Alteration in Serum Lipid Profile in Oral Squamous Cell Carcinoma

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Abstract

Background: Alteration in the pattern of serum lipid profile has been associated with a variety of cancers and precancerous conditions. Low levels of serum lipid serve as a prognostic marker in the early detection of oral precancerous and cancerous conditions because lipid plays an important role in new membrane biogenesis and maintains cell integrity.

Aim: The aim of our study is to evaluate the alteration in serum lipid profile in oral squamous cell carcinoma (OSCC) and compared it with control group.

Materials and Methods: A total of 80 subjects were selected from the Department of Oral Pathology and Microbiology, RUHS College of Dental Sciences, Jaipur (GDC-Jaipur). Among 80 subjects, 40 individuals were diagnosed with squamous cell carcinoma and other 40 individuals were taken in healthy control group selected randomly from other departments. The total parameters assessed include total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), very LDLc, and triglycerides (TGLs). Statistical analysis was carried out by Chi-square and one-way ANOVA test to evaluate parameters.

Results: There was a significant decrease in TC, HDLc, and TGL in the oral cancer group as compared with the control group.

Conclusions: There was an inverse relationship between serum lipid profile and OSCC. The lower serum lipid status may be considered a useful prognostic biochemical indicator for initial changes occurring in the neoplastic proliferating cell.

Key words: Control group, Histologically diagnosed case of oral cancer, Serum lipid profile

INTRODUCTION

Oral cancer (OC) is the sixth most common cancer worldwide. Malignant neoplasms are major causes of fear, morbidity, and mortality all over the world. OC is one of the most mutilating diseases afflicting the humankind.[3] In Southeast Asia, more than 100,000 new cases are reported every year. The main contributing cause of OC in India is the form of quid and smoking. It is most commonly preceded by clinically definable premalignant lesions and conditions. Around 0.3–25% of leukoplakia and 7–12% of oral submucous fibrosis cases will undergo malignant transformation.[3]

In cancer, the newly proliferating cells would need many basic components well above the normal limits, used in physiological process. One such component is lipids which form major cell membrane components essential for various biological functions including cell division and growth of normal and malignant tissues. The increased requirement of lipids to fulfill the need of these new cells would be expected to diminish the existing lipid stores.[3]

Altered lipid levels have been consistently associated with coronary heart disease and their relation to different cancers such as breast and colorectal has also been documented[4] However, the reports on altered lipid levels in OC and pre-cancer are few and conflicting. With the above in mind, the present study was conducted to evaluate the implications of altered serum lipid profile in patients with OC. Hypolipidemia can be considered as one of the biochemical markers in early detection of cancer.[6]
Early detection of these lesions can dramatically improve the treatment outcome and prognosis in such patients. Thus, the development of newer diagnostic and predictive approaches that are safe, economical, and amenable to repeated sampling is imperative. Blood-based/serum-based tests offer the aforementioned advantages.

**MATERIALS AND METHODS**

**Sources of Data**

The present study was done in the Department of Oral Pathology and Microbiology, RUHS College of Dental Sciences, Jaipur. Forty patients with clinically and histopathologically proven oral squamous cell carcinoma (OSCC) were taken, the age group between 31 and 70 years. The study subjects comprised two groups as follows:

1. Group 1: OSCC
2. Group 2: Control group

Group 1 comprised 40 patients of OSCC in the age group of 20–70 years.

**Inclusion Criteria**

Patients clinically and histopathologically diagnosed with OSCC were included in the study.

**Exclusion Criteria**

The following criteria were excluded from the study:

- Patients with underlying systemic disease such as diabetes, hypertension, anemia, jaundice liver or kidney disorders, or other systemic diseases
- Patients on drugs that alter the lipid level

Group 2 (control group) comprised 40 healthy subjects in the same age group, sex matched with those of the OSCC group and with no deleterious oral habits and no associated oral lesions.

**Materials**

a. 5 ml of blood sample of each individual
b. Disposable syringe and needle
c. Plain vial
d. Centrifuged machine (Remi-C)
e. Lipid profile reagent kit (coral clinical systemic)
f. Micropipette and holder
g. Semiautomatic biochemistry analyzer (Stat Fax-3000)

**Methods**

The selected patients were explained in detail about the study and the procedure they were subjected. A formal informed written consent was taken. Systemically and
detailed oral cavity examination of the patients were done. Histopathological examination was carried out in all the cases following incisional/excisional biopsy from the affected area of the oral cavity.

Under aseptic condition, in a selected individual with overnight fasting state, 5-ml of venous blood obtained using sterile disposable syringe and collected in a plain vial. Then, serum was separated by centrifugation in centrifuge machine at 3000 rpm for 10–15 min then use lipid profile reagent kit and analyzed for lipid profile.

**Procedure**
The serum was separated by centrifugation. The serum lipid profile was estimated using kits coral clinical system. Lipid analysis was done on a semiautomatic chemical analyzer (Stat Fax-3000) based on the spectrophotometric principle. By using an ultra violet - visible spectrophotometer, the serum lipid profile in the form of total cholesterol (TC), triglycerides (TGL) and high-density lipoprotein (HDL). Very low-density lipoprotein (VLDL) and LDLLDL were calculated using the formula given below.

- VLDL = TG/5
- LDL = TC−VLDL−LDL

Considering these curiosities, we aimed to estimate the serum lipid levels (TC, LDL, HDL, VLDL, and TGLs) in patients with OC and correlated the values with the control group patients. During the procedure, reagents preparation and stability like temperature maintained 37°C (room temperature) before analyzing the samples.

**TC and TGLs**
The serum cholesterol was estimated by taking three separate test tubes with, respectively, 10 µl of distilled water, 10 µl of sample, and 10 µl of cholesterol standard. 1 ml of cholesterol reagents were added to all three test tubes. The mixtures were well mixed and incubated at 37°C for 10 min. Measured the absorbance of the standard and test sample against the blank at 505 nm in the analyzer within 60 min.

**HDL**
Serum HDL cholesterol (HDLC) two working reagents: Reagent-1 – 450 µl and reagent-2 – 150 µl are ready to use. Calibrator was prepared by adding distilled water 10 µl at room temperature (37°C). R-1 reagents were taken 450 µl in three test tubes with added 10 µl sample and calibrator, mixed well, and incubated at room temperature for 5–10 min. After added reagent-2, 150 µl mixed well and again incubated at room temperature for 5–10 min and read the absorbance of the calibrator and sample again blank at 578 nm wavelength in the analyzer.

**RESULTS**

**Statistical Analyses**
Statistical analyses were done using computer software (SPSS trial version 23 and primer). The qualitative data were expressed in proportion and percentages, and the quantitative data expressed as mean and standard deviations (SDs). The difference in proportion was analyzed using Chi-square test and the difference in means among the groups was analyzed using the Student’s t-test for parametric data significance level for tests which were determined as 95% (P < 0.05).

**Table 1: Distribution of the OSCC cases according to age group**

<table>
<thead>
<tr>
<th>Age groups</th>
<th>OSCC case</th>
<th>Control</th>
<th>P-value LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40</td>
<td>14 (35)</td>
<td>18 (45)</td>
<td>0.65 NS</td>
</tr>
<tr>
<td>40–60</td>
<td>20 (50)</td>
<td>17 (42.5)</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>6 (15)</td>
<td>5 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40 (100)</td>
<td>40 (100)</td>
<td></td>
</tr>
</tbody>
</table>

OSCC: Oral squamous cell carcinoma, SD: Standard deviation, NS: Non-significant, LS: Least squares

**Table 2: Distribution of the OSCC cases according to gender**

<table>
<thead>
<tr>
<th>Gender</th>
<th>OSCC case</th>
<th>Control</th>
<th>P-value LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>9 (22.5)</td>
<td>10 (25)</td>
<td>1.0 NS</td>
</tr>
<tr>
<td>Male</td>
<td>31 (77.5)</td>
<td>30 (75)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40 (100)</td>
<td>40 (100)</td>
<td></td>
</tr>
</tbody>
</table>

OSCC: Oral squamous cell carcinoma, NS: Non-significant, LS: Least squares

**Graph 1: Mean age among the oral squamous cell carcinoma cases**

**Graph 2: Distribution of the oral squamous cell carcinoma cases according to gender**
The Observations are Summarized

A total of 80 subjects were taken for the study including two groups as follows:

Among 40 subjects included in the present study Group 1, 31 (77.5%) were male and 9 (22.5%) were female, with an age range of 31–70 years. The OC and control groups were comparing.

Table 1 and Graph 1 shows the age distribution of the study population of oral cancer. Among 40 subjects, 17.5% (n = 7) of the patients were in the age group of <40 years, 65% (n = 26) in the age group of 40–60 years, and 17.5% (n = 7) in the age group of >60 years. The mean age of oral cancer patient was 45.70 years. Maximum numbers of oral cancer patients were in the age group of 41–60 years.

Among 40 subjects included in the present study, 31 (77.5%) were male and 9 (22.5%) were female, with an age range of 31–70 years. The OC and control groups were comparing. Distribution of cases according to gender groups is given in Table 2 and Graph 2.

Serum Lipid Profile in the OC and Control Group

The mean serum lipid profile values of the OC and control groups are represented in Table 3. P = 0.01 suggesting that, statistically, there was a highly significant reduction of mean serum TC, HDL, and TGs in the subjects of OC as compared with the control group. The mean serum values of LDL and VLDL were reduced in the OC group as compared with the control group, but this reduction was not statistically significant.

In Table 3 and Graph 3, comparing the lipid levels of 40 OC patients with the standard reference values, we observed the mean ± SD of TC level as 186.34 ± 12.31 mg/dl (normal = 150–230 mg/dl), the mean ± SD HDL level as 41.95 ± 10.42 mg/dl (normal = 40–60 mg/dl), and the mean ± SD TG level as 110.30 ± 11.98 mg/dl (normal = 150 mg/dl). The mean LDL level as 82.62 mg/dl (normal = 150 mg/dl), the mean VLDL level as 28.83 ± 5.86 mg/dl.

The mean serum TC, HDL, and TG levels showed statistically significant reduction in the OC group as compared with the control group, whereas LDL and VLDL did not show a statistically significant reduction.
the study, we also observed that 88% \((n = 32)\) were using tobacco in the form of gutkha and quid and 12% \((n = 8)\) in the form of without habit of tobacco.

Table 4 and Graph 4 showed the distribution of OSCC cases according to TC (mg/dl) level. The abnormality in TC level (<200 mg/dl) was observed with mean value of 186.34 mg/dl and SD ± 12.51, which showed decrease in TC level in diagnosed case of OSCC. In the control group, the mean value of TC was 203.24 mg/dl with SD ± 10.51. Statistically significant was found in TC level in OSCC diagnosed cases compared to the control group. Significant \(P\) value of total cholesterol < 0.001 and standard normal range of total cholesterol followed 150–230 mg/dl.

Table 5 and Graph 5 showed the distribution of the OSCC cases according to TGL (mg/dl) level. The abnormality in TGL level (>150 mg/dl) was observed with mean value of 110.3 mg/dl and SD ± 11.98 which showed decrease the TGL level in diagnosed case of OSCC. In the control group, the mean value of TGL was 143.16 mg/dl with SD ± 11.94. Statistically significant was found in TGLs level in OSCC diagnosed cases compared to the control group. Significant \(P\) value of triglycerides < 0.001, and standard normal range of triglycerides followed >150 mg/dl.

Table 6 and Graph 6 showed the distribution of the OSCC cases according to HDL (mg/dl) level. The abnormality in HDL level (40–60 mg/dl) was observed with mean value of 41.95 mg/dl and SD ± 10.42 mg/dl. Which showed decrease the HDL level in diagnosed case of OSCC. In the control group, the mean value of HDL was 50.67 mg/dl with SD ± 9.54. Significant \(P\) value of HDL < 0.001, and standard normal range of HDL followed between 40–60 mg/dl [Graph 7].

**DISCUSSION**

Cholesterol and TGLs are important lipid constituents of the cell and are essential to carry out several vital physiological functions. Cholesterol is essential for the maintenance of the structural and functional integrity of all biological membranes. It is also involved in the activity of membrane-bound enzymes and is important for stabilization of the deoxyribonucleic acid (DNA) helix. Cellular uptake and regulation of cholesterol are mediated by lipoprotein receptors, especially located on the surface of the cells. For transport in plasma, TGL and cholesterol are packaged into lipoproteins, which are then taken up and degraded by cells to fulfill the demands for cellular function.\(^7\)

In some malignant diseases, blood cholesterol undergoes early and significant changes. Low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the ongoing process of oncogenesis. The question arises whether hypolipidemia is a predisposing factor or result of cancer. However, earlier studies have reported that hypolipidemia may result due to the direct lipid-lowering effect of tumor cells or some secondary malfunction of the lipid metabolism or secondary to antioxidant vitamins.\(^8,9\)

In our study, we observed the peak age incidence of patients mean age to be 45.7 years with SD ± 12.89, which is in accordance with the observations reported by Sharma et al. (2018). 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.

Cholesterol is an essential constituent of lipoprotein fractions such as HDL, LDL, and VLDL. About 75% of
the plasma cholesterol is transported in the form of LDL. Body cells sequester cholesterol from the LDL fraction of lipoproteins. LDL receptors are necessary for metabolizing circulating LDL levels and nearly 80% of the plasma LDL is cleared by LDL receptors. High activity of LDL receptors attributes for lowering the serum cholesterol levels. Raste and Naik evaluated lipid profile in patients with carcinoma of breast, cervix, esophagus, colon, stomach, and leukemia and concluded that serum total lipids, cholesterol, and HDLC levels were significantly inversely associated with incidence of cancer, whereas TGL levels significantly elevated in cancer patients. Possible Hypotheses for Hypolipidemia in Cancer and Pre-cancer

- Newly forming and rapidly proliferating malignant cells need many basic components such as lipids well above the normal physiological limits, leading to diminished lipid stores.
- Tobacco induces generation of free radicals and reactive oxygen species responsible for high rate of oxidation/peroxidation of polyunsaturated fatty acids, in turn, leading to increased utilization of lipids.
- Lower cholesterol levels before the detection of carcinoma may be due to underlying carcinoma process.
- Association of hypolipidemia with cancer may be secondary to other factors.
- May be due to increased membrane permeability to carcinogens induced by transfatty acids.
- May be due to antioxidant vitamin therapy.
- Lipid peroxidation may play an important role in cancer development as lipid peroxidation product, malondialdehyde, may cross-link DNA on the same and opposite strands through adenine and cytosine. This may in theory contribute to carcinogenicity and mutagenicity in mammalian cells.

In this study, we measured and compared the serum lipid profile value of 40 OC patients with the control group, significant reduction was found in the mean value. The mean value of TC, TGL and HDL found to be decreased significantly. While mean value of LDL and VLDL were not found to significant. According to statistically significant taken value $P < 0.005$.

The several prospective and retrospective studies have shown an inverse association between blood lipid profile and different cancers. Lohe et al. (2010) have observed an inverse relationship between serum lipid profile and OC and oral pre-cancer. Furthermore, some investigators have also found a relation of low serum cholesterol with increased risk of cancer occurrence and mortality. HDLC levels may be a useful indicator, reflecting the initial changes occurring in neoplastic conditions. A drastic reduction in levels of HDLC was observed in some study, which is in accordance with the previous reports 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.

In our study did not reveal any significant difference between the two groups of LDL and VLDL Cholesterol same as in other studies. Furthermore, similar results were found for LDL cholesterol and VLDL cholesterol which were observed in a study conducted by Chawda et al. (2006).

Graph 7: According to Sherubin et al. (2013) abnormality of lipid parameters in oral cancer patients

Lipid peroxidation may be induced by tobacco carcinogens that are known to produce reactive oxygen species and lipid peroxides.

Long et al. studied the lipid metabolism and carcinogenesis. To compare, preclinical cancer studies and clinical trials have revealed the crucial role of lipid metabolism in tumor growth and metastasis.

Lipid metabolism and cell survival or proliferation of cancer share certain common pathways involving numerous proteins as well as various cells, tissues, and organelles. Abnormalities in these pathways lead to tumor growth. Based on these findings, many drugs targeting lipid metabolism have been developed for cancer treatment. However, some inhibitors are able to inhibit cancer cell proliferation and tumor growth, but they induce cytotoxicity of normal cells as well. Thus, it is particularly important to develop a number of drugs with high specificities, thus decreasing toxicities. Abnormalities in these pathways lead to tumour growth. Based on these findings, many drugs targeting lipid metabolism have been developed for cancer treatment.
However, a detailed study of cholesterol carrying lipoprotein transport and the efficiency of the receptor system may help in understanding the underlying mechanisms of regulation of plasma cholesterol concentrations in cancer.

**CONCLUSIONS**

The results of our study show the evidence of statistically significant inverse relationship between the serum lipid profile values of TC, HDLC, TGL, and OC. The findings of this study suggest that serum lipid profile may be used as a biochemical indicator. The lower serum lipid status may be considered a useful indicator for initial changes occurring in the neoplastic cells. 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 OC patients as a useful indicator to assess the course, prognosis, and treatment of the disease.

However, a detailed study of cholesterol carrying lipoprotein transport and the efficiency of the receptor system may help in understanding the underlying mechanisms of regulation of plasma cholesterol concentrations in cancer. Hence, the present findings strongly warrant an in-depth study of alterations in serum lipid profile patterns in patients with OC.

**REFERENCES**


