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## Original Article

# Serum lipid profile in patients with oral cancer and oral precancerous conditions

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## ABSTRACT

**Background:** The present study was undertaken to estimate and compare the levels of plasma total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL) and triglycerides in patients with oral precancerous lesions/conditions, oral cancer and normal subjects.

**Materials and Methods:** The study comprised of 60 patients with oral precancerous lesions/ conditions, 60 patients with oral cancer and a control group of 60 healthy individuals. The diagnosis of oral precancerous lesions/conditions and oral cancer was confirmed histopathologically. Under aseptic condition 5 ml venous blood of overnight fasting patient was withdrawn from each individual. Serum was separated by centrifugation and plasma levels of TC, LDL, HDL, VLDL and triglycerides were estimated. Descriptive statistical analysis has been carried out in the present study. Analysis of variance has been used to find the significance of study parameters between three or more groups of patients, *Post-hoc* test as Tukey has been used to find the pair wise significance. Significance is assessed at 5% level of significance.

**Results:** Statistically significant decrease in levels of plasma TC, LDL, HDL, VLDL and triglycerides was observed in the precancerous and cancerous groups as compared to the control group. On comparison between precancerous and cancerous groups, significant decrease was observed in cancerous group.

**Conclusion:** The change in lipid levels may have an early diagnostic or prognostic role in the oral premalignant lesions/conditions and oral cancer. The presence of decreased plasma lipid profile should increase the suspicion of these lesions to be investigated further.

**Key Words:** High density lipoprotein, low density lipoprotein, oral cancer, oral leukoplakia, oral submucous fibrosis, total cholesterol, triglycerides, very low density lipoprotein

## INTRODUCTION

In recent years, detection of molecular markers is being emphasized. Body fluids such as saliva, blood, urine and others are used for early diagnosis, predicting prognosis and monitoring the progression of diseases. Blood based tests is more appealing; with the view of its ease, economic advantage and possibility to repeat sampling.[1]

Lipids are essential biomolecules for maintenance of various biological functions including stabilization of deoxyribonucleic acid helix, cell growth and division in normal as well as in malignant tissues.[2] The usefulness of variations in blood cholesterol levels in diagnosis and treatment of various diseases have been studied by several workers. An increase in the level of cholesterol is a major risk factor for coronary heart diseases; on the other hand, the decrease in the level of cholesterol has been associated with an increased risk of cancer.

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Oral cancer is one of the most prevalent cancers and is the tenth most common causes of death.[1] Oral squamous cell carcinoma is often preceded by specific potentially malignant disorders; the most common among them are the oral leukoplakia and oral submucous fibrosis (OSMF). Well-known risk factors are consumption of tobacco, areca nut and alcohol, which result in increased free radicals production. Free radicals cause lipid peroxidation, which in turn affects various cellular vital activities including growth, differentiation and gene expression.[3,4]

An inverse association between cancer and serum cholesterol concentrations has been observed in various cancers. This U or J shaped relationship indicates a higher mortality rate in both high as well as low serum cholesterol concentrations.[5] However, literature on serum lipid profiles and its association in the head and neck cancers are few. Thus, the current study attempts to correlate the relationship between serum lipid levels in oral pre-cancer and cancer, which can be of diagnostic and prognostic ideals. Furthermore, this may also depict the pathophysiology for the development of these lesions. The aim of this study was to estimate and compare the levels of plasma total cholesterol (TC), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL) and triglycerides in oral pre-cancer, oral cancer and normal subjects.

## MATERIALS AND METHODS

A total of 180 patients were randomly selected from out-patients visiting the Department of Oral Medicine and Radiology.

### Inclusion criteria
- Patients with clinically evident and histopathologically confirmed with OSMF, oral leukoplakia and oral cancer.
- Patients and subjects without any underlying systemic illness.

### Exclusion criteria
- Overweight patients.
- Patients older than 65 years.
- Patients with family history of hyperlipidemia.
- Patients on topical/systemic medications for leukoplakia/OSMF.
- Patients on cytotoxic drugs or radiotherapy for oral cancer.
- Pregnant patients.

Further the study groups were sorted as follows:
Group I: 60 patients with oral precancerous lesions and conditions.
Group II: 60 patients with oral cancer.
Group III: 60 healthy individuals.

All subjects were interviewed before being clinically examined in the out-patient department. The questionnaire contained data on demographic factors, types of habits frequency duration of habits. A written consent from patients participating in this study was obtained. This study protocol was approved by the institutional review board. Further, the diagnosis of the lesions (Groups I and II) was confirmed histopathologically [Table 1].[6-8]

Under aseptic condition 5 ml venous blood of overnight fasting patient was withdrawn from each individual using sterile disposable syringe. Blood was then transferred to plastic test tube. Then, the serum was separated by centrifugation on Remi centrifuge machine at 3000 rpm for 15 min. Hemolyzed samples were excluded. Serum levels of TC, triglycerides and HDL were detected by spectrophotometric method using fully automated chemistry analyzer, "Biosystems A25." Procedure was performed as per manufacturer's instructions. Analysis for plasma TC, triglycerides, HDL, LDL and VLDL were carried out at the Clumax Diagnostics Bangalore, Karnataka, India.

### Estimation of serum TC by using cholesterol oxidase/peroxidase method
*Principle of the method*
Free and esterified cholesterol in the sample originates by means of the coupled reactions resulting in a colored complex that can be measured by spectrophotometry. Ready to use reagent was used using equipment Biosystems A25. Reference values for TC: Up to 200 mg/dl — Desirable, 200-239 mg/dl — Borderline, >240 mg/dl — High and TC: HDL ratio — <5.

### Estimation of serum triglycerides
Glycerol phosphate oxidase peroxidase method was used. Ready to use reagent was used using equipment Biosystems A25. Reference values for triglyceride: Up to 150 mg/dl.[9]

### Estimation of HDL cholesterol by enzymatic clearance method
*Principle of the method*
The cholesterol from LDLs, VLDLs and chylomicrons is broken down by the cholesterol oxidase in an

**Table 1: Clinical and socio-demographic details of the subjects**

| Characteristics | Oral pre-cancer patients | Oral cancer patients |
|---|---|---|
| Sex | | |
| Male | *n*=60 | *n*=60 |
| Female | *n*=10 | *n*=10 |
| Age | | |
| Mean | 32.60±11.79 years | 50.10±7.66 years |
| Habits | | |
| Areca nut chewers (gutka) | *n*=16 | *n*=5 |
| Betel leaf+arecanut+tobacco | *n*=10 | *n*=5 |
| Active smokers | *n*=20 | *n*=11 |
| Only tobacco chewers | *n*=4 | *n*=15 |
| Smoker and tobacco chewers | *n*=5 | *n*=10 |
| Smoker+tobacco chewers+alcohol | *n*=5 | *n*=14 |
| Sites affected | | |
| Buccal mucosa | *n*=60 | *n*=18 |
| Labial mucosa | *n*=30 | *n*=8 |
| Soft palate and uvula | *n*=25 | *n*=5 |
| Tongue | *n*=15 | *n*=14 |
| Vestibule | *n*=6 | *n*=7 |
| Floor of the mouth | *n*=10 | *n*=13 |
| Clinical diagnosis | Stage I OSMF (*n*=10)*<br>Stage II OSMF (*n*=15)<br>Homogenous leukoplakia (*n*=25)<br>Speckled leukoplakia (*n*=10) | **Stage I (T1N0M0) *n*=23<br>Stage II (T2N0M0) *n*=19<br>Stage III (T3N0-1M0 or T1-2N1M0) *n*=18 |
| Histopathological diagnosis | Oral submucous fibrosis (*n*=25)<br>***No dysplasia (*n*=2)<br>Mild dysplasia (*n*=11)<br>Moderate dysplasia (*n*=9)<br>Severe dysplasia (*n*=13) | SCC poorly differentiated (*n*=22)<br>SCC moderately differentiated (*n*=18)<br>SCC well differentiated (*n*=20) |

SCC: Squamous cell carcinoma; *OSMF: Oral submucous fibrosis classification according to Pindborg[6] **Tumor, Node and MetastasisTNM classification adopted from Burket's Oral Medicine 11th edition.[7] ***According to World Health Organization classification of grades of dysplasia[8]

enzymatic accelerated non color forming reaction. The detergent present in the reagent B solubilizes cholesterol from HDL s in the sample. The HDL cholesterol level is measured spectrophotometrically. Reference value of HDL: 35-60 mg/dl.[10]

### Determination of VLDL and LDL values
VLDL cholesterol was determined from the following formula: VLDL = TG/5. Reference value of VLDL: <30 mg/dl. LDL cholesterol was determined with the following formula: LDL = TC − (HDL + VLDL). Reference value of LDL: Up to 100 mg/dl.

### Statistical methods
Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on mean ± standard deviation (SD) (Min-Max) and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance. Analysis of variance has been used to find the significance of study parameters between three or more groups of patients, *Post-hoc* test due to Tukey has been used to find the pair wise

significance. 3 × 3 Fisher exact test was used to find the significance of study parameters on categorical scale between two or more groups. Significance is assessed at 5% level of significance.

## RESULTS

The control and study groups consisted of 50 (83.3%) males and 10 (16.7%) females. The mean age of the control group was 25.87 ± 5.09 years; precancerous group was 32.60 ± 11.79 years and of oral cancer group was 50.10 ± 7.66 years.

### Comparison of mean lipid profile
Table 2 reveals a mean plasma triglyceride levels of 198.63 mg/dl (SD ± 3.56), 137.93 mg/dl (SD ± 8.21) and 120.73 mg/dl (SD ± 36.85) for the control, precancerous and oral cancerous groups respectively. A statistically significant ($F = 93.681$; $P < 0.0001$) decrease in mean plasma triglyceride level is observed in the precancerous and cancerous groups as compared with the control group.

The mean plasma TC levels were 216.30 mg/dl (SD 10.75) for the control group, 175.40 mg/dl (SD ± 12.36) for the precancerous group and 168.73 mg/dl (SD ± 32.01) for the cancerous group. Statistically significant ($F$ = 46.174; $P$ < 0.001) decrease of plasma TC level is observed in the precancerous and cancerous groups as compared to the control group.

Mean plasma HDL levels were 77.80 mg/dl (SD ± 6.10) for the control group, 53.53 mg/dl (SD ± 4.47) for the precancerous group and 44.67 mg/dl (SD ± 7.12) for the cancerous group. The plasma level of HDL was decreased in the precancerous and cancerous groups as compared to the control group and this difference was statistically significant ($F$ = 245.265; $P$ < 0.001)

The mean plasma LDL levels were 106.33 mg/dl (SD ± 7.72) for the control group, 89.73 mg/dl (SD ± 5.12) for the precancerous group and 89.30 mg/dl (SD ± 26.12) for the cancerous group. Statistically significant ($F$ = 11.054; $P$ < 0.001) decrease of plasma LDL levels is observed in the precancerous and cancerous groups as compared to the control group.

This study reveals a mean plasma VLDL levels of 34.47 mg/dl (SD ± 4.18) 24.70 mg/dl (SD ± 3.51) and 23.10 mg/dl (SD ± 7.41) for the control, precancerous and oral cancerous groups respectively. Decreased levels of plasma VLDL is observed in the precancerous and cancerous groups as compared to the control group, which was statistically significant ($F$ = 40.224; $P$ < 0.001).

Mean plasma cholesterol — HDL ratio were 2.75 (SD ± 0.32), 3.27 (SD ± 0.37) and 3.71 (SD ± 0.61) for the control, precancerous and oral cancerous groups respectively. A statistically significant ($F$ = 33.997; $P$ < 0.001) increase in the plasma cholesterol — HDL ratio is observed in the precancerous and cancerous groups as compared to the control group.

Table 3 reveals a pair wise comparison of the mean serum lipid profile between the study groups. A statistically significant decrease is observed in oral cancerous group, in triglyceride levels ($P$ = 0.014), TC levels ($P$ = 0.012), HDL levels ($P$ < 0.001), VLDL levels ($P$ = 0.475) and TC — HDL ratio ($P$ = 0.001) in comparison with precancerous group. The mean LDL levels on comparison between the precancerous and cancerous groups is not statistically significant ($P$ = 0.994).

## DISCUSSION

Our study group consisted predominantly of males (83.3%). The mean age was higher (50.10, SD ± 7.66) in patients suffering from oral carcinoma. In our study, all patients (100%) with oral precancerous conditions and cancer were tobacco consumers in different forms [Table 1].

### Table 2: Comparison of mean lipid profile between three groups

| Lipid parameters | Controls | Precancerous | Cancerous | Significance |
|---|---|---|---|---|
| Triglycerides (mg/dl) | 198.63±13.56 | 137.93±8.21 | 120.73±36.85 | $F$=93.681; $P$<0.0001** |
| Total cholesterol (mg/dl) | 216.30±10.75 | 175.40±12.36 | 168.73±32.01 | $F$=46.174; $P$<0.001** |
| HDL (mg/dl) | 77.80±6.10 | 53.53±4.47 | 44.67±7.12 | $F$=245.265; $P$<0.001** |
| LDL (mg/dl) | 106.33±7.72 | 89.73±5.12 | 89.30±26.12 | $F$=11.054; $P$<0.001** |
| VLDL (mg/dl) | 34.47±4.18 | 24.70±3.51 | 23.10±7.41 | $F$=40.224; $P$<0.001** |
| Cholesterol-HDL ratio | 2.75±0.32 | 3.27±0.37 | 3.71±0.61 | $F$=33.997; $P$<0.001** |

Suggestive significance ($P$ value: 0.050 < $P$ < 0.10); *Moderately significant ($P$ value: 0.01 < $P$ ≤ 0.05); **Strongly significant ($P$ value: $P$ ≤ 0.01); HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein

### Table 3: Pair wise comparison of mean lipid profile between three groups

| Lipid parameters | Controls versus precancerous | Controls versus cancerous | Precancerous versus cancerous |
|---|---|---|---|
| Triglycerides (mg/dl) | <0.001** | <0.001** | 0.014* |
| Total cholesterol (mg/dl) | <0.001** | <0.001** | 0.431 |
| HDL (mg/dl) | <0.001** | <0.001** | <0.001** |
| LDL (mg/dl) | <0.001** | <0.001** | 0.994 |
| VLDL (mg/dl) | <0.001** | <0.001** | 0.475 |
| Cholesterol-HDL ratio | <0.001** | <0.001** | 0.001** |

*Moderately significant ($P$ value: 0.01 < $P$ ≤ 0.05); **Strongly significant ($P$ value: $P$ ≤ 0.01);ç HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein

The habit of tobacco consumption is a known etiologic factor for development of oral precancerous diseases and head/neck cancer.[3,11] It is believed that tobacco carcinogens induce generation of free radicals and reactive oxygen species, which are responsible for high rate of oxidation/peroxidation of polyunsaturated fatty acids. Lipid peroxidation further releases peroxide radicals. There is substantial evidence that the hydroxyl radical generated, can destruct tissue by initiation and propagation of lipid peroxidation by abstracting hydrogen from unsaturated fatty acids. This affects essential constituents of the cell membrane and might be involved in carcinogenesis/tumorigenesis.[12]

The present study demonstrated a significant reduction in the mean plasma TC ($P < 0.001$) in cancer and pre-cancer patients as compared to the control subjects. The process of carcinogenesis reduces the levels of cholesterol in the proliferating tissues and in blood compartments.[13] An inverse relationship between plasma cholesterol in the oral cancer and pre-cancer patients as compared to the control patients is seen in our study, which was in accordance with the earlier studies.[14]

In the present study, the association between low plasma cholesterol and oral cancer was observed in older age patients rather than the young study participants; this holds up the hypothesis of a "pre-existing cancer effect" i.e., the incidence of cancer is higher in older subjects; with the possible preclinical cancer at baseline in younger age.[5,14]

Our study also showed a significant reduction in the mean plasma LDL ($P < 0.001$) and HDL ($P < 0.001$) in cancer and pre-cancer patients as compared to the control patients. Low HDL is an additional predictor of cancer and it might be a consequence of disease that is mediated by utilization of cholesterol for membrane biogenesis.[15] A significant reduction in the mean plasma VLDL ($P < 0.001$) and triglycerides ($P < 0.001$) in cancer and pre-cancer patients as compared to the control patients was observed in our study.

On comparison between pre-cancer and cancer groups, there was a significant difference in the levels of the plasma TC ($P = 0.012$), VLDL ($P = 0.011$), HDL ($P < 0.001$) and triglycerides ($P = 0.014$). However, LDL levels were not significantly different between the two groups ($P = 0.994$). It has been stated that low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the rapidly dividing cells in malignancies. Lower HDL levels may

be a sign of initial changes occurring in precancerous and neoplastic conditions.[13] Lipid peroxidation grounds excessive utilization of lipids including TC, lipoproteins and triglycerides for new membrane biogenesis.[16] Lipid metabolism in malignant cells is accomplished by either from circulation, by synthesis through the metabolism or from degradation of major lipoprotein fractions such as VLDL, LDL or HDL. Thus, changes in serum cholesterol values may occur before the manifestation or detection of cancer and may be the result of the cancer process. 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.[17] Several prospective and retrospective studies have shown an inverse association between blood lipid profile and different cancers such as colon and breast cancer.[5]

## CONCLUSION

The change in lipid levels may have an early diagnostic or prognostic role in oral premalignant and malignant lesions. 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. The results of this study add to the evidence of an inverse relationship between lower plasma lipid profile and head/neck malignancies and oral precancerous conditions.

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