

ORIGINAL RESEARCH

Evaluation of Serum Lipids and Lipoproteins as Prognosticators in Leukoplakia

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ABSTRACT

Background: Oral cancer is the 8th most common cancer worldwide. Squamous cell carcinomas constitute 94% of all oral malignancies and are often preceded by leukoplakia. Despite many adjunctive techniques to monitor transformation of leukoplakia to oral squamous cell carcinoma (OSCC), the mortality rate is on the rise.

Incidentally, patients diagnosed with oral potentially malignant disorders (OPMDs) and oral cancers manifest with low cholest-erol levels. Given a thought, hypolipidemia may be a useful adjunctive tool as it reflects the initial changes within the neo-plastic cells, thus giving a red alert in malignant transformation of leukoplakia at the earlier stage.

Aim: To evaluate the feasibility of serum lipid profile as an adjunct early marker for malignant transformation of leukoplakia to OSCC.

Objectives: To estimate the serum cholesterol, triglycerides and lipoprotein (HDL, LDL, VLDL) levels in patients with leukoplakia, OSCC and age matched healthy control group.

To compare the serum cholesterol, triglycerides and lipoprotein levels between patients of leukoplakia, OSCC and age matched healthy control group.

Materials and methods: The study group comprised of selected 30 individuals which included 10 each of histopathologically confirmed OSCC, leukoplakia and healthy controls. A written consent was taken from all of them, and a thorough case history was recorded and then venous blood was collected 12 hours post fasting and centrifuged. The serum cholesterol, triglycerides and HDL were estimated by enzymatic and colorimetric methods using commercially available kits—Roche/Hitachi cobas systems. Chemistry assay QC procured from Bio-Rad was used as control. VLDL and LDL were derived from these values. Results were statistically analyzed using ANOVA and post hoc Tukey Test.

Results: Oral squamous cell carcinoma patients demonstrated significantly lower mean serum cholesterol level (151.60 mg/dl)

than the control group (183.70 mg/dl). The mean cholesterol level in leukoplakia patients (173.90 mg/dl) was lower than that of control group (183.70 mg/dl) but higher than that of the OSCC group (151.60 mg/dl) with no statistical significance.

Conclusion: Convenience, universal availability, patient compatibility and simplicity being the merits of serum lipid profile make it a feasible adjunctive prognosticator in leukoplakic patients.

Keywords: Lipid profile, Leukoplakia, Oral squamous cell carcinoma, Prognosticator.

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INTRODUCTION

Chewing betel leaves, areca, tobacco is a very common practice among the people in Asian countries, which is a huge threat for oral cancer that constitutes 40% of all the cancers.¹ Oral squamous cell carcinoma (OSCC) being the most common oral cancer is an important cause of morbidity and mortality worldwide.² Oral squamous cell carcinoma is often preceded by potentially malignant disorders, the most common being leukoplakia.³ Once leukoplakia is diagnosed, a constant follow-up to detect the early neoplastic changes is the burning issue to be addressed and has scope for extensive research. Although several specific markers are proven in this field, there is a lack of practical application due to economic viability, nonavailability and patient incompatibility.

Lipids are a heterogeneous group of compounds of high energy value which act as thermal and electrical insulators. Among them, cholesterol is the parent molecule from which all other steroids in the body are synthesized.⁴ It also plays an indispensable role in regulating the properties of cell membranes in mammalian cells.⁵ These membrane lipids are essential for biological functions ranging from membrane trafficking to signal transduction.⁶ In addition, lipoproteins are important cellular constituents, occurring both in the cell membrane and in the mitochondria, serving also as means of transporting lipids in the blood.⁴ On the contrary, cholesterol is best known for its role in pathogenesis of atherosclerosis

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of vital arteries, causing cerebrovascular, coronary and peripheral vascular disease.⁴

Recently, several studies have provided a significant association between serum lipid profile and cancer of various sites.⁷ Similarly, studies on oral cancer and OPMDs also have revealed changes in serum lipid profile. Most of them found a significant hypocholesterolemia and varied findings on serum lipoprotein levels.⁸⁻¹² Hence, the present study aims to evaluate the feasibility of serum lipid profile as an adjunct marker to detect early malignant changes in leukoplakia.

MATERIALS AND METHODS

The study group comprised of selected 30 individuals who included 10 each of histopathologically confirmed OSCC, leukoplakia and healthy controls. After obtaining a written consent, a thorough case history was recorded with emphasis on smoking and alcohol consumption habits, following which venous blood was collected 12 hours post fasting and centrifuged. The serum cholesterol, triglycerides and high density lipoprotein (HDL) were estimated by enzymatic and colorimetric methods using commercially available kits from Roche/Hitachi cobas systems. Chemistry assay QC procured from Bio-Rad was used as control. The color intensity of the dye formed was measured photometrically.

The very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were calculated as follows:

$$\text{VLDL} = \frac{\text{Triglycerides}}{5}$$

$$\text{LDL} = \text{Total cholesterol} - (\text{VLDL} + \text{HDL})$$

Inclusion criteria were, untreated cases of histopathologically diagnosed OSCC/leukoplakia and healthy individuals free of any major illness that would affect lipid profile (except OSCC), all in the age group of 40 to 80. Also, subjects in all groups were to be fasting for 12 hours.

Data were statistically analyzed using ANOVA and post hoc Tukey Test and the p-value of less than 0.05 was accepted as statistically significant.

RESULTS

A wide age range was noted in OSCC patients, ranging from 41 to 75 years, the mean age being 53.6 years. Mean age of 51.2 years was observed in leukoplakia cases (Table 1 and Graph 1).

Oral squamous cell carcinoma patients group (n = 10) consisted of five male patients and five female patients. Leukoplakia group consisted of six male patients and four female patients, while control group had three males and seven females (*see* Table 1).

The most frequent sites of OSCC were buccal mucosa and tongue, whereas leukoplakia was commonly noted on buccal mucosa.

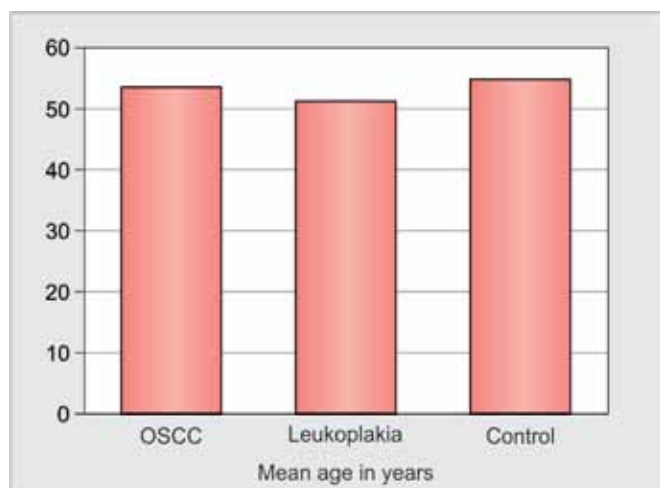
The mean of all serum lipids and lipoprotein values obtained from OSCC, leukoplakia and controls are depicted in Table 2 and Graph 2.

The results of data analysis using ANOVA and post hoc Tukey test are summarized in Table 3.

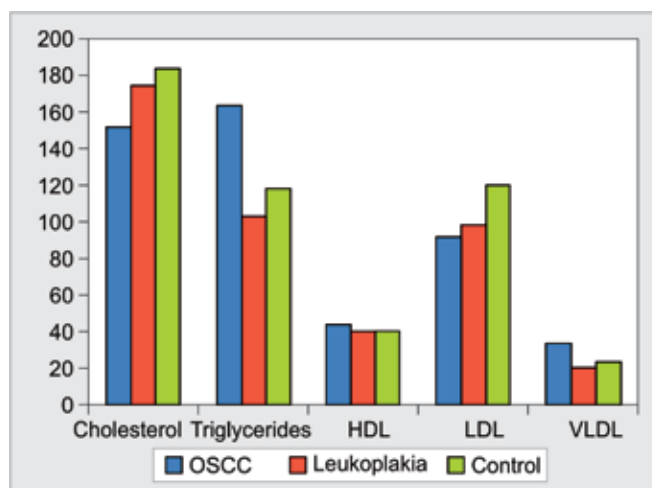
The study results were compared with the standard normal values for serum lipids and lipoproteins as per guidelines by NIH, USA.¹³

The data analysis demonstrated a significant difference in the serum cholesterol levels between the OSCC and controls (p-value = 0.038). The mean cholesterol value was 151.60 mg/dl in OSCC as compared to 183.70 mg/dl in controls. Thus, a hypocholesterolemia in OSCC patients is established. On the other hand, the OSCC group did not demonstrate significant variation in triglycerides, HDL and VLDL values as compared to controls.

Leukoplakia did not demonstrate statistically significant variations in serum lipid profile as compared to either OSCC



Graph 1: Mean age distribution among the OSCC, leukoplakia and control group



Graph 2: Mean lipid and lipoprotein values in OSCC, leukoplakia and controls (mg/dl)

Table 1: Age and gender-wise distribution of samples

Groups	Age (in years)				Gender	
	41-50 (%)	51-60 (%)	61-70 (%)	71-80 (%)	Male (%)	Female (%)
OSCC	50	20	20	10	50	50
Leukoplakia	50	40	10	0	60	40
Control	20	60	20	0	70	30

Table 2: Comparison of mean lipid and lipoprotein values in OSCC, leukoplakia and controls

Groups	N	Cholesterol	Triglycerides	HDL	LDL	VLDL
OSCC	10	151.60 ± 24.40	163.60 ± 127.29	44.03 ± 9.78	91.60 ± 25.44	33.80 ± 24.86
Leukoplakia	10	173.90 ± 31.25	103.00 ± 42.47	39.85 ± 9.85	98.60 ± 34.81	20.50 ± 8.51
Control	10	183.70 ± 26.64	118.40 ± 34.08	40.17 ± 7.61	120.30 ± 28.16	23.70 ± 6.80

Values expressed as mean ± standard deviation (SD) (mg%)

Table 3: Comparison of p-values across the three groups

Groups compared	Cholesterol	Triglycerides	HDL	LDL	VLDL
OSCC with control	0.038	0.427	0.618	0.097	0.335
Leukoplakia with control	0.709	0.903	0.997	0.250	0.892
OSCC with leukoplakia	0.186	0.225	0.569	0.859	0.159

or controls. Notably, a lower serum cholesterol level was evident in leukoplakia as compared to controls and this was higher than that of OSCC group.

Also, a gradual fall in serum LDL level was observed from leukoplakia (98.60 mg/dl) to OSCC (91.60 mg/dl) as compared to control group (120.30 mg/dl), but with no statistical significance.

DISCUSSION

Despite the recent trends in diagnosing and treating OSCC, the mortality rate is alarming with current estimates of age-standardized incidence and mortality being between 6.6/100,000 and 3.1/100,000 in men and 2.9/100,000 and 1.4/100,000 in women respectively.¹⁴ Patient's ignorance of the oral lesion (≤3 months) having no clue of its consequences, is the primary cause for poor prognosis.^{15,16} Therefore, it is important to diagnose oral cancer at the earliest to improve the prognosis necessitating the need for tumor markers which can indicate the presence of OSCC before metastasis.

Ninety percent of oral cancers are estimated to be caused by tobacco use and excessive alcohol consumption.¹⁷ In the present study, a total of 90% of OSCC patients were chronic users of tobacco and/or alcohol. Thus, etiological role of tobacco and alcohol in OSCC is reiterated. In an extensive study by Gupta et al in 1980, they concluded that tobacco use in one form or another was the most common variable relative to the development of leukoplakia.¹⁸ The present study elicited 80% of patients having history of tobacco use.

Leukoplakia is the most common potentially malignant disorder of the oral mucosa.¹⁹ Leukoplakia demonstrates an estimated prevalence of 2% worldwide.²⁰ Risk factor of

developing malignancies is higher in leukoplakic patients (8-10 times).^{21,22} Data from several studies suggest a 4% transformation rate of leukoplakia to OSCC.²

On the other hand, lipids may be defined as compounds which are relatively insoluble in water, but freely soluble in nonpolar organic solvents, like benzene, chloroform, etc.²³ Lipids form the major component of cell membranes and hence are essential for structural and functional integrity of cell membranes, activity of membrane-bound enzymes, stabilization of DNA helix and growth and division of normal and malignant cells.²⁴

Cholesterol, being the major steroid in the body, forms the highest component of cell membranes and the outer layer of plasma lipoproteins. It is an amphipathic lipid transported via lipoproteins, maximum being carried in LDL. Lipids are stored in adipose tissue as triglycerides, which are the esters of the trihydric alcohol glycerol and fatty acids.⁴

Since lipids are insoluble in water, they need the help of carriers in plasma. Therefore, they are complexed with proteins to form lipoproteins namely, chylomicrons, VLDL, intermediate density lipoproteins (IDL), LDL and HDL.²³ Triglyceride is the predominant lipid in chylomicrons and VLDL, whereas cholesterol and phospholipid are the predominant lipids in LDL and HDL.⁴ Triglycerides, cholesterol, HDL, LDL and VLDL constitute the plasma lipid profile.

Rose et al first reported the inverse relation between blood cholesterol level and the risk of cancer.²⁵ This provided the platform for further epidemiological research. The data from studies are confusing because both hypolipidemia and hypercholesterolemia might be linked with malignancy. Not only cancers but also antineoplastic therapies have an influence on lipid profile.²⁶



Hypothesis of Hyperlipidemia and Cancer

A hypothesis that the pathway for cholesterol synthesis may produce various tumorigenic compounds and thus hypertriglyceridemia may also predispose to malignancy is significant (Flowchart 1).²⁷ But, this concept lacks practical application.

On contrary, the trials of lipid lowering interventions done by Kritchevsky et al showed an increase in cancer occurrence of approximately 24% in the cholesterol lowered groups.²⁸ Later several studies reported an inverse association between serum lipid profile and cancer.²⁹⁻³⁴

Possible Mechanism of Association of Hypolipidemia and Cancer

The mechanism of hypolipidemia in cancer remains controversial. Hypolipidemia, whether a cause or an effect of carcinogenesis is still an enigma.^{25,35} Several hypothesis have been proposed on cancer induced hypolipidemia (Flow Chart 2).^{10,30,36-38}

Reports also suggest that the lipid peroxidation product, malondialdehyde, may cross-link DNA on the same and opposite strands via adenine and cytosine. This may in theory contribute to carcinogenicity and mutagenicity in mammalian cells.³⁹⁻⁴² This supports the concept of hypolipidemia inducing a cancer process.

However, return of serum cholesterol levels to normal in responding patients suggested that tumor burden was inversely related to cholesterol levels rather than the propensity of individuals with lowered cholesterol to develop cancer.⁴³ Hence, hypolipidemia as a secondary process during carcinogenesis is more supported.

Various studies on oral cancer and potentially malignant disorders revealed conflicting results on serum lipid and lipoprotein levels. In a famous study by Patel PS et al, a significant decrease in plasma total cholesterol and HDL was observed in head and neck cancer patients as well as in

patients with OPC as compared to the controls. The plasma VLDL and triglycerides levels were significantly lower in oral cancer patients as compared to the plasma VLDL triglyceride levels in patients with OPC and controls.⁸ Study by Vidya K Lohe revealed an inverse relationship between serum lipid profile and oral cancer and precancer.¹⁰

Our study demonstrated statistically significant hypocholesterolemia in OSCC patients as compared to controls. Also, a clinically significant reduction in serum cholesterol levels was noted in leukoplakia patients as compared to controls. Although, serum cholesterol levels were lower in OSCC cases as compared to leukoplakia patients, it was not statistically significant. On the other hand, a significant alteration in levels of triglycerides, HDL and VLDL was not seen in both OSCC and leukoplakia groups. A smaller sample size might have caused any minor changes in these parameters to go unnoticed. In addition, a gradual fall in serum LDL level was observed from leukoplakia to OSCC as compared to control group, but with no statistical significance.

IMPLICATIONS OF LOWER SERUM LIPIDS

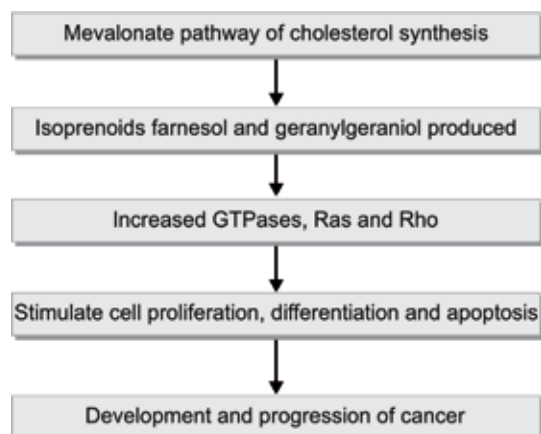
An interesting finding of lower concentration of cholesterol and HDL in patients having widespread disease than in patients with localized tumors was noted in a study by Haltonet et al.⁴⁴ Thereby, serum lipid levels seem to serve as adjunctive prognosticator.

Increased risk of cancer occurrence and mortality in patients with low serum cholesterol is found in some studies.^{29,45,46} One such study by Rose and Shipley reported 66% higher mortality rate in cancer patients with lowest plasma cholesterol than those with highest plasma cholesterol.²⁵

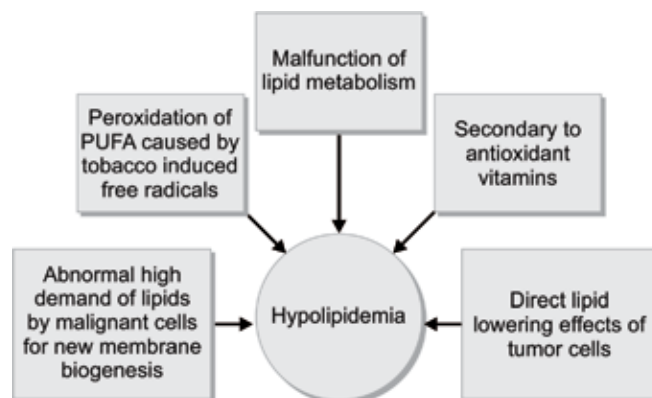
FUTURE STRATEGIES

It is evident from recent studies that both leukoplakia and OSCC cause alterations in lipid metabolism. Thus a molecular level research in this field might reveal changes specific

Flow Chart 1: Schematic diagram depicting hyperlipidemia causing cancer



Flow Chart 2: Schematic diagram on hypolipidemia as cancer effect



to lipid metabolic pathways denoting carcinogenesis. This in future might emerge as an efficient prognosticator of leukoplakia. Hence, an extensive research in this regard is much desired.

CONCLUSION

Statistically significant hypocholesterolemia in OSCC patients as compared to controls is an appreciable finding of our study. Furthermore, OSCC patients showed clinically significant lower cholesterol levels than those with leukoplakia. Also, a gradual fall in serum LDL level was observed from leukoplakia to OSCC as compared to control group. Hence, serum lipid profile may be an adjunctive prognosticator in leukoplakic patients, the advantages being its simplicity, universal availability, patient compatibility. Thus, the present study encourages the use of simple blood-based test like serum lipid profile as an adjunctive prognosticator in leukoplakia patients.

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