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A Study on Alteration in Serum Lipid Profile Pattern in Oral Submucous Fibrosis

KEYWORDS: Cholesterol, oral submucous fibrosis, lipid profile

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ABSTRACT *Introduction: Oral submucous fibrosis (OSMF) is a chronic disease of the oral cavity. Alteration in lipid profile has been associated with malignancies as lipids are required for maintenance of structural and functional integrity of cell membrane.*

Material and Methods: The study was conducted on 45 clinically and histopathologically diagnosed cases of OSMF and 45 age and sex matched controls. Lipid profile including Total cholesterol, Triglycerides, HDL cholesterol, LDL cholesterol and VLDL cholesterol were estimated.

Results: There was significant reduction in serum lipid levels in the patients with OSMF when compared with the controls. The reduction in lipid levels was maximum in stage 3 OSMF when they were compared among the different stages. The present study demonstrated that the level of serum lipids decreased with progression of the disease.

Conclusions: From these findings, it appears that the lower serum lipid levels may be considered as a useful indicator in diagnosis of initial changes occurring in oral premalignant condition like OSMF.

Introduction

Oral submucous fibrosis (OSMF) is a chronic disease of the oral cavity, which is characterized by an epithelial and subepithelial inflammatory reaction followed by fibroelastic changes in the submucosa which was first described by Schwartz in 1952.[1] This disease occurs most commonly in South East Asia but cases have been reported worldwide in countries like Kenya, China, UK, Saudi Arabia and other parts of the world.[2] Over the years, the incidence of OSMF has increased in various parts of the Indian subcontinent.[3] Its prevalence ranges up to 0.4% in Indian rural population and it had been shown by Hazarey V, Erlewad D, Mundhe K, Ughade S (2007) that malignant transformation rate is 7.6%.[4]

The etiological factors to date are chewing of areca nut, capsaicin in chillies, genetic and immunologic processes, micronutrient deficiencies of iron, zinc and essential vitamins. These factors derange the repair of the inflamed oral mucosa, leading to defective healing and resultant scarring.[5] Betel quid chewing is seen almost exclusively in the Indian subcontinent, South East Asia and western Pacific. Tilakaratne, Klinikowski, Saku, Peters, Warnakulasuriya (2006) reported that areca nut is the main etiological factor for OSMF.[6]

The pathogenesis of the disease is not well established, but the cause of OSMF is believed to be multifactorial. Pathogenesis is believed to involve juxta-epithelial inflammatory reaction and fibrosis in the oral mucosa, probably due to increased cross-linking of collagen through up-regulation of lysyl oxidase activity. Fibrosis, or the buildup of collagen, results from the effects of areca nut, which increases collagen production (e.g., stimulated by arecoline, an alkaloid) and decreases collagen degradation. Thus, OSMF is now considered a collagen metabolic disorder. [7]

Also the excessive use of areca nut may also induce the production of free radicals and reactive oxygen species, which are responsible for high rate of oxidation/ peroxidation of polyunsaturated fatty acids which affect essential constituents of cell membrane and might be involved in tumorogenesis.[8,9] Because of the lipid peroxidation, there is a greater utilization of lipids for new membrane biogenesis. Cells fulfill these requirements either from circulation, by synthesis through the metabolism or from degradation of major lipoprotein fractions like very low density lipoprotein (VLDL), low density lipoprotein (LDL) or high density lipoprotein (HDL).[10] Researchers have reported association of plasma/serum lipids and lipoproteins with different cancers[11,12] as cholesterol is essential for maintenance of structural & functional integrity of all biological membranes including cell growth and division of normal and malignant tissues.

The association of hypolipidemia in cancer at the time of diagnosis is a causative factor or a consequence of cancer is not yet known.[13] However, there are only a few studies available on plasma lipid profile in OSMF.[14,15,16] Therefore in our study, we are attempting to evaluate the various biochemical lipid parameters such as total cholesterol (TC), triglyceride (TG), HDL, LDL and VLDL levels in 3 groups of oral submucous fibrosis.

Material and Methods

This study was conducted on 45 clinically and histopathologically diagnosed cases of OSMF. All the patients underwent recording of detailed history, and clinical diagnosis was made on the presence of characteristic features like vesicles, ulceration, mucosal blanching, burning, stiffness of oral mucosa, presence of characteristic fibrous bands and progressive inability to open the mouth. OSMF was divided clinically and functionally into three stages according to the criteria reported by Haider *et al.* [17], based on the presence of fibrous bands at various anatomical sites, and functional staging was based on the degree of mouth opening.

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Clinical Staging:
- Stage 1: Faucial bands only
- Stage 2: Faucial and buccal bands
- Stage 3: Faucial, buccal and labial bands

Functional Staging:
- Stage 1: Mouth opening >20 mm
- Stage 2: Mouth opening 11-19 mm
- Stage 3: Mouth opening <10 mm

45 healthy individuals, matched for age and sex, had no adverse habits or oral lesions, or any other major illness in recent past, were included as controls.

Exclusion criteria includes patients under treatment for OSMF, oro-mucosal disorders with clinical features same as OSMF and any systemic disorders causing similar symptoms. Informed consent was obtained from patients in both the case and control groups. The study was approved by the institutional ethical committee.

After the confirmation of OSMF, 5 ml of fasting (12-14hrs) blood sample was collected in a sterile bottle and allowed to clot for about an hour at 37°C. The serum was then separated by centrifugation at 3000 rpm for 5 min and stored at 4°C. Serum total cholesterol was estimated by CHOD-PAP (Cholesterol Oxidase – Peroxidase) [18], the serum triglycerides by the GPO-PAP (Glycerol-3-phosphate Oxidase – Peroxidase)[19] and HDL-Cholesterol by enzyme selective protection method of Williams P et al.[20] The LDL Cholesterol level was calculated by the Friedewald's equation. [21]

VLDL= Triglycerides/5
LDL=Total Cholesterol- HDL-VLDL.

Statistical analysis
Statistical analysis of the data was carried out with SPSS, version 19. The data was reported as mean ± SD. Student's t-test was used to compare serum lipid profile between the two groups. Analysis of variance [ANOVA] was used to assess the statistical significance of differences between the stages of OSMF and the control group. 'p' value <0.05 was considered as statistical significant.

Results
In the present study, the control group comprised of 45 subjects, out of which 35(77.7%) were males and 10(22.3%) were females with an age range of 22-47 years and mean age 32.77 ± 6.38 years. The OSMF group comprised of 38(84.4%) were males and 7(15.6%) were females out of total of 45 patients with an age range between 25 – 49 years and mean age of 31.57 ± 6.08 years. Majority of the patients (25; 55.6%) were in their second decade of life with a male predominance. In the third and fourth decade patients averaged 33.3% and 11.1% respectively.

Table 1. Age and sex wise distribution of controls and OSMF cases

Details	Controls	OSMF cases
Number of subjects	45	45
Mean age (Mean ± Standard deviation)	32.77 ± 6.38 years	31.57 ± 6.08 years
Age range	22 - 47 years	25 – 49 years
Males	35(77.7%)	38(84.4%)
Females	10(22.3%)	7(15.6%)

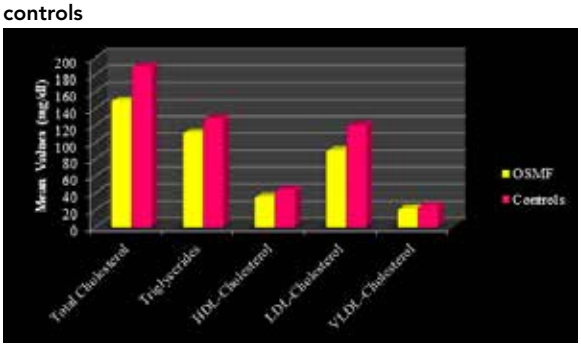
The mean serum TC, serum TG, serum HDL cholesterol, serum LDL, serum VLDL levels in OSMF group were 150.64 **± 18.78 mg/**dL, 112.34 **± 36.74 mg/**dL, 36.69 **± 6.88 mg/**dL, 91.48 **± 16.05** mg/dL and 22.46 **± 7.34 mg/** dL respectively. However, in the control group the corresponding values were 190.12 **± 29.96** mg/dL, 128.25 **± 39.04** mg/dL, 44.04 **± 6.44** mg/dL, 120.42 **± 28.41** mg/dL and 25.65 **± 7.8** mg/dL respectively. (Table 2) A statistically significant reduction [P<0.05] in the mean values for all lipid parameters was noted in OSMF cases when compared with the control group.

Table 2. Comparison of mean serum lipid profile among control group and OSMF cases.

	OSMF (n=45)	Controls (n=45)	't' value	P value
TC	150.64 **± 18.78**	190.12 **± 29.96**	7.4899	<0.0001**
TG	112.34 **± 36.74**	128.25 **± 39.04**	1.9908	0.0496*
HDL	36.69 **± 6.88**	44.04 **± 6.44**	5.2320	<0.0001**
LDL	91.48 ± 16.05	120.42 **± 28.41**	5.6765	<0.0001**
VLDL	22.46 **± 7.34**	25.65 **± 7.8**	1.9980	0.0488*

**** p < 0.001; Highly significant, *p<0.05; Significant**

Figure 1: Mean lipid profile levels between OSMF and controls



In different stages of OSMF i.e stage 1, stage 2 and stage 3, the mean values for TC were 171.12 ± 16.43 mg/dL, 147.74 ± 3.57 mg/dL and 133.06 ± 6.89 mg/dL; for TG were 127.69 **± 38.89** mg/dL, 118.96 ± 38.57 mg/dL and 90.36 ± 20.45 mg/dL; for HDL were 39.31 ± 5.07 mg/dL, 36.57 ± 8.55 mg/dL and 34.19 ± 6.01 mg/dL; for LDL were 106.27 ± 15.23 mg/dL, 87.37 ± 10.47 mg/dL and 80.80 ± 9.64 mg/dL; for VLDL were 25.53 ± 7.79 mg/dL, 23.79 ± 7.71 mg/dL and 18.07 ± 4.09 mg/dL for TC, respectively. A gradual fall in the mean values of lipid profile was seen with disease progression i.e from OSMF stage 1 to stage 3 (Table 3). Also these values were lower in the different stages of OSMF when compared with the controls.

Table 3. Mean serum lipid profile among control group and different stages of OSMF.

	Group I (Stage I) n=15	Group II (Stage II) n=15	Group III (Stage III) n=15	Group IV (Stage IV) **n=45 controls**
TC	171.12 ± 16.43	147.74 ± 3.57	133.06 ± 6.89	190.12 **± 29.96**

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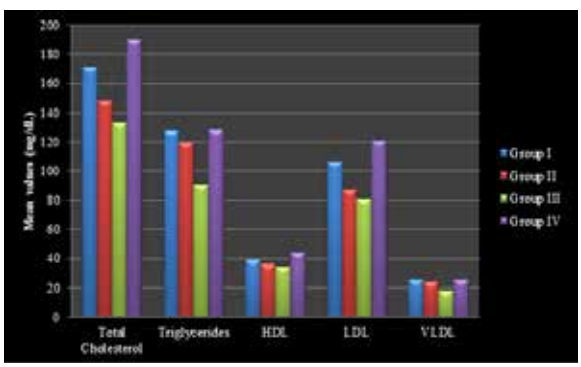
	Group I (Stage I) n=15	Group II (Stage II) n=15	Group III (Stage III) n=15	Group IV (Stage IV) **n=45 controls**
TG	127.69 ± **38.89**	118.96 ± 38.57	90.36 ± 20.45	128.25 ± **39.04**
HDL	39.31 ± 5.07	36.57 ± 8.55	34.19 ± 6.01	44.04 ± **6.44**
LDL	106.27 ± 15.23	87.37 ± 10.47	80.80 ± 9.64	120.42 ± **28.41**
VLDL	25.53 ± 7.79	23.79 ± 7.71	18.07 ± 4.09	25.65 ± **7.8**

Figure 2: Mean lipid profile levels among different stages of OSMF and controls.



ANOVA test for inter-group and intra-group comparisons of serum lipid showed a statistically significant difference in non-homogeneity for the means of four groups. (Table 4).

Table 4. ANOVA test for serum lipid profile among various groups.

		Sum of squares	Df	Mean square	F value	P value
TC	Between groups	46123.536	3	15374.512	29.971	<0.0001
	Within groups	44116.737	86	512.985		
	Total	90240.273	89			
TG	Between groups	17136.278	3	5712.093	4.275	0.007
	Within groups	114917.263	86	1336.247		
	Total	132053.541	89			
HDL	Between groups	1414.258	3	471.419	10.897	<0.0001
	Within groups	3720.446	86	43.261		
	Total	5134.704	89			
LDL	Between groups	24098.700	3	8032.900	16.600	<0.0001
	Within groups	41615.375	86	483.900		
	Total	65714.075	89			
VLDL	Between groups	685.472	3	228.491	4.278	0.007
	Within groups	4592.948	86	53.406		
	Total	5278.420	89			

Post Hoc Tukey test was done for pairwise comparison between the different groups. For TC pairwise differences were statistically significant except Group II & III. For both TG and VLDL pairwise difference for Group II & IV and Group I & III were statistically significant. For HDL pairwise differences were statistically significant for Group II & IV and Group III & IV. For LDL pairwise differences were statistically significant for Group II & IV, Group III & IV and Group I & III (Table 5).

Table 5. Multiple comparison of serum lipid profile among various groups using Post-Hoc Tukey test.

Groups		TC	TG	HDL	LDL	VLDL
I-IV	Mean Difference	19.00	0.55	4.73	14.15	0.11
	P value	<0.05*	>0.05^{NS}	>0.05^{NS}	>0.05 ^{NS}	>0.05 ^{NS}
II-IV	Mean Difference	42.38	9.28	7.47	33.05	1.85
	P value	<0.05*	>0.05 ^{NS}	<0.05*	<0.05*	>0.05 ^{NS}
III-IV	Mean Difference	57.06	37.89	9.85	39.62	7.57
	P value	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*
I-II	Mean Difference	23.38	8.72	2.74	18.90	1.74
	P value	<0.05*	>0.05 ^{NS}	>0.05 ^{NS}	>0.05^{NS}	>0.05 ^{NS}
I-III	Mean Difference	38.06	37.33	5.12	25.47	7.46
	P value	<0.05*	<0.05*	>0.05 ^{NS}	<0.05*	<0.05*
II-III	Mean Difference	14.67	28.60	2.38	6.57	5.72
	P value	>0.05 ^{NS}	>0.05 ^{NS}	>0.05 ^{NS}	>0.05 ^{NS}	>0.05 ^{NS}

NS: p > 0.05; Not Significant, * p < 0.05; Significant

Discussion

Oral submucous fibrosis is one of the most poorly understood and challenging precancerous condition with high prevalence in India. An estimated 2.5 million people suffer from this disease in India. [22] The importance of this disease lies in its inability to open the mouth and dysplasia giving rise to malignancy. In general younger the patient's age, more rapid is the disease progression. [23] In this study, serum lipid profile levels were analyzed in patients with various stages of OSMF and compared with normal healthy individuals, thereby providing a marker and prognostic indicator in the early detection of oral cancer.

Our study showed that OSMF was most prevalent in second decade of life (56.66%) with a male predominance (84.4%). The mean age of our study patients was 31.57 ± 6.08 years. These observations were supported by the studies done earlier which state that the disease mainly occurs between 20 to 40 years of age [24, 25] and male predominance [14, 26, 25] although some studies have shown female predominance as well. [27, 28]

The results of the present study shows that the serum TC, TG, HDL, LDL and VLDL were significantly reduced in OSMF patients group when compared with the control group (Figure 1).

This was in accordance with a study done by Ajai, Panat, Aggarwal, Agarwal, Upadhyay, Joshi (2014) where the lipid levels were significantly lower in OSMF patients than in controls.[29]

Patel, Shah, Jha, Raval GN (2004) showed similar observations in patients with oral squamous cell carcinoma where there was a significant decrease in plasma TC, HDL, VLDL and TG but LDL cholesterol levels did not reveal any significant difference, [13] whereas in our study significant decrease in LDL levels in OSMF was seen as compared to controls.

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Similarly, Lohe, Degwekar, Bhowate, Kadu, Dangore (2010) reported a significant decrease in TC, and HDL in oral precancerous conditions, however LDL, VLDL, and triglyceride did not reveal any significant difference. [30] Other studies [31, 32] also showed a significant decrease in plasma total cholesterol, HDLC, and triglycerides in the patients with the precancerous lesions and conditions as compared to the controls.

Lipids are major cell membrane components required for growth and division of normal as well as malignant cells. Cholesterol is an essential constituent of lipoprotein fractions like LDL, HDL, and VLDL and 75% of the plasma cholesterol is transported in the form of LDL. In some malignancies, serum cholesterol undergoes early and significant changes. Low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the rapidly dividing cells in malignancies. An inverse trend is observed between lower serum cholesterol and premalignant conditions also. [31] This may be due to greater utilization of lipids including total cholesterol, lipoproteins and triglycerides for new membrane biogenesis as well as accumulation of esterified cholesterol in tumoral tissues.[13,32]

There are three main competing hypotheses to explain the inverse association between cholesterol concentrations and the incidence of cancer. Firstly, lower cholesterol values, even before the manifestation or detection of cancer, may be a result of the cancer process. Secondly, lower cholesterol values may precede the development of cancer but the association with cancer is secondary, i.e. cholesterol serves as a marker for some other causal set of variables. Thirdly, lower cholesterol values may precede the development of cancer and may be causally associated with the occurrence of some forms of cancer. [33]

However in contrast to our study, Goyal, Vani, Srikanth, Lalitha (2013) found no statistical significance in the lipid parameters of patients with oral precancerous lesion when compared with controls. [34]

In the present study, serum lipid profile in OSMF patients was compared with controls after clinical and functional grading. A significant reduction in the levels of TC, TG, HDL, LDL and VLDL was seen as the grade of OSMF advanced (Figure 2). This was in accordance to studies done by Kanthem and Guttikonda (2015) where similar findings were observed. [35]

The possibility of not having any significant variation in serum lipid profile levels when pairwise comparision was done among various grades of OSMF may be due to patients who are on the verge of precancer conversion from one grade to the next or might represent patients who are at an initial stages of precancer, which might not have caused the lipid changes in the blood.

Conclusion

The findings in our study show the evidence of an inverse relationship between lower serum lipid profile levels & oral submucous fibrosis. The lower serum lipid profile parameters may be considered as a useful indicator for predicting early neoplastic changes occurring in OSMF, which can be used to prevent further progress of the disease. Lipid profile may serve as an additional prognostic or diagnostic indicator for early diagnosis of premalignant and malignant lesions. The present findings are drawn by a smaller sample size but the findings strongly warrant an in-depth study on a larger sample size.

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