INTRODUCTION

Malignant neoplasms are major causes of fear, morbidity, and mortality all over the world. Oral cancer is one of the most mutilating diseases afflicting mankind[1] Globally, oral cancer is the sixth most common cause of cancer related death. In south East Asia, more than 100,000 new cases are reported every year. Based on cancer registry data in India, 75,000 to 80,000 new cases of oral cancers are reported annually and it ranks number one among men and number three among women. Tobacco in the form of quid and smoking has been a main contributing cause for oral cancer.[2] The biological activity of oral Squamous Cell Carcinoma (SCC) is evaluated and descriptively categorized as highly, moderately, and poorly differentiated. This system was primarily developed by Broders.[3]

In recent years, emphasis has been placed on detecting molecular markers from body fluids such as saliva, urine, and others, for detecting, predicting prognosis, and monitoring the oral cancer progression. The idea of screening and following patients with malignancy by blood-based tests is appealing from several points of view including its ease, economic advantage, non-invasiveness, and possibility of repeated sampling. Lipoproteins are clusters of proteins and lipids, all tangled up together to carry lipids in our blood.[4]

During the last three decades, interest in association between the serum cholesterol and ischemic heart diseases has generated
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numerous studies. Analyzing these studies, researchers observed an interesting inverse relationship between serum cholesterol levels and the occurrence of cancer. Based on this, several cohort studies were carried out on this aspect and as a result, various authors reported a significant inverse relationship between serum cholesterol and various cancers such as carcinoma pharynx, carcinoma lung, and leukemia. Some investigators have even found an increased incidence of mortality as the serum cholesterol level declined.

Lipids are major cell membrane components essential for various biological functions including cell growth and division of both normal and malignant cells. The increased utilization of lipids by the highly proliferating malignant cells for new membrane biogenesis results in the depletion of total cholesterol (TC) levels. However, observations on plasma lipid levels in oral cancer are few.

It is also believed that tobacco carcinogens induce generation of free radicals and reactive oxygen species which is responsible for high rate of oxidation or peroxidation of poly-unsaturated fatty acids. This peroxidation further releases peroxide radicals. This affects the essential constituents of the cell membrane and might be involved in carcinogenesis. Apart from this tobacco-induced lipid peroxidation, there is also greater utilization of lipids by the malignant cells for its new membrane biogenesis. Cells fulfill these requirements either from circulation or synthesis through metabolism from degradation of major lipoprotein fractions such as low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), or high-density lipoprotein (HDL) resulting in the depletion of plasma lipid levels.

Considering these curiosities, we aimed to estimate the plasma lipid levels (TC, LDL, HDL, VLDL, and triglycerides [TG]) in patients with oral cancer and correlated the values with the histopathological grades of the lesion.

MATERIALS AND METHODS

Population studied

- The study included 50 Indian patients with squamous cell carcinoma of oral cavity with habit of using tobacco, betel quid, and alcohol, who had visited the Department of Oral Medicine and Radiology during the period of September 2005 to July 2007.
- The selected study population included 28 females and 22 males.
- The age of the patients ranged from 20 to 60 years.

Methodology

A formal ethical clearance was obtained from the Ethical Committee of the institution. The patients were explained in detail about the study and the procedure they were subjected to. A formal informed written consent was obtained. A comprehensive history was obtained with special reference to their habits, its nature, duration, and frequency of use which was followed by a thorough clinical examination. The patients with oral cancer were grouped clinically according to Tumour Node Metastasis (TNM) staging given by American Joint Committee on Cancer. After establishing the clinical diagnosis, the patients were subjected to incisional biopsy. The biopsy specimens were histopathologically examined and graded according to Broder’s classification. Following the histopathological confirmation (squamous cell carcinoma) of the lesion, 3 ml of blood sample was obtained from the median cubital vein for lipid analysis. The obtained blood sample was transferred into sterile ethylenediaminetetraacetic acid coated vacutettes and was sent to laboratory. The plasma lipids were estimated in a fully automated Siemens centaur auto analyzer using cholesterol kits obtained from biosystems. The procedure is as follows.

Lipids analysis

The samples collected from the subjects were centrifuged and the collected plasma was stored at 80°C until analyzed.

- TC levels were estimated using cholesterol kits. Five micro liters of plasma sample was mixed with 500 μl of working reagent that contained cholesterol oxidase, cholesterol esterase, peroxidase, 4-amino phenazone, surfactant, phenol, buffer, preservatives, and stabilizer. The mixture was then incubated at 37°C for 10 min and absorbance was read at 505 nm.
- Plasma HDL cholesterol levels were estimated using cholesterol kits. 0.3 ml of precipitating reagent was mixed with 200 μl of plasma and the mixture was incubated for 10 min in room temperature. The incubated mixture was centrifuged at 2000 rpm for 15 min. The supernatant obtained was mixed with 10 μl of working cholesterol reagent. The mixture was incubated at 37°C for 10 min and absorbance was read at 505 nm.
- The TG were estimated using gold kits. Ten micro liters of sample was mixed with 1,000 μl of TG assay agent containing pipes buffer, lipase, 4-chlorophenol magnesium ion, ATP, lipase, peroxidase, glycerol kinase, sodium azide, 4 amino antipyrene, glycero 3 phosphate oxidase, and detergents. The mixture is incubated at 37°C for 10 min and absorbance was read at 505 nm.
- Very low-density lipoprotein cholesterol (VLDLC) and low-density lipoprotein cholesterol (LDLC) were calculated using the formula given below:

\[ \text{VLDLC} = \frac{\text{TG}}{5} \]

\[ \text{LDLC} = \text{TC} - \text{VLDLC} - \text{LDLC} \]

Exclusion criteria

- Patients with underlying systemic diseases such as diabetes, hypertension, anemia, jaundice, liver or kidney
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disorders or other systemic diseases, and malignancies elsewhere in the body.

- Patients on drugs that alter the lipid levels.

RESULTS

The observations are summarized

The study included 50 patients with oral cancer. Among that, 56% \((n = 28)\) of the patients were females and 44% \((n = 22)\) were males [Figure 1].

Figure 2 shows the age distribution of the study population. Among 50 study patients, 6% \((n = 3)\) of the patients were in the age group of 21-30 years, 28.0% \((n = 14)\) in the age group of 31-40 years, 34% \((n = 17)\) in the age group of 41-50 years, 20% \((n = 10)\) in the age group of 51 to 60 years, and 12% \((n = 6)\) in the age group of above 60 years. The mean age of patients was 47.30 years. Maximum numbers of patients were in the age group of 41-50 years.

In the study, we also observed that 88% \((n = 44)\) were using tobacco in the form of quid and 12% \((n = 6)\) in the form of smoking.

The involvement of site varied among the patients with 86% \((n = 43)\) of the patients showing involvement of buccal mucosa, followed by tongue in 6% \((n = 3)\), floor of mouth in 4.0% \((n = 2)\), alveolus in 2.0% \((n = 1)\), and soft palate in 2.0% \((n = 1)\).

The observation of the histopathological examination of the 50 oral cancer patients [Figure 3] reveals that 44.0% \((n = 22)\) of the patients showing well differentiation, 44.0% \((n = 22)\) showing moderate differentiation, and 12.0% \((n = 6)\) of the patients showing poor differentiation.

Comparing the lipid levels of 50 oral cancer patients with the standard reference values, we observed the mean TC level as 125.48 mg/dl (normal = 150‑230 mg/dl), the mean HDL level as 25.76 mg/dl normal = 35 mg/dl), the mean LDL level as 82.62 mg/dl (normal = 150 mg/dl), the mean VLDL level as 15.33 mg/dl (normal = 15‑40 mg/dl), and the mean TG level as 76.34 mg/dl (normal = 150 mg/dl).

The abnormality in TC level (>200 mg/dl) was observed in 4.0% \((n = 2)\) of the patients, whereas the abnormality in HDL level (<35 mg/dl) was observed in 94.0% \((n = 47)\) of the patients and the abnormality in LDL level (>150 mg/dl) was observed in 2.0% \((n = 1)\) of the patients. No significant abnormality was found in triglyceride levels [Figure 4].
We correlated the lipid levels with its histopathological differentiation [Table 1]. The mean TC in patients with well-differentiated lesion is 132.95 mg/dl, moderately differentiated lesion is 121.00 mg/dl, and poorly differentiated lesion is 114.50 mg/dl. The mean HDL in patients with well-differentiated lesion is 24.41 mg/dl, moderately differentiated lesion is 27.27 mg/dl, and poorly differentiated lesion is 25.17 mg/dl. The mean LDL in patients with well-differentiated lesion is 95.41 mg/dl, moderately differentiated lesion is 70.77 mg/dl, and poorly differentiated lesion is 79.17 mg/dl. The mean VLDL in patients with well-differentiated lesion is 15.51 mg/dl, and poorly differentiated lesion is 17.27 mg/dl. The mean triglyceride in patients with well-differentiated lesion is 73.09 mg/dl, moderately differentiated lesion is 77.54 mg/dl, and poorly differentiated lesion is 83.83 mg/dl.

**DISCUSSION**

Globally, oral cancer constitutes one of the most prevalent cancers with a very high incidence in the developing countries. Significant geographic variation is noted in the incidence of oral cancer with highest rates reported in the Indian sub-continent and parts of Asia. In India, oral cancer is the second most common cancer. The majority of oral cancers are squamous cell cancers. Other malignant diseases such as tumors of salivary glands, thyroid gland, lymph nodes and bone and soft tissues do occur in the head and neck region; however, these are much less common.  

In industrialized countries, oral cancer affects men two to three times more often than woman largely because of their greater indulgence in the most important risk factors such as heavy alcohol and tobacco consumption. However, in high incidence areas such as India, where chewing and sometimes smoking are also common among women, the incidence of carcinoma tongue and other intraoral sites for woman can be greater than or equal to that of men although varies considerably from region to region. This holds true in our study where among the study population of 50, 56% (n = 28) of the patients were predominantly females. This similar observation of female predominance was also reported by Landish and Murray in their study.

The incidence of oral cancer increases with age. In the west, 95% of patients are above 40 years of age. However, in many cases, patients are less than 35 years old owing to heavy abuse of various forms of tobacco. Furthermore, it is now clear that in many western countries, there has been alarming rise in the incidence of oral cancer during the past two or three decades particularly among young men. This is reflected in rising mortality rates among young men. In this study, we observed the peak age incidence of patients to be 47.30 years which is in accordance with the observations reported by Mashberg and Silverman. This age-related incidence suggests that time-dependent factors result in the initiation and promotion of genetic events that result in malignant change and the diminished immune surveillance seen in the older age group.

Although the etiology of oral cancer is certainly multifactorial, tobacco chiefly used for smoking in the form of cigarettes, cigars, and pipe or in the form of quid and alcohol are acknowledged risk factors for oral cancer. Moreover, more than a single factor is needed to produce such a malignancy. In the study, the chief etiological factor which we observed was tobacco with 88% of the patients using in the form of quid.

Oral cancer may occur at any intraoral site. However, the most common site of involvement we observed in our study was buccal mucosa, 86% which is in accordance with the results reported by Nag who also observed this site to be the most common site of involvement. The predominant occurrence of oral cancer in buccal mucosa is probably due to the constant contact of the quid in the buccal mucosa where the carcinogens in the quid act as contact carcinogens. The nicotine contributes to the carcinogenic potential of tobacco serving as a precursor to many tobacco-specific nitrosamines such as nitrosomornicotine and 4-methyl nitrosamino-1-(3-pyridyl-butanoine) which have been proved for their carcinogenicity in experimental animals.

In this study, we histopathologically examined the lesions and observed 44% (n = 22) of the lesions were well differentiated, 44% (n = 22) were moderately differentiated, and 12% (n = 6) were poorly differentiated. This observation coincides with the findings of authors Fang and Kao who also observed fewer incidences of poorly differentiated lesions (8%) in their study conducted on 150 primary oral squamous cell carcinomas.

**Table 1: Plasma lipid levels in oral cancer patients according to the differentiation results are presented in mean±SD**

| Lipid parameters (mg/dl) | Normal values (mg/dl) | Type of differentiation | P value |
|--------------------------|-----------------------|-------------------------|--------|
| TC                       | 150-230               | 132.95±43.03            | 121.00±43.56 | 114.50±24.92 | 0.508 |
| HDL                      | 35                    | 24.41±6.72              | 27.27±22.34 | 25.17±6.01 | 0.836 |
| LDL                      | 150                   | 95.41±38.36             | 70.77±41.38 | 79.17±16.09 | 0.111 |
| VLDL                     | 15-40                 | 14.62±3.01              | 15.51±3.24 | 17.27±2.88 | 0.178 |
| TG                       | 150                   | 73.09±15.01             | 77.54±16.19 | 83.83±15.87 | 0.302 |

TC: Total cholesterol, VLDL: Very low density lipoprotein, LDL: Low density lipoprotein, HDL: High density lipoprotein, TG: Triglycerides
Analyzing the plasma lipid levels in these patients, we observed a significant decrease in the level of all lipid parameters including TC, HDL, LDL, VLDL, and TG. This finding is not in coincidence with the reports of Patel PS and Shah MH who observed only a decrease of TC and HDL in their study conducted on 121 oral cancer patients. This decrease in the level of plasma lipids can be explained by the fact that 75% of the plasma cholesterol is transported in the form of LDL. Body cells sequester cholesterol from LDL fraction of lipoproteins. LDL receptors are necessary for metabolizing circulatory LDL and nearly 80% of the plasma LDL is cleared by LDL receptors. LDL receptor activity is several folds higher in rapidly proliferating malignant cells than non-dividing cells. This high activity of LDL receptors in proliferating tissues attributes for the lowered plasma LDL and TC levels due to their increased utilization for new membrane biogenesis.

In this study, we also compared the plasma lipid levels of these patients with the histopathological grades of the lesion. We observed a progressive declination of TC levels with the lowest levels seen in poorly differentiated lesions. Krontiras and Roye state that this differential utilization of plasma lipids by the malignant cells can be explained by the fact that the enzyme fatty acid synthase which is necessary for the synthesis of fatty acid increases as the differentiation of the cell decreases. The enzyme fatty acid synthase and its activity are highly elevated in biosynthetically altered anaplastic cells of poorly differentiated lesions as compared to moderate- and well-differentiated lesions. However, in contrary to this statement, we observed the lowest level of VLDL, HDL, and TG in well differentiated lesions and the lowest level of LDL in moderately differentiated lesions. Therefore, the observations of this study do not support the hypotheses put forward by these authors.

CONCLUSION

In this study, we evaluated the relationship between the plasma lipid levels and oral cancer and we studied its significance on the histopathological grades. The incidence of oral cancer was higher in females. The peak incidence of age was fourth decade. Tobacco was the prime etiological factor observed with majority of the patients using tobacco in the form of quid. The most common site of involvement was buccal mucosa. Analyzing the lipid levels in these patients, we observed an inverse relationship between the plasma lipid levels and oral cancer. Finally, correlating the lipid levels with the histopathological grading of the lesion, we observed a differential utilization of lipids by different grades of the lesions. In a study conducted on a group of cancer patients, Rose et al., observed 66% higher mortality rate in patients with lowest plasma cholesterol than in the highest plasma cholesterol. Our results add to this evidence of an inverse relationship between lower plasma lipid profile and oral cancer. Hence, the result of our study strongly warrants an in-depth research on this aspect with larger samples and a longer follow-up to consider the low plasma lipid status in oral cancer patients as a useful indicator to assess the course and prognosis of the disease.

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