDL values were significantly lowered in the tobacco habituates when compared to the healthy controls (57), and passive smoking was reported to be a significant risk factor for decreased HDL(58). Contrarily our study suggests that smoking was an insignificant risk factor for HDL& LDL, so future study considering a larger sample could yield some better difference if at all it exists.

Areca nut chewing alone:

Chewing correlated with lipid profile was significant in our case control study. We found that all the lipid component values were significant ($p < 0.0001$) which is in accordance with the study, which reported a dose-dependent relationship between areca nut use and triglyceride(59). Contrarily current and former areca nut chewers had an elevated triglyceride level, more than normal values (1.7 mM) while a significantly lower level of HDL was observed in current areca nut chewers (60). A rise in the levels of TG, LDL and VLDL with a decrease in the HDL level was observed when the lipid profile of tobacco chewers was analyzed (61). The chief alkaloid in areca nut arecoline undergoes nitrosation and gives rise to N-Nitrosamine, which may have cytotoxic effect on the cells. These carcinogens induce generation of free radicals and reactive oxygen species, which are accountable for high rate of oxidation ∕ peroxidation of polyunsaturated fatty acids releasing peroxide radicals. In the course of areca nut chewing, ROS produced, attacks salivary proteins; alters the structure of oral mucosa, activating an inflammatory response (62). Cross-sectional studies have shown that smokeless tobacco use seems to have an adverse effect on lipid profiles, wherein smokeless tobacco users had 2.5 times the adjusted risk of hypercholesterolemia compared to nonusers (63).

Smoking and alcohol:

Studies indicate a multiplicative effect of tobacco consumption and alcohol containing more than 300 carcinogenic chemicals including polycyclic aromatic hydrocarbons with respect to frequency and duration of OSCC (64). We understand from our study that all the lipid component values were significant ($p < 0.0001$) when correlated with smoking and alcohol risk factor. This can be explained with the consideration that tobacco smoke increases the ethanol acetaldehyde in the mouth (65) and alcohol consumption promotes carcinogenic effects of tobacco smoke and they confer a synergistic risk for oral cancer (66).

Alcohol and chewing:

In our study, it was observed that the lipid components TC, HDL and LDL showed significance (< 0.0001) when correlated with risk factors alcohol and chewing, but TG and VLDL did not show any significance. The effect of alcohol along with tobacco has deleterious effects on the epithelium thereby enhancing carcinogenesis (66).

Chewing and smoking:

Tobacco chewing, a significant risk factor of oral cancer in India is due to consumption of betel quid (67), and the carcinogens present in tobacco and areca nut enhance breakage of cellular structural blocks such as lipids due to lipid per oxidation (68). In our study the lipid components TC, HDL, LDL, VLDL & TG were found to be significantly reduced in alcohol and chewing cases compared to controls, and statistically significant when correlated with the risk factors, which is partly in accordance with a study (69).

Smoking, chewing and alcohol:

Well-known risk factors for OSCC are areca nut chewing, sniffing of tobacco and alcohol consumption in various forms, which results in increased free radicals formation causing lipid peroxidation, affecting diverse cellular vital activities like growth, differentiation and gene expression (70). Our study recorded that all the lipid components (TC, HDLC, LDLC, VLDLC and TG) were statistically significant in correlation to combination of all the three risk factors - smoking, chewing and alcohol. In India, where the habits of chewing tobacco with betel nut, reverse smoking and alcohol usage are extensive, there is a striking incidence of oral cancer which accounts for as many as 30% of all cancers (71).

No Habits:

In our study, a statistical significance in correlation to lipid profile was observed in cases and controls without any habits of alcohol, chewing or smoking. Contrarily, no significance was reported in studies which considered the no habits group (33, 44).

Summary and conclusions:

Newly forming and rapidly proliferating malignant cells necessitate various crucial components such as lipids, well above the normal physiological limits leading to reduced lipid stores (72). Hence, increased de novo synthesis of fatty acids occurs due to intensive tumor growth (49) may perhaps signify overutilization of lipids during transformation from oral precancer to cancer (38). Intracellular and comprehensive cholesterol concentrations are tightly regulated by the balance between *de novo* biosynthesis, uptake, efflux, and storage, and metabolic alterations in lipid pathways supporting hypotheses that the lower cholesterol values, even before the manifestation or detection of cancer; maybe the result of carcinogenesis. Secondly, lower cholesterol values may head the development of cancer, and serve as biomarkers and might be a promising approach for cancer detection and therapeutics. Cancer cells harness lipid metabolism to generate components for biological membranes, and signaling molecules required for proliferation, survival, invasion and metastasis. Dysregulation in lipid metabolism is among the main important metabolic alterations in cancer. Taking into consideration the above facts, the importance of serum lipid levels as a diagnostic marker of oral cancer, should be noted. This study was designed to evaluate the changes in serum lipid levels in OSCC cases and healthy volunteers, in correlation to risk factors which might give an insight into the significant prognostic indicators in diagnosis of oral cancer. Changes to lipid organization that result in cancer initiation and progression contribute to the understanding of carcinogenesis and identification of potential therapeutic targets.

Comparative study of lipid profile in oral squamous cell carcinoma

Table 1 Clinical Characteristics of the OSCC Cases & Healthy controls

Clinical Characteristics	n = 100 (Cases)	n = 100 (Controls)
Gender Males Females Mean age & Range Males Mean age & Range Females	45(45%) 55 (55%) 50.53/9-87 55.27/30-75	55(55%) 45 (55%)
Age Distribution		
26-45	28 (28%)	22(22%)
46-65	60 (60%)	70(70%)
66 and above	12 (12%)	8 (8%)
Habitual Risk Alcoholics	-	3 (3%)
Smokers	6(6%)	13 (13%)
Tobacco chewing	31(31%)	30 (30%)
Alcohol + Smoking	14 (14%)	6 (6%)
Alcohol + Tobacco chewing	26 (26%)	13(13%)
Smoking + Tobacco chewing	2 (2%)	4 (4%)
Alcohol + Smoking + Tobacco chewing No Habits	10(10%) 11(11%)	6 (6%) 25 (25%)
Site of Diagnosis Tongue	23(23%)	
Buccal mucosa (BM)	35 (35%)	
Mandible	12 (12%)	
Oral Cavity	10 (9%)	
Retromolartrigone	8(8%)	
Floor of mouth	4(4%)	
Lip	3(3%)	
Base of tongue	2 (2%)	
Maxilla	2 (2%)	
Palate	1 (1%)	
Staging Stage 1	6 (6%)	
Stage 2	21 (21%)	
Stage 3	39 (39%)	
Stage 4	34(34%)	

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437

Table 2 Lipid profiles according to gender wise distribution

S.No	MEAN ±SD (Patients)			F Statistics	P-value	MEAN ±SD (Control subjects)			F Statistics	P-value
	Males (N=45)	Females (N=55)	Total (N=100)			Males (N=55)	Females (N=45)	Total (100)		
TC	146.2±20.5	149.6±24.6	148.1±22.8	.560	0.0001	178.29±22.04	185.31±25.23	181.45±23.67	1.3	0.34
HDL	42.5±10.1	47.9±11.3	45.52±11.1	6.181	0.0001	47.83±10.66	48.02±10.64	47.92±10.60	1.0	0.9
VLDL	17.5±6.2	18.3±6.0	18±6.0	.478	0.0001	28.41±7.58	29.82±8.9	29.05±8.19	1.37	0.2
LDL	86.1±18.1	83.4±16.9	84.69±17.4	.592	0.0001	96.69±16.57	101±18.58	98.86±17.58	1.2	0.4
TG	87.6±30.7	91.3±30.3	89.7±30.4	.371	0.0001	142.21±37.53	149.57±44.4	145.53±40.73	1.39	0.23

SD= Standard deviation; n=number of cases; F-statistics=ANOVAs.

Table 3 Lipid profiles according to tumor degree of differentiation.

S.No	WD SCC (N=64)	MD SCC (N=31)	PD SCC (N=5)	F-Statistics	P-value (Paired Samples Test)
TC	148.1±21.6	148.5±25.17	137.0±24.8	0.621	0.0001
HDL	45.5±10.8	44.9±10.5	46.8±19.0	0.089	0.0001
VLDL	18±6.1	17.9±6.1	15.8±4.9	0.353	0.0001
LDL	84.69±17.0	85.7±18.8	74.4±13.3	0.93	0.0001
TG	89.7±31.0	89.6±30.3	78.6±25.1	0.357	0.0001

WD= Well differentiated; MD= Moderately differentiated; PD= Poorly differentiated

Comparative study of lipid profile in oral squamous cell carcinoma

Table 4: Correlation of risk factors of OSCC with Lipid Profile

Habits in cases	TC MEAN ±SD	HDL MEAN ±SD	VLDL MEAN ±SD	LDL MEAN ±SD	TG MEAN ±SD
Smoking	150.33 ± 20.50	46.00 ± 10.88	19.33 ± 6.44	85.00 ± 22.32	97.00 ± 31.46
Alcohol	No cases	No cases	No cases	No cases	No cases
Chewing	140.66 ± 16.67	40.19 ± 10.40	17.44 ± 6.09	83.03 ± 10.80	87.38 ± 30.49
Smok + Alch	140.43 ± 18.05	39.71 ± 7.64	17.29 ± 5.78	85.57 ± 12.16	86.29 ± 29.01
Alch + Chew	145.15 ± 26.31	45.38 ± 13.12	17.93 ± 7.53	82.88 ± 22.76	89.54 ± 37.53
Chew + Smok	133.5 ± 54.45	41.5 ± 28.99	14.5 ± 2.12	77.5 ± 27.58	72.5 ± 12.02
All three habits	137.0 ± 20.57	43.7 ± 7.53	17.4 ± 4.25	75.9 ± 14.04	86.7 ± 20.91
No habits	148.2 ± 27.30	43.5 ± 11.87	18.8 ± 6.43	85.9 ± 20.75	94.4 ± 31.31
Habits in control	**TC MEAN ±SD**	**HDL MEAN ±SD**	**VLDL MEAN ±SD**	**LDL MEAN ±SD**	**TG MEAN ±SD**
Smoking	172.38 ± 19.31	43.85 ± 11.22	32.15 ± 8.64	93.23 ± 11.60	161 ± 43.41
Alcohol	165.67 ± 23.80	39 ± 10.58	35.67 ± 4.16	91 ± 18.36	178.67 ± 20.53
Chewing	181 ± 24.20	51 ± 10.92	36 ± 9.57	94 ± 16.65	180 ± 47.44
Smok + Alch	227 ± 19.50	71 ± 9.38	33 ± 2.66	96 ± 12.90	166 ± 13.30
Alch + Chew	210 ± 20.79	59 ± 9.92	22 ± 6.63	109 ± 15.85	108 ± 32.98
Chew + Smok	210 ± 21.51	54 ± 6.61	29 ± 2.65	127 ± 22.02	143 ± 12.37
All three habits	195 ± 23.45	53 ± 8.26	28 ± 5.25	114 ± 20.49	140 ± 26.04
No habits	130 ± 26.59	36 ± 10.69	25 ± 9.00	69 ± 20.31	124 ± 44.70

Samata *et al*

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439

Table 5: Statistical correlation for significance of risk factors with lipid profile:

LIPIDS		Smoking MEAN ±SD	Alcohol MEAN ±SD	Chewing MEAN ±SD	Smok + Alch MEAN ±SD	Alch + Chew MEAN ±SD	Chew + Smok MEAN ±SD	Chew+smok+alch MEAN ±SD	No habits MEAN ±SD
TC	Case	150.33 ± 20.50	No cases	140.66 ± 16.67	140.43 ± 18.05	145.15 ± 26.31	133.5 ± 54.45	137.0 ± 20.57	148.2 ± 27.30
	Control	172.38 ± 19.31	165.67 ± 23.80	181 ± 24.20	227 ± 19.50	210 ± 20.79	210 ± 21.51	195 ± 23.45	130 ± 26.59
	95% CI	16.49-27.60		34.54-46.13	62.73-110.40	58.23-71.46	64.95-88.04	51.84-64.15	-25.71—-10.68
	t-Stat	7.83		13.72	7.16	19.33	13.06	18.59	-4.77
	p-value	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HDL	Case	46.00 ± 10.88	No cases	40.19 ± 10.40	39.71 ± 7.64	45.38 ± 13.12	41.5 ± 28.99	43.7 ± 7.53	43.5 ± 11.87
	Control	43.85 ± 11.22	39 ± 10.58	51 ± 10.92	71 ± 9.38	59 ± 9.92	54 ± 6.61	53 ± 8.26	36 ± 10.69
	95% CI	0.76-6.93		7.83-13.78	28.90-33.67	10.37-16.86	6.63-18.36	7.09-11.50	-10.65—-4.34
	t-Stat	2.46		7.16	25.86	8.28	4.2	8.3	-4.69
	p-value	>=0.014		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
VLDL	Case	19.33 ± 6.44	No cases	17.44 ± 6.09	17.29 ± 5.78	17.93 ± 7.53	14.5 ± 2.12	17.4 ± 4.25	18.8 ± 6.43
	Control	32.15 ± 8.64	35.67 ± 4.16	36 ± 9.57	33 ± 2.66	22 ± 6.63	29 ± 2.65	28 ± 5.25	25 ± 9.00
	95% CI	10.69-14.94		16.32-20.79	14.45-16.96	2.09-6.04	13.83-15.16	9.26-11.93	4.01-8.38
	t-Stat	11.87		16.36	24.69	4.05	42.72	15.69	5.6
	p-value	<0.0001		<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
LDL	Case	85.00 ± 22.32	No cases	83.03 ± 10.80	85.57 ± 12.16	82.88 ± 22.76	77.5 ± 27.58	75.9 ± 14.04	85.9 ± 20.75
	Control	93.23 ± 11.60	91 ± 18.36	94 ± 16.65	96 ± 12.90	109 ± 15.85	127 ± 22.02	114 ± 20.49	69 ± 20.31
	95% CI	3.26-13.19		7.05-14.88	6.93-13.92	20.65-31.58	42.54-56.45	33.20-42.99	-22.62—-11.17
	t-Stat	3.27		5.52	5.88	9.41	14.02	15.33	-5.8
	p-value	0.001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
TG	Case	97.00 ± 31.46	No cases	87.38 ± 30.49	86.29 ± 29.01	89.54 ± 37.53	72.5 ± 12.02	86.7 ± 20.91	94.4 ± 31.31
	Control	161 ± 43.41	178.67 ± 20.53	180 ± 47.44	166 ± 13.30	108 ± 32.98	143 ± 12.37	140 ± 26.04	124 ± 44.70
	95% CI	53.42-74.57		81.49-103.74	73.41-86	8.6-28.31	67.09-73.90	46.71-59.88	18.83-40.36
	t-Stat	11.93		16.42	24.97	3.69	40.87	15.96	5.42
	p-value	<0.0001		<0.0001	<0.0001	0.0003	<0.0001	<0.0001	<0.0001

Conflicts of interest:

All authors declare no conflict of interest.

Comparative study of lipid profile in oral squamous cell carcinoma

References:

1. Johnson DE, Burtness B, Leemans CR, Lui VW, Bauman JE, Grandis JR, Head and neck squamous cell carcinoma. *Nature Reviews Disease Primers*, 6 (2020) 1.

2. Aggarwal V, Tuli H, Varol A, Thakral F, Yerer M, Sak K, , Varol M , Jain A, Khan MA & Sethi G, Role of reactive oxygen species in cancer progression: Molecular mechanisms and recent advancements. *Biomolecules*, 9(2019)735.

3. Ashakumary L & Vijayammal PL, Effect of nicotine on lipoprotein metabolism in rats. *Lipids*, 32 (1997) 311.

4. Kunkel GT, Maceyka M, Milstien S & Spiegel S, Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond. *Nature Reviews Drug Discovery*, 12 (2013) 688.

5. Wong MCS, Fung FDH, Leung C, Cheung WWL, Goggins WB & Ng CF, The global epidemiology of bladder cancer: a joinpoint regression analysis of its incidence and mortality trends and projection. *Sci Rep* ,8 (2018) 1129.

6. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet,* 388(2016)1459

7. Thompson LDR, World Health Organization classification of tumours: Pathology and genetics of head and neck tumours. *Ear, Nose & Throat Journal*, 85 (2006) 74.

8. de Abreu PM, Có ACG, Azevedo PL, do Valle IB, de Oliveira KG, Gouvea SA, Cordeiro-Silva MF, Louro ID, de Podestá JRV, Lenzi J, Sena A, Mendonça EF& von Zeidler SLV, Frequency of HPV in oral cavity squamous cell carcinoma. *Cancer*, 18(2018)324.

9. Noone, A. M., Cronin, K. A., Altekruse, S. F., Howlader N, Lewis D R, Petkov V I & Penberthy L, Cancer Incidence and Survival Trends by Subtype Using Data from the Surveillance Epidemiology and End Results Program, 1992-2013. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* , 26(2017)632.

10. Jemal A, Simard EP, Dorell C, Noone AM, Markowitz LE, Kohler B, Eheman C, Saraiya M, Bandi P, Saslow D, Cronin KA, Watson M, Schiffman M, Henley SJ, Schymura MJ, Anderson RN, Yankey D &Edwards BK, Annual Report to the Nation on the Status of Cancer, 1975-2009, featuring the burden and trends in human papillomavirus(HPV)-associated cancers and HPV vaccination coverage levels. *J Natl Cancer Inst,* 105(2013)175.

11. Brouwer AF, Eisenberg MC & Meza R, Age effects and temporal trends in HPV-related and HPV-unrelated oral cancer in the United States: A multistage carcinogenesis modeling analysis. *PLOS ONE*, 11 (2016) e0151098.

12. Braakhuis BJM, Brakenhoff RH & René Leemans C, Treatment choice for locally advanced head and neck cancers on the basis of risk factors: Biological risk factors. *Annals of Oncology*, 23 (2012) 173.

13. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A & Bray F, Estimating the global cancer incidence and mortality in 2018: Globocan sources and methods. Int J Cancer, 144(2019) 1941.

14. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA & Jemal A, Global cancer

Current Trends in Biotechnology and Pharmacy
Vol. 16 (4) 429 - 444, Oct 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.4.77

441

statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 68 (2018) 394.

15. Muzaffar J, Bari S, Kirtane K & Chung C.H, Recent Advances and Future Directions in Clinical Management of Head and Neck Squamous Cell Carcinoma. *Cancers*, 13 (2021) 338.

16. Canning Madison, Guo Gang, Yu Miao, Myint Calvin, Groves Michael W, Byrd James Kenneth & Cui Yan, Heterogeneity of the Head and Neck Squamous Cell Carcinoma Immune Landscape and Its Impact on Immunotherapy. *Frontiers in Cell and Developmental Biology*, 7 (2019) 52.

17. Mehanna H, Kong A & Ahmed SK, Recurrent head and neck cancer: United Kingdom National Multidisciplinary Guidelines. *The Journal of Laryngology & Otology*, 130 (2016)181.

18. Ferlay J, Ervik M, Lam F, Colombet M, Mery L & Piñeros M, Global Cancer Observatory: Cancer Tomorrow. Lyon, France:International Agency for Research on Cancer, 2020.

19. Gupta S, Gupta R, Sinha DN & Mehrotra R, Relationship between type of Smokeless Tobacco & Risk of cancer: A systematic review. *Indian Journal of Medical Research*, 148 (2018) 56.

20. Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, Scotti L, Jenab M, Turati F, Pasquali E, Pelucchi C, Galeone C, Bellocco R, Negri E, Corrao G, Boffetta P, & La Vecchia C, Alcohol consumption and site-specific cancer risk: A comprehensive dose–response meta-analysis. *British Journal of Cancer*, 112(2014)580.

21. Han AY, Kuan EC, Mallen-St Clair J, Alonso JE, Arshi A & St John MA, Epidemiology of squamous cell carcinoma of the lip in the United States. *JAMA Otolaryngology–Head & Neck Surgery*, 142 (2016) 1216.

22. Rigel DS, Cutaneous ultraviolet exposure and its relationship to the development of skin cancer. *Journal of the American Academy of Dermatology*, 58 (2008)129.

23. Tandon P, Dadhich A, Saluja H, Bawane S & Sachdeva S, The prevalence of squamous cell carcinoma in different sites of oral cavity at our rural health care centre in Loni, Maharashtra – A retrospective 10-year study. *Współczesna Onkologia*, 2 (2017) 178.

24. Adelstein D, Gillison ML, Pfister DG, Spencer S, Adkins D, Brizel DM, Burtness B, Busse PM, Caudell JJ, Cmelak AJ, Colevas AD, Eisele DW, Fenton M, Foote RL, Gilbert J, Haddad RI, Hicks WL, Hitchcock YJ, Jimeno A, Leizman D, Lydiatt WM, Maghami E, Mell LK, Mittal BB, Pinto HA, Ridge JA, Rocco J, Rodriguez CP, Shah JP, Weber RS, Witek M, Worden F, Yom SS, Zhen W, Burns JL, Darlow SD, NCCN guidelines insights: Head and neck cancers. *J Natl Compr Canc Netw*, 15(2017)761.

25. Sujir N, Ahmed J, Pai K, Denny C & Shenoy N, Challenges in early diagnosis of oral cancer: Cases series. *Acta Stomatologica Croatica*, 53 (2019) 174.

26. Dissanayaka WL, Pitiyage G, Kumarasiri PV, Liyanage RL, Dias KD & Tilakaratne WM, Clinical and histopathologic parameters in survival of oral squamous cell carcinoma. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology*, 113(2012) 518.

27. Koontongkaew S, The tumor microenvironment contribution to development, growth, invasion and metastasis of head

Current Trends in Biotechnology and Pharmacy
Vol. 16 (4) 429 - 444, Oct 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.4.77

442

and neck squamous cell carcinomas. *Journal of Cancer*, 4 (2013) 66.

28. Petersen PE, Oral cancer prevention and control – the approach of the World Health Organization. *Oral Oncology*, 45 (2009) 454.

29. Mehrotra R & Gupta DK, Exciting new advances in oral cancer diagnosis: Avenues to early detection. *Head & Neck Oncology*, 3 (2011)1.

30. Poorey VK & Thakur P, Alteration of lipid profile in patients with head and neck malignancy. *Indian Journal of Otolaryngology and Head & Neck Surgery*, 68 (2015) 135.

31. Eichholzer M, Stähelin HB, Gutzwiller F, Lüdin E & Bernasconi F, Association of low plasma cholesterol with mortality for cancer at various sites in men: 17-y follow-up of the prospective basel study. *The American Journal of Clinical Nutrition*, 71 (2000) 569.

32. Mehrotra R, Pandya S, Chaudhary A K, Singh H P, Jaiswal R K, Singh M, Gupta S C, & Singh M, Lipid profile in oral submucous fibrosis. *Lipids in Health and Disease*, 8 (2009) 29.

33. Singh S, Ramesh V, Premalatha B, Prashad KV & Ramadoss K, Alterations in serum lipid profile patterns in oral cancer. *Journal of Natural Science, Biology and Medicine*, 4 (2013) 374.

34. Kitahara CM, Berrington de González A, Freedman ND, Huxley R, Mok Y, Jee SH & Samet J. M, Total cholesterol and cancer risk in a large prospective study in Korea. *Journal of Clinical Oncology*. 29 (2011) 1592.

35. Chawda JG, Jain SS, Patel HR, Chaduvula N & Patel K, The relationship between serum lipid levels and the risk of oral cancer. *Indian Journal of Medical and Paediatric Oncology*, 32 (2011) 34.

36. Samata Gadde, Sudhakar Poda, Suryanarayana Veeravilli & Lakshmi Addala, Lack of the brafv600e mutation in oral squamous cell carcinoma. *Journal of Medical Science And Clinical Research*, 04 (2016) 14912.

37. Wilms T, Boldrup L, Gu X, Coates PJ, Sgaramella N & Nylander K, High levels of low-density lipoproteins correlate with improved survival in patients with squamous cell carcinoma of the head and neck. *Biomedicines*, 9 (2021) 506.

38. Lohe VK, Degwekar SS, Bhowate RR, Kadu RP & Dangore SB, Evaluation of correlation of serum lipid profile in patients with oral cancer and precancer and its association with tobacco abuse. *Journal of Oral Pathology & Medicine*, 39 (2010) 141.

39. Kumar P, Augustine J, Urs AB, Arora S, Gupta S & Mohanty VR, Serum lipid profile in oral cancer and leukoplakia: Correlation with tobacco abuse and histological grading. *Journal of Cancer Research and Therapeutics*, 8 (2012) 384.

40. Nydegger Urse & Bütler Rene, Quantitative lipoprotein and Lipid determinations in patients with various malignancies. *Protides of the Biological Fluids*, (1972) 311.

41. Patel PS, Shah MH, Jha FP, Raval GN, Rawal RM, Patel MM, Patel JB & Patel DD, Alterations in plasma lipid profile patterns in head and neck cancer and oral precancerous conditions. *Indian J Cancer*, 41 (2004) 25.

42. Mehta R, Gurudath S, Dayansoor S, Pai A & Ganapathy K S, Serum lipid profile in patients with oral cancer and oral precancerous conditions. *Dental research journal*, 11 (2014) 345.

43. Sachdev R, Garg K, Singh G & Mehro-

tra V, A comparative study to assess the independency of lipid profile and blood sugar levels as a diagnostic marker in oral cancer and precancerous disorders. *Indian Journal of Dental Sciences*, 12 (2020) 187.

44. Ghosh G, Jayaram KM, Patil RV & Malik S, Alterations in serum lipid profile patterns in oral squamous cell carcinoma patients. *The Journal of Contemporary Dental Practice*, 12 (2011) 451.

45. Ray G & Husain SA, Role of lipids, lipoproteins and vitamins in women with breast cancer. *Clinical Biochemistry*, 34 (2001) 71.

46. Sherubin EJ, Kannan KS, Kumar DN & Joseph I, Estimation of plasma lipids and its significance on histopathological grades in oral cancer: Prognostic significance an original research. *Journal of Oral and Maxillofacial Pathology*, 17 (2013) 4.

47. Rajendran R & Sivapada Sundaram B; Benign and malignant tumors of the oral cavity. *Shafer's Textbook of Oral Pathology*, (Elsevier New Delhi), 2009, 101.

48. Hohneck AL, Rosenkaimer S, Hofheinz R-D, Akin I, Borggrefe M & Gerhards S, Blood cholesterol and outcome of patients with cancer under regular cardiological surveillance. *Current Oncology*, 28 (2021) 863.

49. Guo C-bin, Cui L-hui, Yu G-yan, Liu D-xin, Meng S-cong & Song Q, Endogenous fatty acid synthesis in squamous cell carcinomas of the oral cavity. *British Journal of Oral and Maxillofacial Surgery*, 38 (2000) 506.

50. Germain N, Dhayer M, Boileau M, Fovez Q, Kluza J & Marchetti P, Lipid metabolism and resistance to anticancer treatment. *Biology*, 9 (2020) 474.

51. Naik PP, Ghadge MS & Raste AS, Lipid profile in leukemia and hodgkin's disease. *Indian Journal of Clinical Biochemistry*, 21 (2006) 100.

52. Jahanshahi G & Sabaghian M, Comparative immunohistochemical analysis of angiogenesis and mast cell density in oral normal mucosa and squamous cell carcinoma. *Dental Research Journal*, 9 (2012) 8.

53. Sharma B & Agnihotri N, Role of cholesterol homeostasis and its efflux pathways in cancer progression. *The Journal of Steroid Biochemistry and Molecular Biology*, 191 (2019) 105377.

54. Beevi SS, Rasheed AM & Geetha A, Evaluation of oxidative stress and nitric oxide levels in patients with oral cavity cancer. *Japanese Journal of Clinical Oncology*, 34 (2004) 379.

55. Cerutti PA, Prooxidant states and tumor promotion. *Science*, 227 (1985) 375.

56. Singh M, Singh AB, Singh A & Agarwal SP, Correlation of plasma lipid levels and histopathological grading in patients with oral squamous cell carcinoma and its pre-cancerous lesions. *International Journal of Otorhinolaryngology and Head & Neck Surgery*, 4 (2018) 1436.

57. Kritchevsky SB, Wilcosky TC, Morris DL, Truong KN & Tyroler HA, Changes in plasma lipid and lipoprotein cholesterol and weight prior to the diagnosis of cancer. *Cancer Research*, 51 (1991) 3198.

58. Neufeld EJ, Mietus-Snyder M, Beiser AS, Baker AL & Newburger JW, Passive cigarette smoking and reduced HDL cholesterol levels in children with high-risk lipid profiles. *Circulation*, 96 (1997) 1403.

59. Guh J-Y, Chuang L-Y & Chen H-C, Betel-quid use is associated with the risk of the metabolic syndrome in adults. *The American Journal of Clinical Nutrition*, 83

(2006) 1313.

60. Lee B-J, Chan M-Y, Hsiao H-Y, Chang C-H, Hsu L-P & Lin P-T, Relationship of oxidative stress, inflammation, and the risk of metabolic syndrome in patients with oral cancer. *Oxidative Medicine and Cellular Longevity*, (2018).

61. Rao S & Subash EY, The effect of Chronic Tobacco Smoking and Chewing on the Lipid Profile. *J Clin Diagn Res*, 7 (2013) 31.

62. Jeng JH, Ho YS, Chan CP, Wang YJ, Hahn LJ, Lei D, Hsu CC &Chang MC, Areca nut extract up-regulates prostaglandin production, cyclooxygenase-2 mrna and protein expression of human oral keratinocytes. *Carcinogenesis*, 21 (2000) 1365.

63. Tucker LA, Use of smokeless tobacco, cigarette smoking, and hypercholesterolemia. *American Journal of Public Health*, 79 (1989) 1048.

64. Parkin DM, Bray F, Ferlay J & Pisani P. Global cancer statistics, 2002. *CA: A Cancer Journal for Clinicians*, 55 (2005) 74.

65. Salaspuro V & Salaspuro M, Synergistic effect of alcohol drinking and smoking onin vivo acetaldehyde concentration in saliva. *International Journal of Cancer*, 111 (2004) 480.

66. Castellsagué X, Quintana MJ, Martínez MC, Nieto A, Sánchez MJ, Juan A, Monner A, Carrera M, Agudo A, Quer M, Muñoz N, Herrero R, Franceschi S & Bosch FX, The role of type of tobacco and type of alcoholic beverage in oral carcinogenesis. *International Journal of Cancer*, 108 (2004) 741.

67. Proia NK, Smoking and smokeless tobacco-associated human buccal cell mutations and their association with Oral Cancer--A Review. *Cancer Epidemiology Biomarkers & Prevention*, 15 (2006) 1061.

68. Subbulakshmi AC, Mohan N, Thiruneervannan R & Naveen S, Comparative evaluation of serum lipid profile in patients with oral submucous fibrosis and oral squamous cell carcinoma with that of control subjects: A case control study. *Journal of Pharmacy and Bioallied Sciences*, 9 (2017) 191

69. Thabusum D, Reddy R, Ramesh T & Rajesh N, Lipid profile as a marker of pre-stage cancer and oral cancer in tobacco users. *International Blood Research & Reviews*, 3 (2014) 26.

70. Gurudath S, Ganapathy KS, D. S, Pai A, Ballal S & Asha ML, Estimation of superoxide dismutase and glutathione peroxidase in oral submucous fibrosis, oral leukoplakia and oral cancer - A comparative study. *Asian Pacific Journal of Cancer Prevention*, 13 (2012) 4409.

71. Patil PB, Bathi R & Chaudhari S, Prevalence of oral mucosal lesions in dental patients with tobacco smoking, chewing, and mixed habits: A cross-sectional study in South India. *J Family Community Med*, 20 (2013) 130.

72. Neerupakam M, Sathish S, Polisetty N, Alaparthi RK, Katta SA & Damera S, Alterations in plasma lipid profile patterns in oral cancer. *Journal of Indian Academy of Oral Medicine and Radiology*, 26 (2014) 274.