



Study of serum lipid profile in patients with oral submucous fibrosis and oral squamous cell carcinoma and their comparative analysis

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Abstract

Background & Aims: The aim of this study was to evaluate serum lipid profile and compare their levels between the patients with oral submucous fibrosis (OSMF) and oral squamous cell carcinoma (OSCC).

Materials & Methods: This descriptive study was done in two groups of patients; OSMF and OSCC patients. There were forty-five participants in each group. Serum samples obtained from centrifugation of 12 hour fasting blood samples was analyzed by fully automated analyzer Beckman coulter AU-680 for estimation of the lipid levels (cholesterol, triglycerides (TGL), and high-density lipids (HDL)) using colorimetric methods. Low-density lipid (LDL) values were obtained by calculation.

Results: The comparison of lipid profile between Oral Submucous Fibrosis and Oral Carcinoma cases shows statistically significant results for TC, HDL and LDL.

Conclusion: The change in lipid levels may have an early diagnostic or prognostic role in oral premalignant and malignant lesions.

Keywords: Oral Submucous Fibrosis; Oral Squamous Cell Carcinoma; Serum Lipid Profile

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Introduction

Early detection is the key factor for oral cancer control, and one of the major reasons behind the high mortality rate of oral cancer is the delay in diagnosis of the potentially malignant disorders, the precursors of oral cancer. There are various biochemical markers available for precancerous and cancerous patients, out of which along with tumor markers is the patient's serum lipid profile.

The possible influence of lipids in the pathogenesis of malignancies could be attributable to the influence lipids on the metabolism of malignant cells in terms of proliferation and incorporation in the membranes of neoplastic cells as well as on intercellular messengers and mediators of the inflammatory reaction (1).

Earlier studies have shown that hypolipidemia may result primarily due to the direct lipid-lowering effect of tumor cells and secondarily due to malfunction of the lipid metabolism and/or antioxidative vitamins. These

studies have shown that lower blood lipids have been associated with various cancers (2-5).

To add to the curiosity of the unanswered question whether hypolipidemia is a cause or effect of cancer, this study was aimed to analyze the serum lipid profile in Oral submucous Fibrosis (OSMF) and Oral Squamous Cell Carcinoma (OSCC) patients and conducted to understand the role of these lipids in their pathogenesis. Statistically evaluating their pathological levels in comparison to physiological levels helps us to establish the role of serum lipid markers in diagnosis and prognosis of Oral Squamous Cell Cancer and Oral Submucous Fibrosis.

Materials & Methods

The study was done at the Department of Biochemistry, SMS Medical College and Hospital, Jaipur, India, in collaboration with the Department of Oncology, SMS Medical College and Hospital, Jaipur, India. The study was initiated after obtaining necessary permissions from Research Review Board, Ethics Committee, and Department of Medical Oncology, SMS Medical College, Jaipur, India. A comparative study was then done for serum lipid profile levels in patients OSMF and OSCC with 45 patients in each group.

Patients were selected from the outpatient department of oncology diagnosed with OSMF and OSCC with the age range of 30-65 years with no history of prior chemotherapy and radiotherapy who were willing to participate and give written informed consent for the study.

Patients with family history of hyperlipidemia, suffering from febrile diseases, major illness in the recent past, and systemic disorders like uncontrolled diabetes, hypertension, and thyroid disorders were excluded from the study. Pregnant females and subjects who were obese were also excluded from the study.

Sample collection and analysis: The blood samples of the patients were taken in plain vials in the morning after 12 hour fasting. Serum was separated after centrifugation at 3000 to 4500 rpm and analyzed then by fully automated analyzer Beckman coulter AU-680. Serum samples free from hemolysis were chosen.

Sampling Technique: For selection of the subjects, simple random sampling technique was used by selecting every eligible subject.

Reagents: Serum Total cholesterol, HDL, and triglyceride levels were estimated by enzymatic colorimetric method using in vitro diagnostic reagents. The reagents were ready to use. The unopened reagents were stable until the expiry date printed on the label when stored at 2–8 °C. Opened reagents (routine) are stable for 90 days when stored in the refrigerated compartment of the analyzer.

Estimation of Total cholesterol: Enzymatic method (CHOD-PAP) (6-9)

Reagent composition: Buffer (ph 7.5), Cholesterol Oxidase, Cholesterol Esterase, Peroxidase, Chromogen, Stabilizers, inactive ingredients and surface-active agents.

Principle: Cholesterol esters are hydrolyzed by cholesterol esterase (CHE) into cholesterol and fatty acids. Cholesterol oxidase (CHO) catalyzes oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. Catalyzed by peroxidase (POD), hydrogen peroxide oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye, which has maximum absorbance at 510 nm. The intensity of red color is proportional to the amount of cholesterol in the specimen.

Estimation of Serum HDL-Cholesterol: (6-9)

Reagent composition:

Reagent 1 Good's Buffer, Cholesterol Oxidase, Peroxidase, Preservative, N,N-bis (4-sulphobutyl)-m toluidine disodium (DSBmT), Accelerator.

Reagent 2 Good's Buffer, Cholesterol Esterase, 4-AAP, Detergent, Restrainer, Preservative, Ascorbic Acid Oxidase.

Calibrator: Lyophilized human Serum, Sodium Azide

Principle: The Method is in a two reagent format and depends on the properties of a unique detergent, as illustrated. This method is based on accelerating the reaction of cholesterol oxidase with non-HDL un-esterified cholesterol and dissolving HDL selectivity using a specific detergent.

In the first reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the generated peroxide is consumed by a peroxidase reaction with DSBmT yielding a colorless product. The second reagent consists of a detergent capable of solubilizing HDL specifically, cholesterol esterase and chromogenic coupler to develop color for the quantitative determination of HDL cholesterol. This may be referred as the Accelerator Selective Detergent methodology.

Estimation of Serum Triglycerides: Enzymatic method (GPO-PAP method) (6-9)

Reagent composition: Triglycerides Enzyme Reagent, buffer (pH 7.5), Lipoprotein Lipase, Glycerol Kinase, Glycerol Phosphate Oxidase, Ascorbate Oxidase, Peroxidase, ATP, 4-Aminoantipyrine.

Principle: Triglycerides are hydrolyzed by Lipoprotein Lipase into glycerol and fatty acids. Catalyzed by Glycerol Kinase, glycerol is phosphorylated to glycerol-3-phosphate. Glycerol-3-Phosphate is oxidized to dihydroxyacetone phosphate and hydrogen peroxide, in presence of Glycerol Phosphate Oxidase. Catalyzed by Peroxidase, Hydrogen

Peroxidase causes oxidation of phenolic chromogen (4 aminoantipyrine) and p-chlorophenol to a red-colored compound. The intensity of the red color is proportional to the amount of triglycerides in the serum.

Estimation of Serum VLDL-cholesterol and LDL-cholesterol: (6-9)

VLDL was estimated by TG/5 based on the average ratio to cholesterol in VLDL

Serum LDL was estimated from the Friedwald and Fredrickson's formula (1972), which is $LDL = \text{Total Cholesterol} - [HDL + VLDL]$

Results

Statistical Analysis: Quantitative data was analyzed in the form of percentage, mean, standard deviation, and one-way ANOVA with Bonferroni correction and Scheffe post hoc at 95% confidence interval. Collected data was then submitted to Microsoft Excel 2007 worksheet in the form of master chart. These data were classified & analyzed by Microsoft Excel 2007 worksheet; statistical analysis was done with by Primer software. Levels of statistical significance were set at a Pvalue < 0.05.

Table 1. Age and gender distribution of OSMF and OSCC patients

| Parameter | OSMF patients (n=45) | OSCC patients (n=45) |
|-----------------------|----------------------|----------------------|
| Age (years) | 50.06 ± 8.62 | 51.13 ± 8.56 |
| Gender (Male: Female) | 35:10 | 35:10 |

There was a significant difference between the means for OSMF and OSCC patients in total cholesterol, high-density cholesterol, and low-density cholesterol

levels. There was no significant difference between means in triglycerides and VLDL levels (Table 2 and Figure 1).

Table 2. Comparison of Mean Lipid profile levels between Oral Submucous fibrosis and Oral Squamous Cell Carcinoma Cases

| Lipids (mg/dl) | OSMF patients (n=45) | OSCC patients (n=45) | P value |
|-------------------|----------------------|----------------------|------------|
| Triglycerides | 91.40 ± 22.46 | 84.18 ± 22.22 | 0.064 (NS) |
| Total Cholesterol | 131.42 ± 23.30 | 113.24 ± 26.11 | < 0.01 (S) |
| HDL | 40.31 ± 6.31 | 34.2 ± 6.37 | < 0.01 (S) |
| LDL | 72.83 ± 25.31 | 62.21 ± 27.94 | < 0.01 (S) |
| VLDL | 18.28 ± 4.49 | 16.84 ± 4.44 | 0.061 (NS) |

*P-value as obtained on applying Student T test (S at p<0.05)

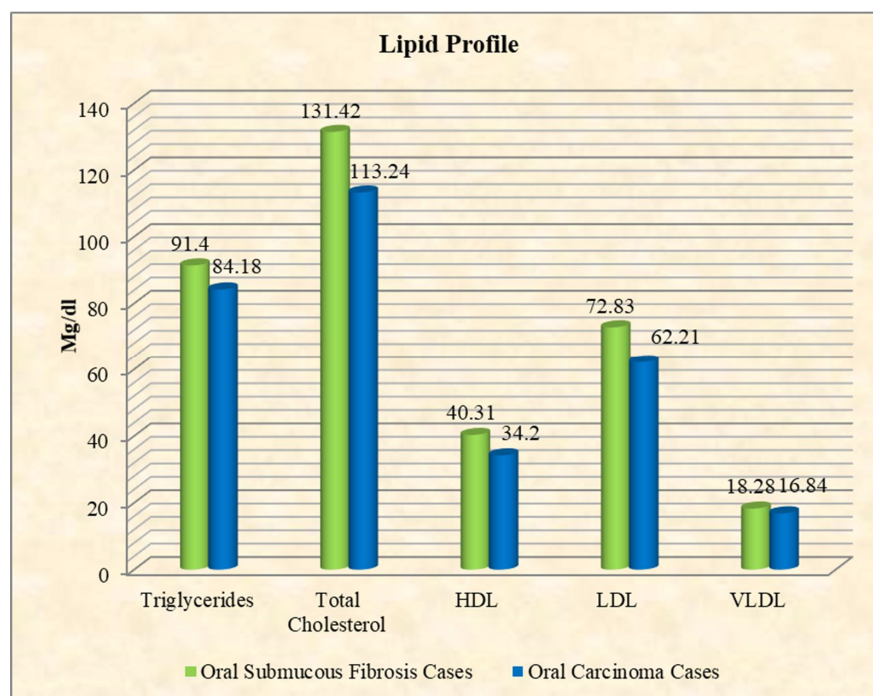


Fig. 1. Comparison of Lipid Profile Comparison between Oral Submucous fibrosis and Oral Carcinoma Cases

Discussion

Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion and sometimes metastasis. These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasize (10).

Oral submucous fibrosis (OSMF), an insidious chronic disease which reported mainly in Indians with high use of areca nut, is a precancerous condition and have a significant tendency to develop to oral and esophageal cancers (11). Premalignant lesions and conditions usually precede oral cancer (12). Early detection is the key for oral cancer control.

Incidence of oral potentially malignant and malignant lesions is increasing, thereby escalating the burden of cancer globally. OSCC has become a global health problem with increasing incidence and mortality rates with variation in incidence in each geographic location in relation to age, gender, and habits (13). It has

been well established by researchers that virtually all oral cancers are preceded by visible clinical changes in the oral mucosa in the form of white or red patches which may or may not be associated with additional features of significant discomforts (13). They could have a higher risk of malignant transformation unless diagnosed early and treated.

In some malignant diseases, blood lipid levels undergo early and significant changes. Lowered levels of blood cholesterol in the proliferating tissues and in blood compartments may be due to the ongoing process of oncogenesis. The question arises whether hypolipidemia is considered to be a predisposing factor or a consequence of malignancy? However, earlier studies have shown that hypolipidemia may result primarily due to the direct lipid-lowering effect of tumor cells and secondarily to either malfunction of the lipid metabolism or antioxidative vitamins.

With this background, the present study was done to validate serum lipid changes as a potential biological

marker for Oral malignant disorders and Oral squamous cell carcinoma.

In our study, OSCC was seen between the age group of 4th to 6th decades of life with the mean age of 51 years. Similarly, OSMF was also seen most commonly affecting age group of 5th decade with mean age of 50 years in our study. These results are well supported by other studies such as Misra et al. in 2009 who reported a mean age of 53.15(14). Findings of majority of the studies such as by Singh MP (2016) (15), Abdulla R (2018) (16), Tandon A (2018) (17), and Kumar GK (2019) (18) are consistent with our finding.

In the study done by Tandon A et al. (2018) (17), increased incidence of OSCC was found in males compared to females with a male-to-female ratio of 3.26:1, which is consistent with other North Indian studies on oral cancer as well as with our study. Males are more commonly affected compared to females by OSCC in both developed (male: female ratio 2.5:1) and developing (male: female ratio 3:1) countries, which may be due to easy acceptance of habits by males (19). However, in recent time, this difference in gender distribution is reducing in the developed countries due to increment intaking up tobacco-related habits including smoking by females (19).

In the present study, the values evidently show that oral cancer patients have significantly lower serum cholesterol, lower serum HDL, and lower serum LDL values compared with OSMF patients. This result is consistent with the result of the study of Lohe et al. in 2010 (19). It was postulated that low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the process of carcinogenesis (20).

Lipids are the most important cell membrane parts that are required for various biological functions such as maintaining cell integrity, cell growth, and division of normal and malignant cells. Changes in the lipid profiles have been observed both among precancerous disease group and oral cancer group (12). There are three main competing hypotheses to explain the relation between low cholesterol and oral cancer. (a): Low cholesterol may be an indicator of cancer process even before

cancer manifests clinically. (b): Low cholesterol serves as a marker for some other causal sets of variables and its association with oral cancer may be secondary even though it precedes cancer. (c): Low cholesterol levels may precede the development of cancer and may be causally associated with some forms of cancer (21). Lohe et al. (20) in their study stated that serum lipid levels were inversely associated with the development of precancerous and cancerous lesions.

In a study conducted by Fu-Chuan Chao et al. (22), it was stated that hypolipidemia is a result of direct lipid lowering effect of tumor cells as these neoplastic cells directly utilize cholesterol for their own metabolism.

In another study conducted by Min-Ah Choi et al. (23), it was suggested that hypocholesterolemia is secondary to decreased levels of serum antioxidative vitamins. Decrease in the level of antioxidative vitamins in serum results in increased number of free radicals which causes increased lipid peroxidation.

Desai et al. (24) proposed that free cholesterol within the tumor cells is preferentially channeled into storage as cholesterol esters rather than being released from the cells as circulating HDL. This mechanism explains the decreased levels of HDL in cancer patients. In the present study, a significant decrease was noticed in serum HDL levels in OSCC patients compared to the OSMF patients.

Hypertriglyceridemia may also predispose to malignancy. Elevated triglyceride levels have been demonstrated in the patients with several different types of cancer (25). However, we found a non-significant difference in serum triglycerides between OSMF and cancer patients.

The diagnostic implications of assessing lipid profile in smokers as well as tobacco and areca nut chewers might be that alteration in lipid profile may be the indication that the changes in the oral mucosa are occurring and if such changes in lipid levels be seen in precancerous patients (OSMF) may alert us to further investigation using different diagnostic aids. Thus, the estimation of lipid levels appears to be an easier and faster investigative method that should be included in routine diagnostic pathology services.

Conclusion

We conclude that the serum total cholesterol, serum HDL, and serum LDL levels are significantly lowered in patients with oral squamous cell carcinoma (OSCC) compared to oral submucous fibrosis (OSMF) patients and this reduction may be due to the significant changes in the cell integrity in OSCC patients.

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Conflict of interest

The authors have no conflict of interest in this study.

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Nil

Ethical Statement

The study was initiated after obtaining necessary permissions from Research Review Board, Ethics Committee, and Department of Medical Oncology, SMS Medical College, Jaipur, India. Written informed consent was obtained from all patients who participated in the study.

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