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Original Article

Risk factors, lipid profile, and histopathological study of oral cancers in Kolar district: A case-control study

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ABSTRACT

Aims: To estimate serum lipid profile in oral squamous cell carcinoma and correlate the risk factors and lipid profile with oral squamous cell carcinoma.

Materials and Methods: Lipid profile was done in agriculturists/laborers in the age group of 30-70 years; 56 subjects (cases = 28, control = 28) were included. Study was carried out for a duration of four months; statistical analyses applied were mean, standard deviation, and independent 't' test. $P < 0.05$ was considered statistically significant.

Results: Eleven cases had buccal mucosa cancer, nine had tongue carcinoma, and eight had gingivobuccal sulcus carcinoma. Lipid profile such as total cholesterol, triglycerides, LDL cholesterol, non-high-density lipoprotein (non-HDL) cholesterol, and very-low-density lipoprotein (VLDL) were marginally and slightly elevated in cases compared to controls. HDL was grossly decreased in cases compared to controls.

Conclusions: There was a significant association between HDL and squamous cell carcinoma; maximum number of SCC had a history of smoking in the range of 10-19 years, irrespective of other lipid parameters, constrained to the fact that lipids are genetically determined, have geographical variation, and are highly skewed.

KEY WORDS: Histopathology, lipid profile, oral squamous cell carcinoma, reduced HDLT

INTRODUCTION

Enhanced medical healthcare has increased the longevity of individuals; consequently, non-communicable diseases such as coronary heart disease and cancer are on the rise. In India, among all cancers, oral squamous cell carcinomas are the most common cancer in males and the third most common cancer in females.[1] Estimated incidence of oral squamous cell carcinoma is 12.48 cases in males per 1,00,000 population and 5.52 cases in females per 1,00,000 population.[2] In our reference population, cancer of oral cavity constitutes 29.6% of all cancers.[3]

In India, tobacco is consumed in the form of betel quid, tobacco with lime, tobacco powder, smoking, and reverse smoking (keeping the lit end in the mouth). These habits of tobacco consumption are an essential causative factor in the development of oral precancerous diseases and squamous cell carcinomas.[4-6] It is hypothesized that tobacco carcinogens induce the generation of free radicals and reactive oxygen species (ROS), which are responsible for high rate of oxidation and peroxidation, which, in turn, release peroxide radicals. These radicals are generated in bursts and constitute the chief form of damage brought to the genetic material resulting in non-lethal mutations, eventually leading to frank malignant lesions.[7]

Polyunsaturated Fatty Acids (PUFA) are the major class of biomolecules that are susceptible to ROS-induced oxidative damage, and the process is known as lipid peroxidation.[8] Due to this lipid peroxidation, there is an increased utilization of lipids including total cholesterol, lipoproteins, and triglycerides for new membrane biogenesis. These requirements of the cells are met either from circulation, by synthesis through metabolism, or from degradation of major lipoprotein fractions such as very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).[9-11] Genetic mutations in cancer cells alter the receptor-initiated signal pathways and lead to the constitutive active uptake and metabolism of nutrients, including lipids that promote cancer and their growth. Lipids have multiple key roles in cells. These alterations in circulating lipoproteins have been found to be associated with breast cancer and colorectal cancer.[12] However, there are few reports of plasma lipid profile alterations in head and neck cancers in South Indian population.

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Even though biopsy is considered to be the gold standard in the diagnosis of oral cancers, it has its own limitations as it is painful, has higher chances of bleeding as carcinomas are highly vascular, and biopsy may sometimes be superficial warranting a repeated biopsy.

The nature of effect of cancer on lipid levels need to be further explored. The idea of lipid profile test for screening and follow-up of patients with oral cancers is appealing due to the simplicity of the test and its cost-effectiveness. This prompted us to take up this study to analyze and review the risk factors, lipid profile, and histopathological variants in oral cancer patients confirmed by histopathology.

AIMS AND OBJECTIVES

- To estimate serum lipid profile in different histological grades of oral malignancies
- To correlate risk factors and lipid profile with different histological grades of oral cancers.

MATERIALS AND METHODS

This is a case-control study comprising men and women who are agriculturists/laborers in the age group of 30-70 years. We included 56 subjects (cases = 28, controls = 28). The study duration was four months (May–August 2012). The study group was selected from inpatients and outpatients presenting to the Otorhinolaryngology department of our hospital.

Inclusion criteria

All cases of oral malignancies confirmed on histopathology admitted and attending the Otorhinolaryngology outpatient department of our hospital.

Exclusion criteria

Factors and diseases that are known to alter the serum lipid levels, patients on hypolipidemic drugs, obese, hypothyroid, diabetic individuals, and those suffering from ischemic heart disease were excluded.

Controls

- Age- and gender-matched healthy volunteers with no history of oral precancerous lesions were taken as controls
- The controls were selected after considering the inclusion and exclusion criteria
- Those in the control group were screened for the same parameters, as done for the cases.

Ethical clearance was obtained from our institutional ethical committee before the start of the study.

After obtaining informed consent from both the control and case groups, 4 ml of venous blood was collected under aseptic precautions into red-topped plain vacutainers for lipid profile estimation. Blood was collected after an overnight fasting of minimum of 8 hours (Overnight fasting sample ensured that no alteration of lipid profile occurred because of diet). Proper aseptic venepuncture procedure using adequate pressure to transfer blood into vacutainers prevented hemolysis of sample). Sample was then centrifuged at 3,000 rpm for 2 minutes to separate the serum.

Lipid profile was done *Using Vitros* on a 250 dry chemistry autoanalyzer from Johnson and Johnson, which works on the principle of "reflectance photometry".

Sample was analyzed for the following parameters:

- Total Cholesterol by Cholesterol Oxidase-Peroxidase method[13]
- Triglycerides by Glycerol Peroxidase method[14]
- HDL-Cholesterol by Precipitation method[15]
- Calculation of LDL by Friedewald's Formula[16]
- Calculation of VLDL[16]
- Calculation of Non-HDL Cholesterol.[17]

The samples were analyzed in batches, routine Bio-Rad internal quality control samples were run, and quality was assured throughout.

Biopsy was taken from the oral cavity in patients with suspicion of cancers under aseptic precautions. Tissue was then fixed in 10% formalin, processed routinely, embedded, and cut. These cuts sections were then stained with Hematoxylin and Eosin (H and E) stain. The stained sections were then categorized into well-differentiated, moderately differentiated, and poorly differentiated based on the degree of differentiation of malignant cells.

Statistical analysis

The values of parameters collected were tabulated. Mean and standard deviation was obtained, and independent 't' test was performed to compare the mean values of the parameters. $P < 0.05$ was considered to be statistically significant.

RESULTS

Table 1 depicts the clinical characteristics of the patients suffering from oral cancers. The sites of tumor in the oral cavity are as follows: out of 28 cases, buccal mucosa cancer was noted in $n = 11$ (39%), tongue carcinoma in $n = 9$ (32%), and gingivobuccal sulcus carcinoma in $n = 8$ (29%). With regard to the patterns of tobacco consumption in the subjects [Table 2], we observed chewing of tobacco in $n = 20$ cases (71%); smoking alone was not observed in our study, but $n = 8$ (29%) of the cases were both smokers and tobacco chewers. When these data were compared with controls, $n = 1$ (4%) were smokers, $n = 4$ (29%) were tobacco chewers, and $n = 2$ (14%) were both tobacco chewers and smokers. Non-tobacco consumers were $n = 21$ (75%). The occurrence of oral cancers in smokers was most commonly observed in the frequency distribution of 10-19 yrs ($n = 5$) and >20yrs (three cases). We could not find any cases in the frequency distribution of 5-9 years. A similar finding was also observed with regard to tobacco

chewing. The maximum number of cases ($n = 15$) with oral cancers had a history of tobacco chewing for 10-19 years, and $n = 13$ had a history of tobacco chewing for 5-9 years.

The lipid profile when compared between cases and controls shows that total cholesterol, triglycerides, LDL cholesterol, non-HDL cholesterol, and VLDL were marginally and slightly elevated in cases compared to controls, which was statistically not significant. However, we observed HDL to be grossly decreased in cases compared to controls, with a significant 'p' value [Table 3]. Irrespective of the mode of tobacco consumption, all 28 cases had squamous cell carcinoma. Histological grading showed $n = 21$ (75%) to be suffering from well-differentiated squamous cell carcinoma [Figure 1] and $n = 7$ (25%) with poorly differentiated squamous cell carcinoma [Figure 2].

DISCUSSION

Cancer of oral cavity is highly challenging and an unresolved problem for the human population as on date, particularly with regard to Indian population. Oral cancer constitutes about 3-4% of all cancers in western industrialized countries, mainly affecting the middle-aged and elderly population, and is more common in males than females.[18]

Studies have reported that, in India, the incidence of oral cancer accounts for as many as 30-40% of all cancers.[19,20] Over-production of lipid peroxidation products and depleted

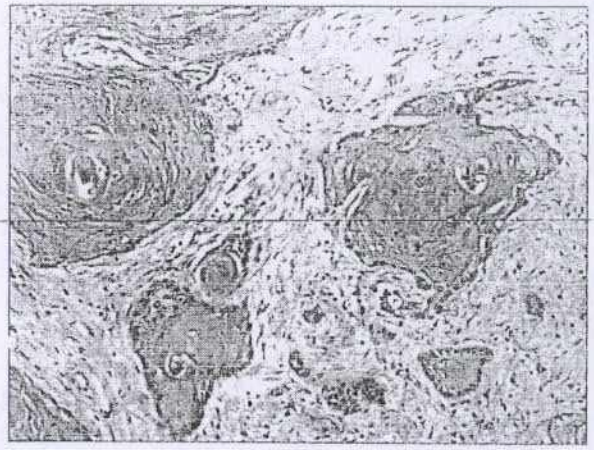

Figure 1: Section studied shows features of well-differentiated squamous cell carcinoma. (Hematoxylin and Eosin stain, ×100)

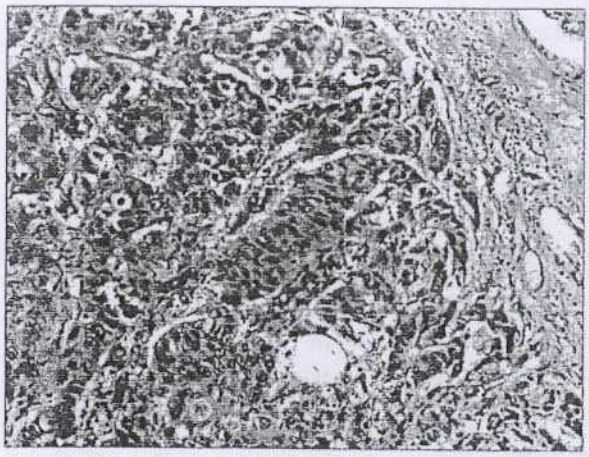

Figure 2: Section studied shows features of poorly differentiated squamous cell carcinoma. (Hematoxylin and Eosin stain, ×100)

Table 1: Characteristics of patients suffering from oral cancers (n=28)

Median age of patients in years (range)	58.5 (30-70)
Gender	
Male	14 (50%)
Female	14 (50%)
Site of tumor in oral cavity	
Buccal mucosa	11 (39%)
Tongue	09 (32%)
Gingivobuccal sulcus	08 (29%)
Histopathological examination	
Squamous cell carcinoma	28
Others	-
Histopathological grade	
Well-differentiated squamous cell carcinoma	21 (75%)
Poorly differentiated squamous cell carcinoma	07 (25%)

Table 2: Pattern of tobacco consumption in subjects

	Cases (n=28)	Controls with history of tobacco consumption (n=07) Without history of tobacco consumption (n=21)
Type of tobacco consumption		
Tobacco chewing	20 (71%)	04 (29%)
Smoking	-	01 (4%)
Both tobacco chewing and smoking	08 (29%)	02 (14%)
None	-	21 (75%)
Smoking duration		
5-9 yrs	-	-
10-19 yrs	05	02
>20 yrs	03	01
Chewing duration		
5-9 yrs	13	-
10-19 yrs	15	05
>20 yrs	-	01

Table 3: Comparison of lipid profile between cases and controls

Parameters (mg/dl)	Mean+SD		't' value	'P' value
	Cases (n=28)	Controls (n=28)		
Total cholesterol	178+28.7	168.7+36.4	1.068	0.29
Triglycerides	146.29+77.6	138+57.49	0.45	0.654
HDL	33.64+5.87	39.4+5.58	-3.64	<0.05*
LDL	115.11+15.14	102+37.08	1.722	0.091
Non-HDL cholesterol	144.64+29.58	129.5+37.28	1.684	0.098
VLDL	29.32+15.5	28.14+11.224	0.326	0.746

*Statistically significant, LDL = Low-density lipoprotein, HDL = High-density lipoprotein, VLDL = Very-low-density lipoprotein

antioxidants have an important role in development of cancer.[18] Lipid peroxidation and antioxidant defense mechanism may relate to preventive and curative chemotherapeutic efficacy and management.[18]

Exposure to tobacco in any form is a well-established risk factor for head and neck squamous cell carcinoma (HNSCC).[21,22] Substantial evidence show that tobacco consumption increases the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) either directly or through activation of inflammatory cascade.[23,24] It has been reported that when antioxidants are produced excessively or during deficient antioxidant defense, it can alter protein structure/function, initiate lipid peroxidation, and cause oxidative DNA damage.[25] ROS and ROS-mediated lipid peroxidation have been demonstrated as putative mediators in oral cancer development.[26]

Association of lipid with coronary artery disease is a well-established fact; however, studies defining its association with cancers or oral precancerous condition (OPC) is scarce, and it has been documented that patients with OPC show a significant tendency to develop cancer.[27] It is believed that tobacco carcinogens induce generation of free radicals and ROS, which are responsible for the high rate of oxidation and/or peroxidation of polyunsaturated fatty acids (PUFA). This peroxidation further releases peroxide radicals. This affects the essential constituents of the cell membrane and might be involved in carcinogenesis/tumorigenesis.[28] Because of lipid peroxidation, there is a greater utilization of lipids including total cholesterol (TC), lipoproteins, and triglycerides (TG) as new membrane biogenesis of cells meet these requirements either from circulation or by synthesis through the metabolism or degradation of major lipoproteins fractions such as VLDL, LDL, or HDL. Studies conducted by Choi *et al.* have shown that antioxidant vitamins have protective effects against lipid peroxidation.[9] It has been documented that in case of various cancers, blood lipids are on lower limit of normal[27] Some investigators have also observed positive correlation between low serum cholesterol with increased risk of cancer occurrence and mortality.[29-32]

The altered serum or plasma lipid levels are not due to dietary intake or utilization and biosynthesis because it is continuously recycled in and out of blood stream. The question whether hypolipidemia in cancer is a cause or consequence remains unanswered despite considerable advancements in the cancer diagnostics approach. These factors made us curious to evaluate the plasma lipid profile such as total cholesterol (TC), LDL cholesterol (LDLc), HDL cholesterol (HDLc), non-HDLc, VLDL cholesterol, and triglycerides in HNSCC patients, patients with OPC, and healthy individuals. The association between altered plasma lipid profile and habit and forms of tobacco consumption, correlation of plasma lipids with staging, and type of oral cancers were concentrated in our study.

Compared to previous studies, the median age of cases in this study was in the range of 30-70 years. The significance observed in this selected population was that no difference was noted in the incidence of oral cancers between males and females. It is well documented that tobacco consumption in any form over a period of 10-19 years may lead to increased incidence of oral cancers. This was found to be true in our study as well. Although several retrospective and prospective studies have shown that lipid levels decline in oral squamous cell carcinomas, to our surprise, even with proper, standard sample protocol for sample analysis, we observed a mild increase in serum lipid levels in cases compared to controls; however, this was not statistically significant. Nevertheless, our study is on par with the study by Alexopoulos *et al.*, who observed a similar trend.[33]

In our study, HDLc was significantly decreased in cases as compared to controls. This finding may be due to lipid peroxidation, which is an essential biochemical process involving the oxidation of PUFA, an important component of cell membranes. Tobacco carcinogens generate ROS and lipid peroxides, leading to tissue injury due to elevated lipid peroxidation, further damaging the cellular structural blocks such as lipids, proteins, and DNA. Thus, lipid peroxidation may play a role in endogenous formation of exocyclic DNA adducts and low HDLc in cancer patients. Marginally elevated lipid profile, particularly TC, TG, LDLc, non-HDLc, and VLDL, in cases compared to controls may be attributed to the exposure to tobacco carcinogens, which hampers the antioxidants' defense, leading to accelerated lipid peroxidation. Most cancers on histopathological examination had squamous cell carcinoma and the histopathological grade showed 75% with well-differentiated squamous cell carcinoma and the remaining 25% with poorly differentiated squamous cell carcinoma.

In conclusion, we could derive with the facts that in our population, there was significant association between HDL and squamous cell carcinoma; maximum number of SCC had a history of smoking in the range of 10-19 years, irrespective of other lipid parameters, constrained to the fact that lipids are genetically determined, have geographical variation, and are highly skewed. Moreover, the antioxidants vitamins and other antioxidants parameters might have led to marginal elevation of lipids other than HDLc. However, to what extent their dietary habits, nutritional status, and other physical activities led to these changes in lipids needs to be documented.

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