ORIGINAL RESEARCH

Alterations in serum lipid profile patterns in patients with oral submucous fibrosis

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Abstract

Aim: Altered lipid profile patterns have been associated with pre-malignancies and malignancies because lipids play a vital role in the maintenance of cell integrity. The present study evaluated alterations in serum lipid profile in untreated patients with oral submucous fibrosis (OSF) and compared the changes in serum lipid profiles among various histological grades of OSF.

Materials and methods: The study included 30 patients with oral submucous fibrosis and 19 healthy controls. Serum lipids, including (i) total cholesterol, (ii) LDL cholesterol (LDLC), (iii) HDL cholesterol (HDLC), (iv) VLDL cholesterol (VLDLC) and (v) triglycerides, were analyzed using spectrophotometry kits.

Results: A significant decrease in serum total cholesterol (TC) levels, TC:HDLC ratios(p = 0.005, p = 0.001 respectively) were observed in oral submucous fibrosis patients as compared to the control group. The serum lipid profiles in different histologic gradings of OSF namely early and moderately advanced OSF showed no significance. Our data strengthens the evidence of an inverse relationship between serum lipid levels and oral submucous fibrosis.

Conclusion: Our findings suggest that decrease in total cholesterol in patients with OSF could be due to the greater utilization of lipids including total cholesterol by the cells for new membrane biogenesis. Our findings strongly warrant an in depth study of alterations in serum lipid profile in patients with OSF. The lower serum lipid levels may have a diagnostic or prognostic role in the early diagnosis of Oral premalignant and malignant lesions.. Low levels of cholesterol could be due to the rapidly dividing cells in premalagnancies and malignancies

Introduction

Oral submucous fibrosis (OSF) is a chronic, progressive, scarring disease associated with the chewing of areca nut, an ingredient of betel quid and predominantly affects the people of South- East Asian origin. This condition was described first by Schwartz (1952) while examining five Indian women from Kenya, to which he ascribed the descriptive term 'atrophia idiopathica (trpoica) mucosae oris,. Later in 1953, Joshi from Bombay (Mumbai) redesignated the condition as oral sub mucous fibrosis ,implying predominantly its histologic nature. The disease is characterised by burning sensation in the mouth while consuming spicy food, appearance of vesicles in the cheek and palate and fibrosis of the oral mucosa resulting in difficulty in mouth opening. The WHO definition of an oral precancerous condition –"a generalized pathological stage of the oral mucosa associated with a significantly increased risk of cancer," describes well the characteristics of OSF.[1]

Lipids are major cell membrane components essential for various biological functions, including cell growth and division of normal and malignant tissues. Usefulness of variations in tissue/blood cholesterol levels in diagnosis and treatment of various diseases has been studied by several workers.[2,3,4] There is compelling evidence to implicate the habitual chewing of areca nut in the development of OSF.[1] The major alkaloid in areca nut arecoline undergoes nitrosation and gives rise to N-Nitrosamine, which might have cytotoxic effect on the cells.[5] This may induce the production of free radicals and reactive oxygen species which are responsible for high rate oxidation/ peroxidation of polyunsaturated fatty acids, this peroxidation further releases peroxide radicals which affect essential constituents of the cell membrane and might be involved in tumorigenesis. Because of the lipid peroxidation, there is a greater utilization of

lipids, including total cholesterol, lipoproteins and triglycerides for new membrane biogenesis. Cells fulfil these requirements either from circulation by synthesis through the metabolism or from degradation of major lipoprotein fractions, like VLDL, LDL or HDL.[6] Lower blood lipid levels have been associated with various cancers.[7,8,9,10]

However , only a few studies have been carried out on serum lipid profiles in precancerous conditions Considering this, the present study was aimed at evaluating the serum lipid profile, including total cholesterol, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), very low-density lipoprotein cholesterol (VLDLC) and triglycerides in oral sub mucous fibrosis patients and comparing it with values among control groups and also among the various histological grades of OSF.

Subjects and Methods

The study was conducted at the Department of Oral Medicine and Radiology, Department of Oral and Maxillofacial pathology, Meenakshi Ammal Dental College and Hospital, Chennai

The study subjects comprised 3 groups as follows:

1. Group 1: Control Group.
2. Group 2: OSF Group.
3. Group 3: Subjects with different histologic grades of OSF.

Selection of subjects

Group 1(Control group) comprised of healthy individuals in the age group of 20-65 years, sex matched with those of the OSF group and with no deleterious oral habits and no associated oral lesions. Those with systemic diseases and disorders such as chronic heart disease, diabetes mellitus and AIDS, and bleeding dyscrasias were excluded. Patients on drugs that alter lipid profile were also excluded.

Group 2 (OSF group) comprised of patients in the age group of 20-65 years and those who were clinically diagnosed to have oral submucous fibrosis lesions which were confirmed histopathologically. Those with systemic diseases and disorders such as chronic heart disease, diabetes mellitus and AIDS, and bleeding dyscrasias were excluded. Patients on drugs that alter lipid profile were also excluded.

Method of examination and confirmation of clinical diagnosis

The patients were explained in detail about the study and the procedure they were subjected to .A formal informed written consent was obtained. Examination of the patients was carried out using a mouth mirror and probe under artificial light. The clinical photographs were obtained to complete the clinical records. Routine hematological examinations (to ascertain bleeding time, clotting time, fasting blood sugar levels, hemoglobin count and

erythrocyte sedimentation rate) and blood pressure estimation were done for all subjects to rule out any systemic diseases. A comprehensive history was obtained from the patients with reference to their habits and patients with burning sensation, difficulty in mouth opening and palpable fibrotic bands were clinically diagnosed as OSF and were finally confirmed by histopathology.

Collection of Venous Blood

Fasting blood samples were collected under all aseptic precautions by vein puncture. Two cc of venous blood was withdrawn with the help of a 2 ml disposable syringe and a 24 gauge disposable needle, into plain vacuettes. These samples were allowed to clot for 30 minutes and then centrifuged for 15 minutes to get a clear serum sample which is separated from the clot and transferred to a disposable vial for assay. The estimation was performed within 3 hours of receiving the samples by using an appropriate kit (Genuine Biosystem) and quantitated for total serum cholesterol, LDL, VLDL, HDL and triglycerides using a computerized semiautomated biochemistry analyzer.

Serum cholesterol levels were estimated using cholesterol kits obtained from Genuine Biosystem, Chennai, India. Briefly, 10 µl of plasma sample was mixed with 500 µl of working reagent that contained cholesterol oxidase, cholesterol esterase, peroxidase, 4-aminophenazone, surfactant, phenol, buffer, preservatives and stabilizer. The mixture was incubated at 37°C for 10 minutes and absorbance was read at 505 nm.

Serum HDL cholesterol levels were also estimated using cholesterol kits. Briefly, 10 ml plasma sample was mixed with 0.3 ml precipitating reagent (PEG 6000, stabilizer and preservative), followed by 10 minutes incubation at room temperature. The mixture was centrifuged at 2000 rpm for 15 minutes. The supernatant obtained was mixed with working cholesterol reagent. After incubation of 10 minutes 37°C, absorbance was read at 505 nm.

Quantitative serum triglyceride levels were estimated using reagent kits. Briefly, 10 µl plasma sample was mixed with 1000 µl of triglycerides assay reagent containing pipes buffer, lipase, 4-chlorophenol, Mg ion, ATP, lipase, peroxidase, glycerol kinase, sodium azide, 4-aminoantipyrene, glycerol-3-phosphate oxidase and detergents. The mixture was then incubated for 10 minutes at 37°C and absorbance was read at 505 nm.

VLDLC and LDLC levels were calculated as given below:

VLDLC = Triglycerides/5
LDLC = Total cholesterol - (VLDLC) - (HDLC).

Statistical analysis

The statistical analysis consisted of the student's t-test, which was performed to compare mean values of the parameters. The SPSS 15.0 for Windows was used to ascertain the results. p-value < 0.05 was considered to be statistically significant.

Results

Among the 30 patients diagnosed clinically as OSF, all were males (Table 1).This included the age group ranging from 20-60 yrs. The lesions were mostly (60%) seen in the age group of 20-30 years,) and least (3%) in the 50-60 years age groups (Table 2). The control group consisted of 19 patients who were matched with age and sex with those of the OSF group. Majority of them (50%) had the habit of areca nut with tobacco chewing. Among 30patients with OSMF, 10 had habit of chewing areca nut alone (33%), 5(17%) had the habit of smoking, alcohol consumption and areca nut chewing. However, smoking or alcohol consumption alone was associated with lesions in OSF group (Table 3). Based on their histological diagnosis, we found only early OSF and moderately advanced OSF in our study. Majority (56.7%) had the moderately advanced type of OSF (Table 4).

Table 1. Gender distribution among patients in the oral submucous fibrosis group

S No	GENDER	TOTAL	PERCENTAGE
1	MALE	30	100
2	FEMALE	0	0

Table 2. Age distribution among patients in the oral submucous fibrosis group

S.No	Age Group	Total	Percentage
1	20-30	18	60%
2	31-40	9	30%
3	41-50	2	07%
4	51-60	1	03%
	Total	30	100%

Table 3. Distribution of habits among patients in the oral submucous fibrosis group

S. No	Habits	Total	Percentage
1	Areca nut + tobacco Chewing	15	50%
2	Areca nut chewing	10	33%
3	Smoking+alcohol+Areca nut chewing	5	17%
4	Smoking	0	0%
5	Alcohol	0	0%

Table 4. Histological Grading of lesions in patients with oral submucous fibrosis

SNO	Grading	Total	Percentage
1	Early	13	43.3%
2	Moderately Advanced	17	56.7%

Comparison of serum lipid profiles between Groups 1 and 2

The mean serum lipid profiles of control group and oral submucous fibrosis group, at the time of diagnosis, are given in Table 5 and Graph 1. A significant decrease in serum total cholesterol (TC) levels and ratio of TC: HDL (p =0.005, p =0.001 respectively) and a nonsignificant decrease in the serum Triglycerides, low-density lipid cholesterol (LDLC) levels and very low-density lipid cholesterol (VLDLC) levels (p = 0.035, p = 0.014 and p = 0.056 respectively) were observed in oral submucous fibrosis group as compared to the control group.

The serum lipid profile in patients with early OSF and moderately advanced OSF were analysed using independent t test . There was no significant difference in serum lipid profiles between the two groups.

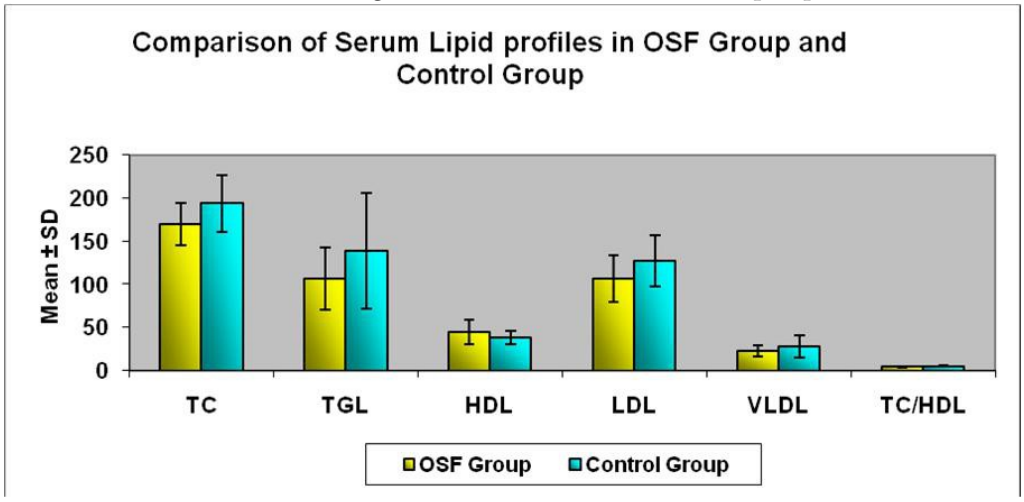

Table 5. Serum lipid profile in patients with oral sub mucous fibrosis and controls

	Group	N	Mean	Std.Deviation	P value
TC	OSF Group Control Group	30 19	169.73 194.16	24.740 32.703	0.005 "S"
TGL	OSF Group Control Group	30 19	107.17 139.21	36.016 67.318	0.035 " NS"

HDL	OSF Group Control Group	30 19	44.70 38.58	14.735 7.669	0.102 "NS"
LDL	OSF Group Control Group	30 19	106.20 127.32	26.958 29.806	0.014 "NS"
VLDL	OSF Group Control Group	30 19	22.37 28.00	6.552 13.515	0.056 "NS"
TC/HDL	OSF Group Control Group	30 19	22.37 28.00	.7493 .8813	0.00 **"S"**

*S= Significant **NS= Not significant

Table 6. Comparison of serum lipid profile with histological grading in patients with sub mucous fibrosis

	Histological Grading	N	Mean	Std.Deviation	P value
TC	Early OSF Moderately Advanced OSF	13 17	175.85 165.06	23.919 25.034	0.243 " NS"
TGL	Early OSF Moderately Advanced OSF	13 17	110.62 104.53	25.102 43.145	0.655 "NS"
HDL	Early OSF Moderately Adv OSF	13 17	39.15 48.94	4.62 18.253	0.071 "NS"

LDL	Early OSF	13	114.69	18.477	0.134 "NS"
	Moderately Adv OSF	17	99.71	30.947	
VLDL	Early OSF	13	22.77	5.659	0.774 "NS"
	Moderately Adv OSF	17	22.06	7.318	
TC/HDL	Early OSF	13	4.508	.5604	0.131 "NS"
	Moderately Adv OSF	17	4.088	.8373	

*S= Significant **NS= Not significant

Discussion

Lipids are major cell membrane components essential for various biological functions including cell growth and division of normal and malignant tissues. The changes in lipid profile have long been associated with cancerous and precancerous conditions because lipids play a key role in maintenance of cell integrity.[1] As lipids may play a role in precancer and cancer, this study was aimed to estimate serum lipid profile in oral submucous fibrosis groups and to *compare* the serum lipid profiles in oral submucous fibrosis groups values with values from control groups. Apart from this comparison of changes in serum lipid profiles among various histolgical grades of OSF were also analysed.

Different methods of histopathological classification are available for oral submucous fibrosis. The recent one is simplified into three grades as early, intermediate and advanced. As it does not define the tissue changes clearly, we adopted the earliest classification of Pindborg *et al* [12]. Our cases comprised of 13 early and 17 moderately advanced OSF cases. Out of 30 cases, all 30 patients were males. Srinivasan M *et al* [13] as well as Ranganathan K *et al* [14] have reported a male predominance in their studies.

In the present study, comparision of the serum lipid profiles in OSF group and control group showed that there was a decrease in TC level (p=0.005) in the OSF group when compared to that of the control group and the results were significant. This

is consistent with the studies done by Patel PS *et al*[11] and Vidya K. Lohe *et al* [15] who also noticed significant decrease in TC levels in their patients.

Further in the present study, comparison of the serum lipid profiles in OSF group and control group revealed that there was decrease in TGL (p=0.035), LDL (p=0.014) , VLDL (p=0.056) in the OSF group when compared to the control group but the results were not significant. This is consistent with the observation by Ravi Mehrotra *et al* [16] In their studies also the TGL, LDL and VLDL levels in OSF patients were not significantly high. However, Patel PS *et al* [11] reported a significant decrease in TGL, LDL, VLDL and HDL in patients with oral precancerous conditions.

Inour patients with OSMF, the levels of HDL were increased when compared to the control group and this is not consistent with the findings of the above authors. The ratio of TC/HDL was also decreased in our patients. This can probably be attributed to reverse cholesterol transport, a key component of cholesterol homeostasis.[17]

The high HDL in our patients and the decreased ratio of TC/HDL is however consistent with the results of the study done by R. Mallika *et al*[18] in which high HDL levels were seen in tobacco consumers. In their study they showed that sustained physical activity in tobacco users increased the HDL and the TC/HDL ratio in tobacco users were low. Apart from this a comparison was also made based on the serum lipid profiles in different histologic gradings of OSF namely early and moderately advanced OSF but the differences were not significant .

The results in our study add to the evidence of an inverse relationship between serum lipid profile and oral submucous fibrosis.The decrease in total cholesterol in patients with OSF could be due to the excessive use of areca nut. In areca nut the major alkaloid arecoline undergoes nitrosation and gives rise to N- nitrosamine, which might have cytotoxic effect on the cells. This may induce the production of free radicals and reactive oxygen species which are responsible for high rate oxidation peroxidation of polyunsaturated fatty acids and this peroxidation further releases peroxide radicals which affect essential constituents of the cell membrane and might be involved in tumorigenesis. Lipid peroxidation leads to greater utilization of lipids including total cholesterol, lipoproteins and triglycerides for new membrane biogenesis. Cells fulfil these requirements either from circulation by synthesis through the metabolism or from degradation of major lipoprotein fractions. [5, 6]

Low levels of cholesterol could be due to the rapidly dividing cells in malignancies. Our findings strongly warrant an in depth study of alterations in serum lipid profile in these patients. In our study, the decrease in total cholesterol levels may be a useful indicator reflecting initial changes occurring

in precancerous and neoplastic conditions. The evidence suggests that in precancerous conditions like OSF cells are able to remetabolize lipids for their growth and to generate phospholipids membranes. Recent progress in molecular biology will assist researchers in the near future to identify the genes and enzymes of lipid metabolic pathways.

Apart from studies on serum lipid levels, the role of iron, hemoglobin, minerals such as copper, selinium and zinc have also been studied in patients with OSF. The changes in the levels of these parameters can be used as surrogate markers in early diagnosis of OSF patients.[19-22]

Conclusion

From the present study, the lower serum lipid levels may have a diagnostic or prognostic role in the early diagnosis of Oral premalignant and malignant lesions.. Low levels of cholesterol could be due to the rapidly dividing cells in premaliganincies and malignancies.

Our findings suggest that decrease in total cholesterol in patients with OSF could be due to the greater utilization of lipids including total cholesterol by the cells for new membrane biogenesis. As there is a change in the serum lipid profile in these patients, we can conclude that the change in lipid levels may have a diagnostic or prognostic role in the early diagnosis of Oral premalignant and malignant lesions.

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