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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

Aims and Objectives: The aim of the study was to study serum lipid profile and compare their levels in patients with oral sub mucous fibrosis (OSMF) and oral squamous cell carcinoma (OSCC).
Materials and Methods: This study was done in two groups of patients - OSMF and OSCC. There were forty five participants in each group. Serum obtained from centrifugation of 12 hour fasting blood samples was analyzed on fully automated analyzer Beckman coulter AU-680 for estimating the lipid levels (cholesterol, triglycerides [TGL], and high-density lipids [HDL]) by colorimetric method. Low-density lipid [LDL] values were obtained by calculator.
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 sub mucous fibrosis (OSMF), oral squamous cell carcinoma (OSCC) and serum lipid profile.

Introduction

Early detection is the key for oral cancer control and one of the major reasons behind the high mortality rate of oral cancer is the delay in early 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 one such tumor marker is serum lipid profile.
The possible influence of lipids in the pathogenesis of malignancies could be attributable to influence on the metabolism of malignant cells in terms of proliferation and incorporation in the membranes of neoplastic cells and lipids' function intercellular messengers or as mediators of the inflammatory reaction [1].
Earlier studies have shown that hypolipidemia Introduction 4 may result primarily due to the direct lipid-lowering effect of tumor cells and secondarily to either malfunction of the lipid metabolism or antioxidant 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 at analyzing the serum lipid profile in OSMF and OSCC patients and this study was conducted to understand the role of these lipids in the oral potentially malignant disorders like Oral leukoplakia, Oral submucous fibrosis and also in Oral Squamous Cell Carcinoma. Hence, by evaluating statistically their pathological levels in comparison to physiological levels helps us to establish the role of serum lipid markers in diagnosis and prognosis of Oral Cancer and Oral Submucous Fibrosis.

Materials and Methods/Study design

The study was done in the Department of Biochemistry, S.M.S. Medical College and Hospital, Jaipur in collaboration with Department of Oncology, SMS Medical College and Hospital, Jaipur. The study was initiated after obtaining necessary permissions from Research Review Board, Ethics Committee and Department of Medical Oncology, SMS Medical College, Jaipur. A comparative study was then done for serum lipid profile levels in patients of oral submucous fibrosis (OSMF) and oral squamous cell carcinoma (OSCC) with 45 patients in each group.
Patients were selected from the outpatient department of oncology diagnosed with oral sub mucous fibrosis and oral squamous cell carcinoma in age group of 30-65 years, with no history of prior chemotherapy and radiotherapy and those 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 or 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 was taken in plain vials in morning after 12 hour fasting. Serum was separated from samples after centrifugation at ~3000-4500 rpm and analyzed on fully automated analyzer Beckman coulter AU-680. Serum samples free from hemolysis is the recommended specimen.

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

Reagents: Serum Total cholesterol, HDL and triglyceride levels are estimated by enzymatic colorimetric method using *in vitro* diagnostic reagents. The reagents are ready to use. The unopened reagents are 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 cholast 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 colour is proportional to amount of cholesterol in specimen.

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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 (CO) 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 peroxide generated 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 (CE) and chromagenic coupler to develop color for the quantitative determination of HDL cholesterol. This may be referred to 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 (LPL) into glycerol and fatty acids. Catalyzed by Glycerol kinase (GK), glycerol is phosphorylated to glycerol-3-phosphate. Glycerol-3-Phosphate is oxidized to dihydroxyacetone phosphate and hydrogen peroxide, in presence of glycerol phosphate oxidase (GPO). Catalyzed by Peroxidase (POD), Hydrogen peroxidase causes oxidation of phenolic chromogen (4 aminoantipyrine) and p-chlorophenol to a red colored compound. The intensity of the red colour 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 Freidwald and Fredrickson's (1972) formula, which is LDL = Total Cholesterol-[HDL+VLDL].

Results

Statistical Analysis: Quantitative data was analyzed in the form of Percentages, mean, standard deviation, and one-way ANOVA with Bonferroni correction and Scheffe post hoc at 95% confidence interval. Data thus collected was submitted to Microsoft excel 2007 worksheet in the form of master chart. These data were classified & analyzed with the help of Microsoft excel 2007 worksheet, statistical analysis was done with the help of Primer software. Levels of statistical significance were set at a P value < 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

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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 $p<0.05$)

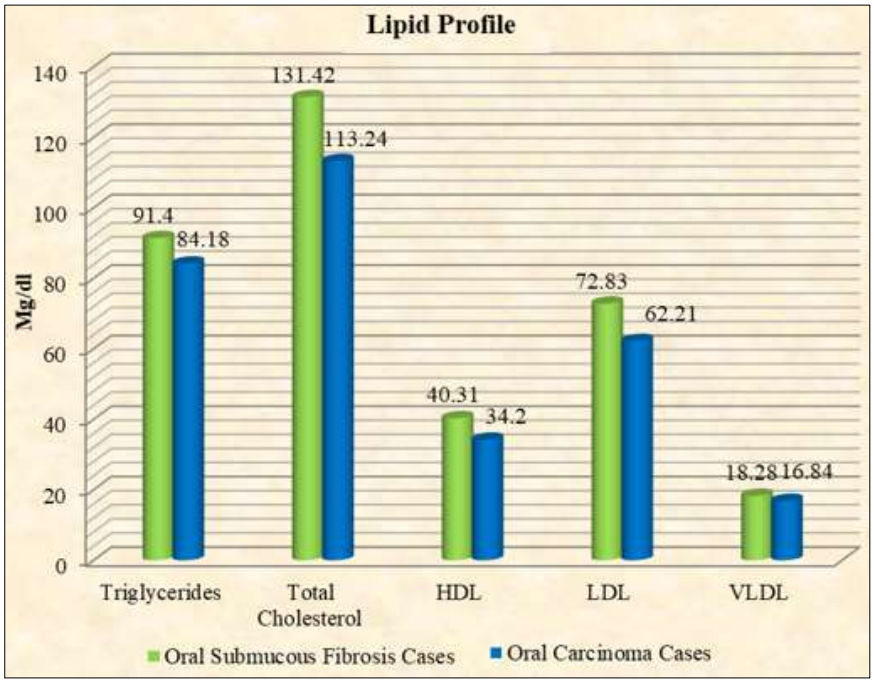

Fig 1: Comparison of Lipid Profile Comparison between Oral Submucous fibrosis and Oral Carcinoma Cases.

There is a significant difference between the means of OSMF and OSCC patients in total cholesterol, high-density cholesterol, and low-density cholesterol levels. There is no significant difference between means in triglycerides and VLDL levels. [Table 2 and Figure 1].

Discussion

Oral submucous fibrosis (OSMF), an insidious chronic disease, reported mainly in Indians associated with the use of areca nut is a precancerous condition and have a significant tendency to develop oral and esophageal cancer [10]. Premalignant lesions and conditions usually precede Oral cancer [11]. Early detection is the key for oral cancer control.

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 [12].

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. 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

discomfort. They 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 antioxidant vitamins.

With this background the present study was done to validate Serum lipid changes as biological marker for Oral Potentially 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 studies such as Mishra *et al*. in 2009 who reported a mean age of (53.15) [13]. Majority of the studies such as by Singh MP (2016) [14], Abdulla R (2018) [15], Tandon A (2018) [16] and Kumar GK (2019) [17] are consistent with our finding.

In the study by Tandon A *et al* (2018) [16], increased incidence of OSCC was found in males than in females with a male-to-female ratio of 3.26:1, which is consistent with other north Indian studies on oral cancer and 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 [18]. However, in recent time, this difference in gender distribution is reducing in the developed countries due to more females taking up tobacco-related habits including smoking [18].

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 when compared with OSMF patients. This result is consistent with the result of studies Lohe *et al*. in 2010 [19]. Was postulated that low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the process of carcinogenesis [19].

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 among precancerous disease group and oral cancer group. 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 if it precedes cancer.
c) Low cholesterol levels may precede the development of cancer and may be causally associated with some forms of cancer [20].

Lohe *et al*. [19]. 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*. [21], 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*. [22], it was suggested that hypocholesterolemia was 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.

S Desai *et al* [23]. Proposed that free cholesterol within the tumor cells, is preferentially channeled into storage as cholesterol esters rather than being released from the cells to

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 in OSCC patients as compared to the OSMF patients.

Hypertriglyceridemia may also predispose to malignancy. Elevated triglyceride levels have been demonstrated in patients with several different types of cancer [24]. However; we found a non-significant difference in serum triglycerides between OSMF and cancer patients.

The diagnostic implications of assessing lipid profile in smokers and 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 such changes in lipid levels if seen in precancerous patients (OSMF) may alert us to further investigate 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

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

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