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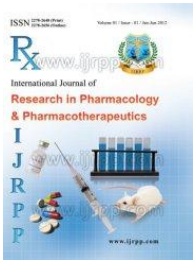

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Plasma lipid profile patterns in oral leukoplakia and oral squamous cell carcinoma

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ABSTRACT

Background

Changes in the lipid profile has been reported to be associated with oral cancer and also in cases of oral premalignant lesions and conditions. This study was done to assess the relationship between plasma lipid profile and oral squamous cell carcinoma (OSCC) and oral leukoplakia (OL). OL is a potentially malignant lesion which could transform into malignancy hence this study evaluates the correlation of cholesterol levels and leukoplakia and also the tobacco abusers.

Aims

to assess the serum lipid levels in OL and OSCC and to look for any relation between incidence of OL and OSCC and lipid profile levels

Settings and design

This study included a total of 126 subjects of which 30 had OL and 30 had OSCC and 66 were age and sex matched controls.

Materials and methods

Analysis of total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), triglycerides (TGL) levels were done using the standard reagents from erba diagnostics, Daman.

Statistical analysis used

SPSS package 14th version and ANOVA was used for multiple group comparison followed by students T test for pair wise comparison.

Results

Plasma levels of HDL, VLDL, TGL and ratio of HDL and LDL was significantly reduced in patients with OSCC and OL. Plasma TC and LDL was significantly reduced in patients with speckled leukoplakia when compared with the rest of the types.

Conclusions
There was a inverse relationship between lipid profile and OSCC and OL.
Keywords: Lipid profile, Oral leukoplakia, Oral squamous cell carcinoma

INTRODUCTION

Cholesterol and triglycerides are the important lipid constituents of the cell and are essential for maintenance of the structural and functional integrity of all biological membranes. They are essential to carry out several vital physiological functions. They are involved in the activity of membrane bound enzymes and are important for stabilization of DNA helix.

Tobacco carcinogens generate reactive oxygen species and lipid peroxides, leading to tissue injury due to elevated lipid peroxidation, further damaging the cellular structural blocks like lipids, proteins, DNA. Thus lipid peroxidation may have a role in endogenous formation of exocyclic DNA adducts. Reduced lipid profile has been reported to be associated with oral cancer and also in cases of oral premalignant lesions and conditions. Incidence of cancer has been reported to be increased in patients with reduced cholesterol levels. Patients with reduced cholesterol and concurrently associated with oral cancer are said to be having a higher mortality.

We carried out a hospital based case control prospective study to assess the relationship between lipid profile and oral leukoplakia and oral squamous cell carcinoma. Association of lipid profile with different types of leukoplakia (homogenous, speckled and verrucous), dysplastic and non dysplastic leukoplakia was studied. Association of lipid profile with types of habits and duration of habit was also studied. Why where all this to be correlated, since the malignant transformation rate for homogenous, speckled and verrucous is different. Hence reduced lipids in speckled leukoplakia cases need to be observed carefully than rest. Same with type of habit and duration, tobacco alone or simple pan or in combination with alcohol may have different effects. Duration of consumption of these substances also effects the lipid profile levels. Hence an effort was made to study their correlation.

MATERIALS AND METHODS

Patients for the study were selected from the outpatient Department of Oral Medicine, Diagnosis and Radiology, Bapuji Dental College and Hospital, Davangere and also from S S Institute of Medical Sciences and Research Center, Davangere. Study included a total of 126 patients, out of which 30 had OL, 30 had OSCC and 66 were age and sex matched controls out of which 33 had habits (either tobacco or alcohol) and 33 were without habits. All cases of OL and OSCC were clinically diagnosed and histopathologically confirmed. After the clinical examination, relevant data were entered into the proforma

Medically compromised patients and individuals on any type of drug that could interfere with the serum lipid profile levels were excluded. OL cases were clinically classified as homogeneous, speckled (non homogeneous) and verrucous form. Patients clinically diagnosed as malignancy and histologically confirmed as oral Squamous cell carcinoma were included in the study. Dysplasia in OL were graded using Smith Pindborg grading system[1] and clinical staging in OSCC was done using TNM classification.[2] Histological grading of OSCC was done using Broders classification.[3] Patients with and without habits were considered in control group as stated. Habits included smokeless and smoked form of tobacco and alcohol users. A detailed history of tobacco use was noted. These information were duly filled in the proforma.

Fresh clean 5ml of blood was collected from patients. This was centrifuged and non hemolyzed serum was extracted. Estimation of plasma lipids were done at Department of Oral Pathology & Microbiology, Bapuji Dental College, Davangere. Plasma levels of TC, LDL, HDL, VLDL and TGL were estimated using *des* kits obtained from Erba Diagnostics, Daman, as per standard protocol and manufacturers recommendations. Statistical analysis were done using SPSS package 14th version and ANOVA was used for multiple group comparison followed by students T test for pair wise comparison.

RESULTS AND OBSERVATION

In this study majority of the patients with OL were in the age range of 41-50 yrs and that of OSCC were in the age range of 31-60 yrs. Majority of males (93.3%) presented with OL than females

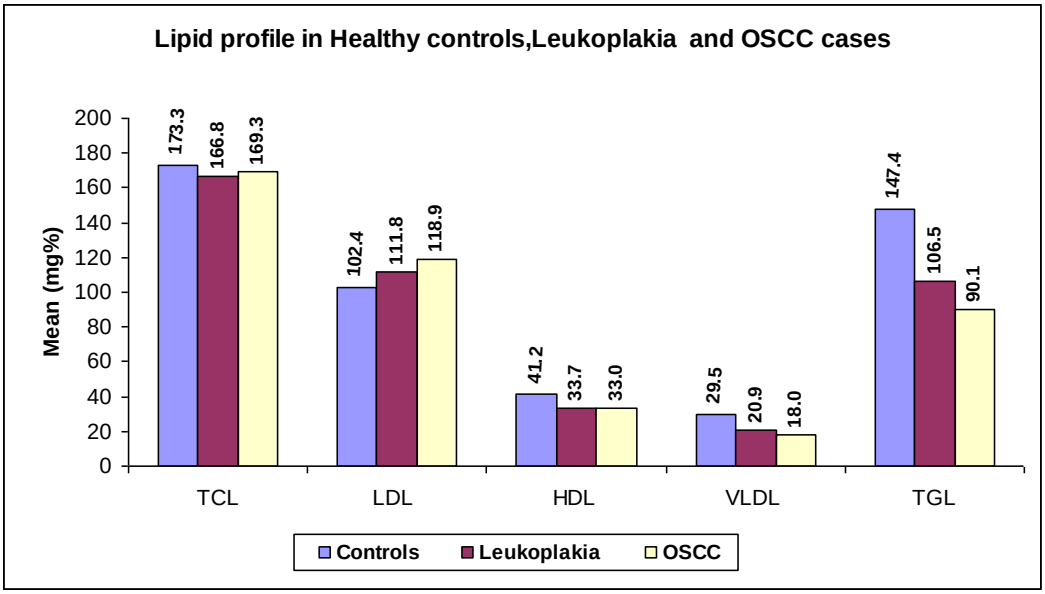
(6.7%). OSCC was predominantly seen in females (57%) than males (43%). Females who presented with OL and OSCC were chewers where as males had a combined form of habit (either smoked / smokeless tobacco / alcohol). Subjects with OL were 100% habit associated and majority (22 cases) had habit of using tobacco for more than 10 years.. In the OSCC group 93.34% had habit where as the rest were habit free. These habits free individuals were females and they all had carcinoma of tongue. Patients with OSCC (80%) used smokeless form of tobacco and majority (18 cases) had habit for more than 10 years. In the OL group 50% of cases were of homogeneous type, 46.7% were of speckled type and 3.3% were of verrucous type. In OL group with a habit of using smoked form of tobacco, 75% of the lesions occurred in the retrocommisure. OL patients using smokeless form of tobacco had 100% of lesions occurring on buccal mucosa and these findings were statistically significant ($p<0.05$). In OL group 23.4% of cases had no dysplasia, 43.3% had mild dysplasia and 33.3% had moderate dysplasia.

Plasma TC was reduced and LDL was increased in subjects with OL and OSCC but the difference was not significant ($p>0.05$). HDL, VLDL, TGL were reduced significantly in OL and OSCC subjects and more so in OSCC subjects and the differences were highly significant ($p<0.001$). Females with OL and OSCC had comparatively higher lipid profile than males but these differences were not significant ($p>0.05$).

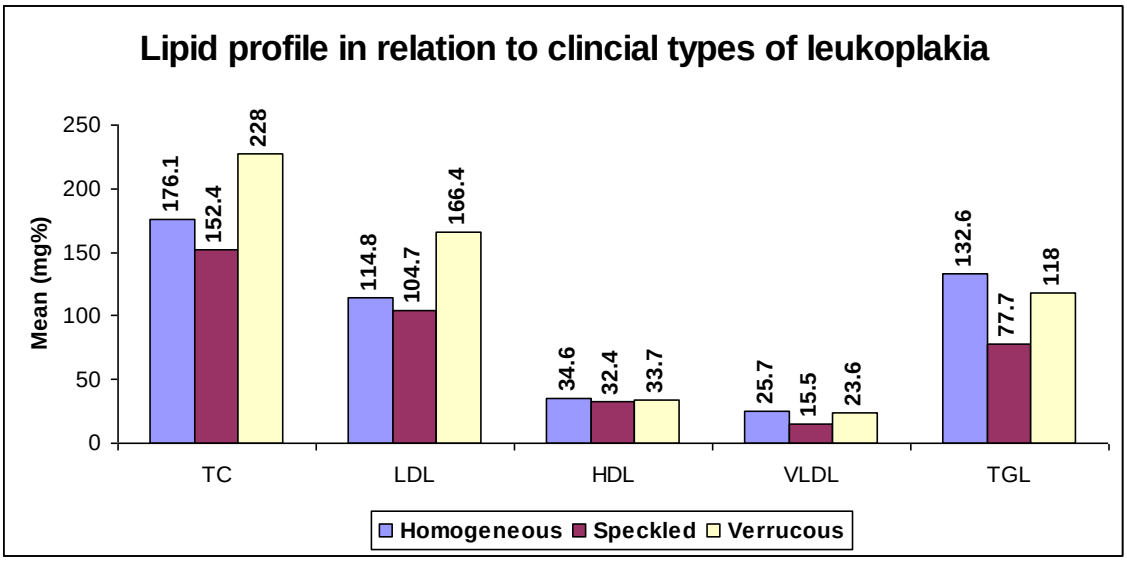
In OSCC group the patients with smokeless form of habit had higher plasma lipid profile values when compared with patients having smoked form of habit but the difference was not significant ($p>0.05$). Also the lipid profile fractions were lowest in stage 2 cancer and highest in stage 3 but the differences were not significant ($p>0.05$).

In OL group, speckled leukoplakia had the lowest lipid profile levels and TC and LDL was maximum in verrucous group. These findings were significant ($p<0.05$) for TC and LDL. In OL group, moderately dysplastic leukoplakia had the lowest TC and LDL, VLDL and TGL was lowest in the dysplastic group when compared with cases having no dysplasia. These finding were significant ($p<0.05$) for plasma VLDL.

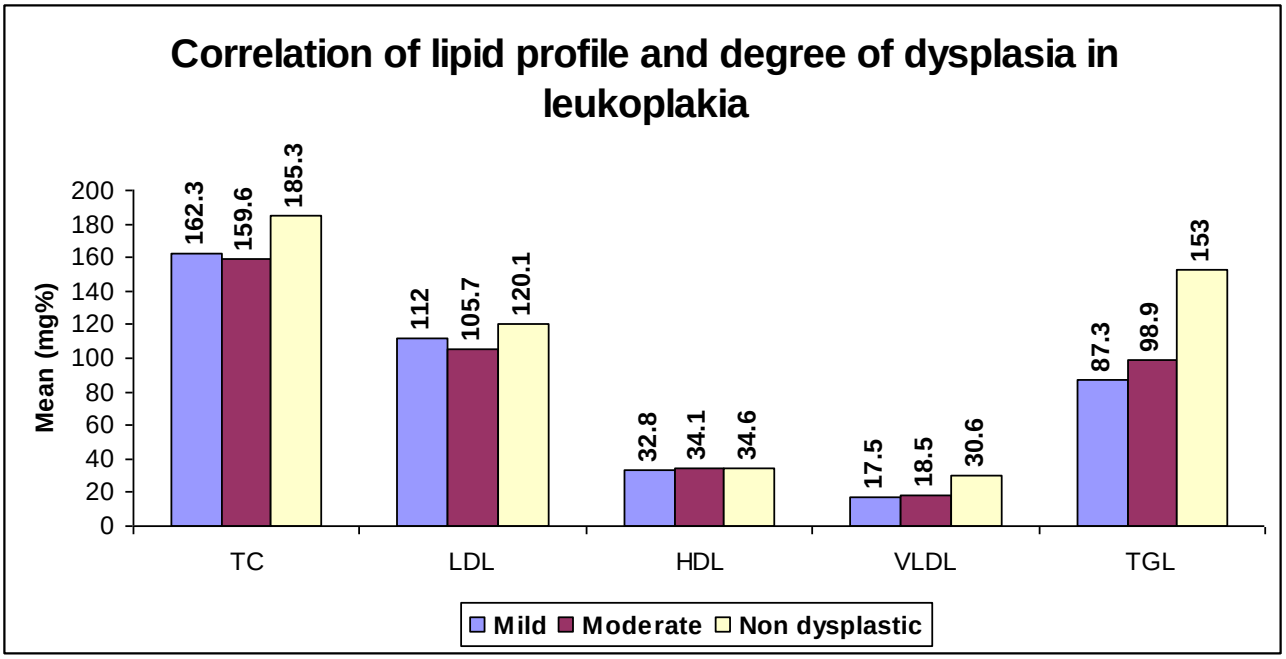
In the control group we observed that those with habits had reduced plasma lipid profile levels than those without any habit. The differences were statistically significant ($p<0.05$) for TC and LDL. TC, VLDL and TGL were less in subjects with habits and LDL, HDL were more in patients without habits comparatively. In OSCC group, patients who had a habit of tobacco use for more than 10 years had reduced TC, LDL, VLDL and TGL except for HDL.



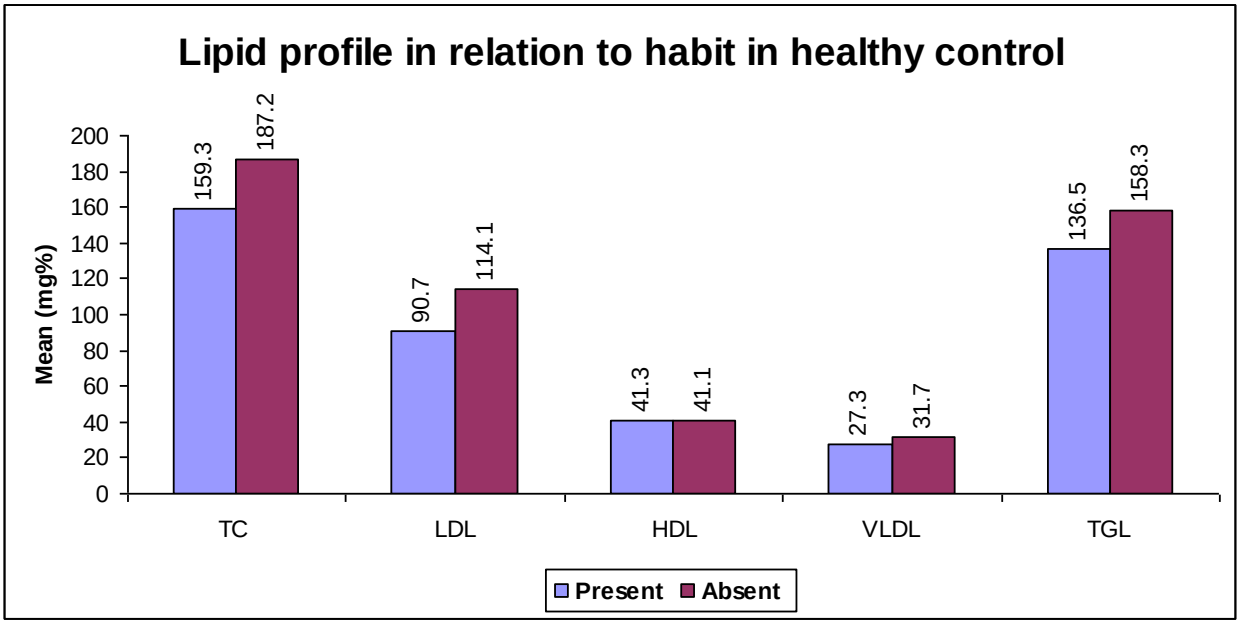
Graph 1 representing the variation of lipid profile in the three study groups.



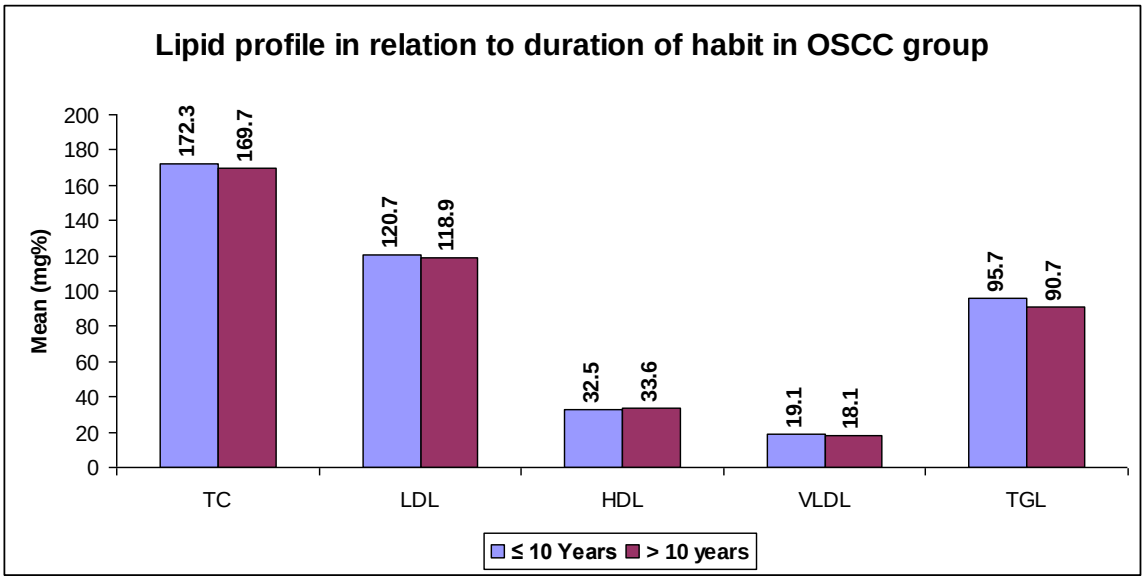
Graph 2 representing the lipid profile levels in the clinical types of leukoplakia and their mean values.



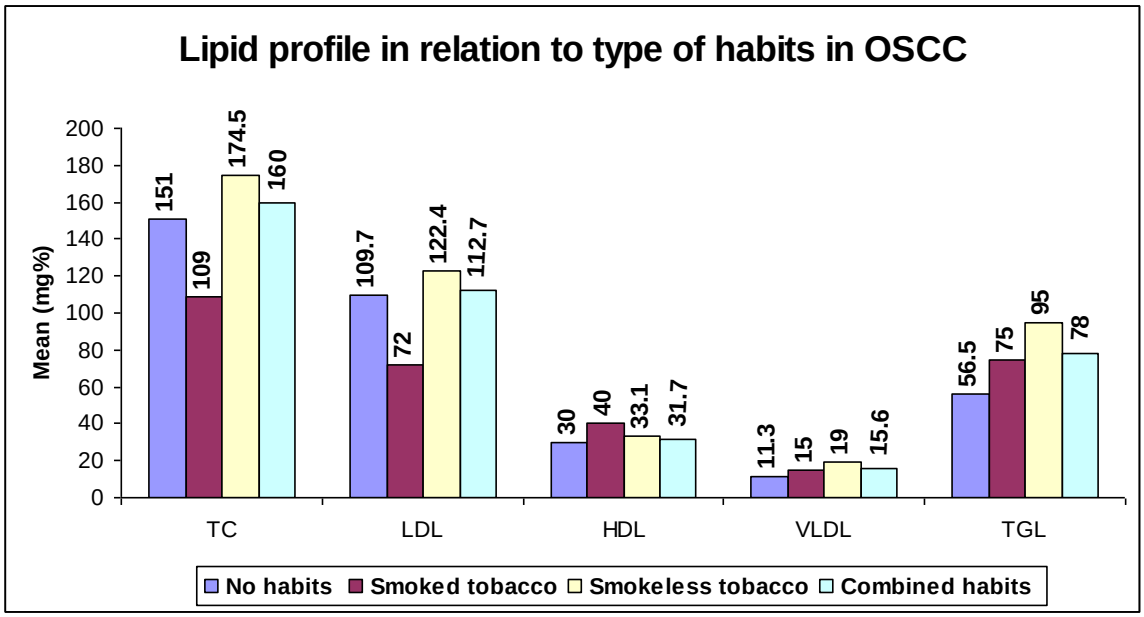
Graph 3 representing the lipid profile in relation to degree of dysplasia in leukoplakia.



Graph 4 Showing lipid profile in relation to habit in healthy controls



Graph 5 Showing lipid profile in relation to duration of habits in OSCC



Graph 6 Showing lipid profile in relation to type of habits in OSCC

Lipid profile in the study groups

Table 1: Table representing the mean values of lipid profile in the three study groups and the statistical data obtained.

Group	Number of cases	TC mg%	LDL mg%	HDL mg%	VLDL mg%	TGL mg%	HDL/ LDL
Controls	66	173.3 ± 42.6	102.4 ±40.9	41.2 ± 2.5	29.5 ± 12.3	147.4 ± 61.3	0.48 ± 0.22
Leukoplakia	30	166.8 ± 30.7	111.8 ± 3.6	33.7 ± 3.8	20.9 ± 11.9	106.5 ± 64.2	0.31 ± 0.08
OSCC	30	169.3 ± 32.9	118.9 ± 6.4	33.0 ± 4.0	18.0 ± 8.8	90.1 ± 44.2	0.29 ± 0.07
ANOVA	F	0.33	2.55	93.4	12.5	11.7	17.0
	P	0.72 NS	0.08 NS	<0.001 HS	<0.001 HS	<0.001 HS	<0.001 HS
Difference between groups	Controls & Leukoplakia	0.72 NS	0.43 NS	<0.01 S	<0.01 S	<0.01 S	< 0.01 S
	Controls & OSCC	0.88 NS	0.08 NS	< 0.01 S	<0.01 S	< 0.01 S	< 0.01 S
	Leukoplakia & OSCC	0.96 NS	0.70 NS	0.71 NS	0.60 NS	0.52 NS	0.83 NS

Table 2: showing lipid profile in relation to clinical diagnosis of leukoplakia

Clinical diagnosis	Number of cases	TC mg%	LDL mg%	HDL mg%	VLDL mg%	TGL mg%	HDL/ LDL
Homogeneous	15	176.1 ± 33.1	114.8 ± 27.5	34.6 ± 4.4	25.7 ± 13.9	132.6 ± 75.7	0.32 ± 0.11
Speckled	14	152.4 ± 17.8	104.7 ± 11.8	32.4 ± 2.6	15.5 ± 7.3	77.7 ± 36.4	0.31 ± 0.04
Verrucous	1	228.0	166.4	33.7	23.6	118.0	0.23

		± 0.0	± 0.0	± 3.8	± 0.0	± 0.0	± 0.0
ANOVA	f	5.54	4.17	2.06	2.99	3.04	0.61
	p	<0.05	<0.05	0.15	0.07	0.06	0.55
		S	S	NS	NS	NS	NS

Tables 3: showing correlation between degree of dysplasia in leukoplakia and lipid profile

Histologic grading	**Number of cases**	**TC mg%**	**LDL mg%**	**HDL mg%**	**VLDL mg%**	**TGL mg%**	**HDL/ LDL**
Mild	13	162.3 ± 23.6	112.0 ± 18.5	32.8 ± 3.8	17.5 ± 8.6	87.3 ± 42.8	0.30 ±0.07
Moderate	10	159.6 ± 39.8	105.7 ± 28.2	34.1 ± 3.4	18.5 ± 10.0	98.9 ± 66.6	0.34 ±0.09
Non dysplastic	7	185.3 ± 23.4	120.1 ± 26.0	34.6 ± 4.7	30.6 ± 15.7	153.0 ± 78.6	0.30 ±0.09
ANOVA	f	1.77	0.75	0.54	3.60	2.80	0.78
	p	0.19 NS	0.48 NS	0.59 NS	< 0.05 S	0.08 NS	0.47 NS

Table 4 showing lipid profile in relation to habit in healthy controls

Habits	**Number of subjects**	**TC mg%**	**LDL mg%**	**HDL mg%**	**VLDL mg%**	**TGL mg%**	**HDL/ LDL**
Present	33	159.3 ± 42.3	90.7 ± 41.3	41.3 ± 2.1	27.3 ± 10.7	136.5 ± 53.6	0.55 ± 0.26
Absent	33	187.2 ± 38.7	114.1 ±37.5	41.1 ± 2.9	31.7 ± 13.5	158.3 ±67.3	0.40 ± 0.14
t value		2.80	2.41	0.29	1.46	1.45	2.92
p value		<0.05 S	< 0.05 S	0.77 NS	0.15 NS	0.15 NS	<0.05 S

Table 5 showing lipid profile in relation to duration of habit in OSCC

Duration of habit	**Number of cases**	**TC mg%**	**LDL mg%**	**HDL mg%**	**VLDL mg%**	**TGL mg%**	**HDL/ LDL**
≤ 10 years	10	172.3 ± 31.2	120.7 ± 22.8	32.5 ±3.7	19.1 ± 10.9	95.7 ± 54.3	0.28 ± 0.07
> 10 years	18	169.7 ± 35.6	118.9 ± 29.5	33.6 ± 4.2	18.1 ± 7.9	90.7 ± 39.5	0.30 ± 0.08
t		0.02	0.17	0.72	0.26	0.26	0.62
p		0.84 NS	0.86 NS	0.48 NS	0.80 NS	0.80 NS	0.54 NS

Table 6 showing lipid profile in relation to type of habits in OSCC

Habits	**Number of cases**	**TC mg%**	**LDL mg%**	**HDL mg%**	**VLDL mg%**	**TGL mg%**	**HDL/ LDL**
No habits	2	151.0 ± 15.6	109.7 ± 22.2	30.0 ± 0.0	11.3 ± 6.6	56.5 ± 33.2	0.28 ± 0.06
Smoked tobacco	1	109.0 ± 0.0	72.0 ±0.0	40.0 ± 0.0	15.0 ±0.0	75.0 ± 0.0	0.56 ± 0.0
Smokeless tobacco	24	174.5 ± 32.7	122.4 ± 26.4	33.1 ± 4.0	19.0 ± 9.5	95.0 ± 47.3	0.28 ± 0.05

Combined habit	3	160.0 ± 26.0	112.7 ± 20.5	31.7 ± 2.9	15.6 ± 3.4	78.0 ± 17.1	0.29 ± 0.03
t		1.73	1.38	1.65	0.57	0.57	9.18
p		0.19 NS	0.27 NS	0.20 NS	0.64 NS	0.64 NS	< 0.05 S

DISCUSSION

Cholesterol and triglycerides are the important lipid constituents of the cell and are essential for maintenance of the structural and functional integrity of all biological membranes. They are essential to carry out several vital physiological functions. They are involved in the activity of membrane bound enzymes and are important for stabilization of DNA helix.[4]

In malignant diseases the blood cholesterol undergoes early and significant changes. Low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the process of carcinogenesis.[4] It is also possible that the association may be secondary to factors such as serum retinol, vitamin E and Beta carotene. [5] Studies involving head and neck cancers have observed an inverse relation between lower serum cholesterol and head and neck as well as esophageal cancers. [6]

Lipid peroxidation is an essential biochemical process that involves the oxidation of polyunsaturated fatty acids, the important components of cell membranes. Tobacco carcinogens generate reactive oxygen species and lipid peroxides, leading to tissue injury due to elevated lipid peroxidation, further damaging the cellular structural blocks like lipids, proteins, DNA. Thus lipid peroxidation may have a role in endogenous formation of exocyclic DNA adducts.[4] It has been reported that smoking alters the serum lipid and lipoproteins by elevation of serum free fatty acids after smoking. It has been suggested that nicotine stimulates secretion of catecholamines, leading to activation of adenylcyclase of adipose tissue, resulting in increased lipolysis, increased concentration of plasma free fatty acids and increased secretion of hepatic triglycerides and VLDL cholesterol into the blood stream.[7] Exposure to tobacco carcinogens hampers antioxidant defense, leading to accelerated lipid peroxidation. There is a strong relationship between vitamin E (a liposoluble antioxidant vitamin) and lipids, especially cholesterol. Vitamin E is co- transported with all forms of cholesterol and contributes to first line defense against lipid peroxidation.[4]

An alcoholic patient would be prone for developing nutritional deficiency and vitamin deficiency which inturn could effect the plasma lipid levels. Vitamin A levels have known to be monitoring cell differentiation and maturation process. However, the hypochoelsterolemia which prevails is due to the cancer effects or is it a cause for cancer occurrence is yet to be answered.[4] Low levels of cholesterol has been associated with increased incidence of cancer and the reverse also has been true where in cancers are associated with low levels of cholesterol.[5]

In our main study groups involving OL, OSCC, we found a reduced TC, HDL, VLDL and TGL when compared with the controls and our findings were similar to that reported by Patel et al[4] and also by Lohe VK et al [8] and also Ghosh G et al. [9] LDL was increased in the OL and OSCC group and is similar to that reported by Manoharan.[10] The enhanced cholesterol levels in these cancer patients can be related to low levels of the micronutrients such as ascorbic acid and vitamin E. These nutrients are known to modulate the pathway for cholesterol biosynthesis and lower plasma cholesterol levels. Both ascorbic acid and vitamin E have a profound effect on the hepatic conversion of cholesterol to bile acids. Various types of tobacco habits and alcohol are known to deplete such essential nutrients which have important effects on lipid and lipoprotein metabolism. [10]

Plasma lipid profile was reduced in aged cancer patients and this could be because individuals with lower lipid profile live longer and hence have more chances of developing cancer than young individuals. [11] It could be related to cumulative effects of DNA adducts.

We found reduced lipid fractions in subjects with a prevalent habit when compared with those who did not have any habit and is similar to that reported by Patel et al.[4] It could be attributed to lipid peroxidation by tobacco.[4] Our study found that

smokeless form of tobacco is associated with enhanced lipid profiles when compared with smoked form of tobacco. Our finding is substantiated by that reported by Tucker.[12] They reported that smokeless form of tobacco had two and half times more chances of hypercholesterolemia and could be due to multiple effects and not a cause and effect.[12] Prolonged use of tobacco results in accumulation of nicotine and hence affects the free fatty acid levels and that could be the reason that our subjects with a habit of tobacco use for more than 10 years had reduced lipids when compared with individuals with habit less than 10 years.

Our study found that speckled leukoplakia had the lowest TC and LDL, it could have been a preliminary finding. Patel et al [4] reported that oral precancerous lesions and conditions in general are associated with reduced lipid profile. Since speckled leukoplakia is associated with highest malignant transformation rate, lowered lipid profile levels could serve as a marker for malignant transformation. Our study also found that moderately dysplastic leukoplakia had the lowest lipid fractions, once again dysplastic leukoplakia has been associated with malignant transformation in about 11-36% of patients. Hence lowered lipid profile in these conditions could assess the malignant transformation. It could have been a preliminary finding in literature.

Lowered lipid profile could assess the prognosis in patients with OSCC, since less of cholesterol in cellular membrane makes the cells hyper-permeable. Such cells are more porous for the carcinogens, which reaches the nucleus and cause DNA adducts. Hence results in malignancy. [13] Defective cholesterol synthesis results in defective T and B lymphocyte functions, which cannot detect the cancer cells and results in loss of immune surveillance. [14]

CONCLUSION

We observed reduced lipid profile in OSCC and OL cases. When individual habits like use of tobacco in smoked / smokeless form is considered to be prime factor for the occurrence of OSCC and OL were considered, we found that smokeless tobacco users had increased lipid profile than smokers and controls. We had OSCC patients who a had a predominant habit of chewing. This was quite contradictory. It could be possible that these two findings were entirely different from each other, since the disease per se and chewing tobacco could be having different effects on lipids and hence their net results were diverting from each other. It could be because tobacco chewing has different association with cholesterol. We found a significant inverse association between lipid levels and OSCC like many other studies which favors our finding. OSCC and OL is associated with reduced lipid profiles and it can be used to assess the prognosis as discussed earlier. Even lipid profiles can be used as a marker for malignant transformation in cases of OL and hence early detection of malignancies in OL so that early treatment can be instituted, that would cure the disease and increase the survival rate in these patients. The association of individual habits of tobacco chewing and smoking on lipid profiles needs to be further studied considering larger samples and this forms the future prospective of the study.

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