Innovations

"Comparative Evaluation of Serum Lipid Profile in Oral Squamous Cell Carcinoma and in Leukoplakia Patients"

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Abstract: Background: *Determining hematologically changed lipid profile in patients with OSCC(oral squamous cell carcinoma) and leukoplakia andrelate serum lipid profile of OSCC patients with oral leukoplakia patients.* ***Subjects And Methods:*** *Different study groups: Group (I) 25 cases of oral Leukoplakia (with dysplasia),Group (II) 25 cases of OSCC, Group (III) 25 cases of control group individual without medical, dental, and habit history.5 ml fasting blood sample was collected under sterile conditions in plain vacutainer and allowed to clot for 1hr. Centrifugation of Vacutainer was done for 5 minutes at 3000 rpm. Serum was analyzed for lipid profile using fully automatic biochemistry analyser, based on principal of photometry.* ***Results & Statistical Analysis Used:*** *Data were summarized as Mean ± SE. Groups were compared by one way analysis of variance (ANOVA) and significance of mean difference between groups was done by Tukey's HSD (honestly significant difference) post hoc test after determining normality by Shapiro-Wilk's test and similarity of variance by Levene's test. Discrete groups were compared by chi-square ($\chi 2$) test. Two-tailed ($\alpha=2$) p value lower than 0.05 ($p<0.05$) was considered statistically significant. Analyses performed on SPSS software.Overall lipid profile show; as severity of disease increases, lipid content decreases and There is inverse relationship between serum lipid profile, OSCC and Leukoplakia.* ***Conclusions:*** *Detailed study on larger*

sample size can provide insights-into-the-inverse relationship between serum lipid profiles,leukoplakia and OSCC.

Introduction: Oral squamous cell carcinoma (OSCC) constitutes about 90% of total head and neck cancers[1], Oral squamous cell carcinoma is preceded by the occurrence of premalignant lesions or conditions. Leukoplakia is a premalignant lesion associated with development of oral cancer, It occurs predominantly in males. Although oral leukoplakia and OSCC are histologically characterized by having loss of cellular and architectural integrity there is increased need of lipids and proteins for proliferating tumor cells. The changes in lipid profile have long been associated with cancer because lipid plays a key role in maintenance of cell integrity[1,2]. They are major cell membrane components essential for various biological functions including cell growth and division of normal cell and malignant tissue.[3,4] Hence aim of the study is to evaluate the status of lipid profile in the blood serum, which is useful indicator of early changes occurring in the malignant cell.

Subjects and Methods

The present study was conducted in the department of oral pathology and microbiology sardar patel post graduate institute of dental sciences and hospital lucknow. The study participant included 25 normal healthy volunteers,25 patients with diagnosis of leukoplakia and 25 patients with diagnosis of oral squamous cell carcinoma.

Inclusion Criteria

Age was 25–60 years irrespective of gender. In Group I, patients with the confirmed diagnosis of leukoplakia were included.Group II, patients with the confirmed diagnosis of oral squamous cell carcinoma were included in the study,and in Group III, healthy volunteers having no oral lesion and not having the habit of tobacco or alcohol use were included.

Exclusion Criteria

Cases with a known history of systemic disorder, Pregnant and lactating women, were excluded; Immunocompromised patient, patient suffering from mental illness, treated cases of leukoplakia, oral squamous cell carcinoma, Patients with other related precancerous lesions and conditions, Patients having OSCC or leukoplakia but not having any habit of consuming tobacco, were also excluded from the study.

Sample collection

The participants were explained in detail about the procedure, and a signed consent form was taken from them.and the procedures followed were in accordance with the ethical standards.

Method

Total 75 subjects were included in the study out of which 25 leukoplakia, 25 oral squamous cell carcinoma (OSCC) and 25 age and gender were matched normal healthy individual having no such lesions were taken as control group (Table 1).

A 5 ml of fasting blood sample was collected under sterile conditions in plain vacutainer and allowed to clot for 1hr. The vacutainer was centrifuge at 3000 rpm for 5 min. Serum was then analyzed for lipid profile using a fully automatic biochemistry analyzer based on the principal of photometry.

Fully Automatic Bio-Chemistry Analyzer

Biochemistry analyzer is a medical laboratory instrument designed to analyze blood bio-chemistry parameters. Blood samples are placed in a rack of test tubes. This rack is rotated through a stepper motor for positioning of blood sample through the measurement chamber of the analyzer.150 Samples and reagents are metered at pre-set intervals into thermostatically controlled travelling cuvettes. The absorbance of the reaction mixture is measured automatically in colorimeter.

System Description

It consists of light source, homochromy device, colorimetric cup, temperature sensor, sampling device, photoelectric converter and microcomputer system and so on. The step motor drives the rotating wheel where there are 8 interferential filters distributed proportionally, one of them is a standby and the others are employed for the measurement.

Autosampler

An auto-sampler is an integral part of this instrument. It is an electromechanical device fitted with different probes to aspirate water, reagents and samples from the test placed in different holes provided in the sampler tray.

Results

Basic characteristics

Tables no.1 and 2 offer description of age and sex in the three groups. The age of Control group, Leukoplakia and OSCC group ranged from 30-72 yrs, 30-70 yrs and 32-75 yrs+respectively with mean (± SE) 49.48 ± 1.77 yrs, 48.08 ± 2.07 yrs and 52.68 ± 2.20 yrs respectively and median 48 yrs, 45 yrs and 52 yrs respectively. The mean

age of OSCC was slightly higher than other groups. In other words, subjects of three groups were age and gender matched and thus comparable and may also not influence the study outcome measure (lipid profile).

Lipid profile

I. TC

The Mean of Total cholesterol TC (mg/dl) of three groups is presented in Table 3. Control, Leukoplakia and OSCC groups mean ranges from (± SE) 177.08 ± 2.14 mg/dl, 156.10 ± 3.26 mg/dl and 137.34 ± 1.18 mg/dl respectively and median 175 mg/dl, 150 mg/dl and 136 mg/dl respectively. (Table 3).Comparing the mean TC of three groups, ANOVA showed significantly different TC among the groups ($F=71.35$, $p<0.001$) (Table 3). Further, comparing the mean difference in TC between the groups, Tukey test showed significantly different and lower TC in both Leukoplakia (11.8%) (177.08 ± 2.14 vs. 156.10 ± 3.26, $q=8.92$, $p<0.001$) and OSCC (22.4%) (177.08 ± 2.14 vs. 137.34 ± 1.18, $q=16.89$, $p<0.001$) as compared to Control (Table 4).

II. HDL

The HDL of Control, Leukoplakia and OSCC group were with mean (± SE) 56.43 ± 1.30 mg/dl, 51.61 ± 1.23 mg/dl and 45.30 ± 0.94 mg/dl respectively and median 59 mg/dl, 50 mg/dl and 45 mg/dl respectively and is further summarized in Table 3. In other words, as severity increases mean HDL decreases. Comparing the mean HDL of three groups, ANOVA showed significantly different HDL among the groups ($F=23.01$, $p<0.001$) (Table 3). Further, comparing the mean difference in HDL between the groups, Tukey test showed significantly different and lower HDL in both Leukoplakia (8.5%) (56.43 ± 1.30 vs. 51.61 ± 1.23, $q=4.14$, $p=0.013$) and OSCC (19.7%) (56.43 ± 1.30 vs. 45.30 ± 0.94, $q=9.57$, $p<0.001$) as compared to Control (Table 5).

III. LDL

The Mean of Low density lipoprotein LDL (mg/dl) of three groups is presented in Table 3. The LDL of Control, Leukoplakia and OSCC groups were with mean (± SE) 87.21 ± 2.85 mg/dl, 77.34 ± 3.26 mg/dl and 68.17 ± 1.45 mg/dl respectively and median 87 mg/dl, 74 mg/dl and 70 mg/dl respectively. In other words, as severity increases mean LDL decreases. Comparing the mean LDL of three groups, ANOVA showed significantly different LDL among the groups ($F=13.05$, $p<0.001$) (Table 3). Further, comparing the mean difference in LDL between the groups, Tukey test showed significantly different and lower LDL in both Leukoplakia (11.3%) (87.21 ± 2.85 vs. 77.34 ± 3.26, $q=3.75$, $p=0.027$) and OSCC (21.8%)(87.21 ± 2.85 vs. 68.17 ± 1.45, $q=7.22$, $p<0.001$) as compared to Control (Table 6).

IV. VLDL

TheMean of VLDL (mg/dl) of three groups is summarized in Table 3. The VLDL of Control, Leukoplakia and OSCC were with mean (± SE) 33.44 ± 0.52 mg/dl, 27.15 ± 0.34 mg/dl and 23.88 ± 0.77 mg/dl respectively and median 87 mg/dl, 74 mg/dl and 70 mg/dl respectively. In other words, as severity increases mean VLDL decreases. Comparing the mean VLDL of three groups, ANOVA showed significantly different VLDL among the groups ($F=72.93$, $p<0.001$) (Table 3). Further, comparing the mean difference in VLDL between the groups, Tukey test showed significantly different and lower VLDL in both Leukoplakia (18.8%) (33.44 ± 0.52 vs. 27.15 ± 0.34, $q=11.05$, $p<0.001$) and OSCC (28.6%) (33.44 ± 0.52 vs. 23.88 ± 0.77, $q=16.80$, $p<0.001$) as compared to Control (Table 7).

V. TG

The Mean of TG (mg/dl) of three groups is further summarized in Table 3. The TG of Control, Leukoplakia and OSCC were with mean (± SE) 167.20 ± 2.61 mg/dl, 135.76 ± 1.70 mg/dl and 119.38 ± 3.83 mg/dl respectively and median 166 mg/dl, 135 mg/dl and 120 mg/dl respectively. In other words, as severity increases mean TG decreases. Comparing the mean TG of three groups, ANOVA showed significantly different TG among the groups ($F=72.74$, $p<0.001$) (Table 3). Further, comparing the mean difference in TG between the groups, Tukey test showed significantly different and lower TG in both Leukoplakia (18.8%) (167.20 ± 2.61 vs. 135.76 ± 1.70, $q=11.03$, $p<0.001$) and OSCC (28.6%) (167.20 ± 2.61 vs. 119.38 ± 3.83, $q=16.78$, $p<0.001$) as compared to Control (Table 8).

Discussion:

Out of the 75 patients included in the study, there were 65 males and 10 females. The distribution of male and female subjects between the three groups is elaborated in [Table 1and 2]. In all three groups, the frequency (%) of males was higher than females with highest being in OSCC. This male predominance (83.3%) was observed by **Mehta R (2014),Mahesh N (2014), Kumar P (2012).** Patients with oral carcinoma had a mean age that was on higher side (50.10, SD ± 7.66). While increasing number of studies suggest that the proportion of oral cavity cancer cases in women increased worldwide especially among young white individuals in western countries **Patel SC (2011)** [5,6,7,15]

In the age estimation done by **Mortazavi H (2014)** lesion is seen most often in middle-aged and older men. Under 30 years of age only less than 1% of males have leukoplakia**,** whereas in other studies the occurrence of OSCC in patients younger than 45 years was reported as ranging from 0.4%-6.7% of all cases However, epidemiologic studies demonstrate an increasing incidence of OSCC young patients worldwide especially among young females **Fan Y.(2014).**[8,9]

I. TC (Total Cholesterol)

In the estimation of lipid profile firstly **TC** was observed among three groups and further summarized in Table 3 and 4. Further, comparing the mean difference in TC between the groups,in table 4 Tukey test showed significantly different and lower TC in both Leukoplakia p<0.001) and OSCC p<0.001) as compared to Control. As severity increases mean TC decreases. The observation of our study was similar to the study done by **BaduniA. (2015), Mehta R. (2014), Kumar P. (2012)** in which an inverse relation was observed between the total cholesterol and disease stage and mortality in various malignancies. Lower level of TC was recommended due to increased consumption by tumor cells.

While the study done by **Goel P. (2014)** was contrary to our study that is increased total cholesterol.[5,7,10,11]

II. HDL (Good Cholesterol)

Table 3 and table 5 show observed HDL value (mg/dl) and that the mean HDL of Control was the highest followed by Leukoplakia and of OSCC the least (Control > Leukoplakia > OSCC) (Table 3). Further, comparing the mean difference in HDL between the groups, Tukey test showed significantly different and lower HDL in both Leukoplakia (p=0.013) and OSCC (p<0.001) as compared to Control (Table 5). Further, the mean HDL also differ and was lower significantly in OSCC as compared to Leukoplakia (p=0.001) (Table 5).

Hence as the severity increases mean HDL decreases. Which was in accordance with study done by **Baduni A (2015), MehtaR (2014), Kumar P (2012) this** is similar to our findings that is the plasma level of HDL was decreased in the precancerous and cancerous groups as compared to the control group and this difference was statistically significant (F = 245.265; P < 0.001).

While in another study revealed by **Goel P (2014**), HDL increased in 15% of patients, thatwhich was contrary to our study.[5,7,10, 11]

III. LDL (Bad Cholesterol)

Table 3, show that the mean LDL of Control was the highest followed by Leukoplakia and OSCC the least (Control > Leukoplakia >OSCC) (Table 10). Further, comparing the mean difference in LDL between the groups, Tukey test showed significantly different and lower LDL in both Leukoplakia (p=0.027) and OSCC (p<0.001) as compared to Control(Table 6). Further, the mean LDL also differ and lower significantly in OSCC as compared to Leukoplakia (p=0.043) (Table 6). In other words, as severity increases mean LDL decreases. These findings were similar to our

present study and also the study done by **Mehta R (2014), Mahesh N (2012)** which showed a significant reduction in the mean plasma LDL (P < 0.001) in cancer and pre-cancer patients as compared to the control patients. While the study done by **Goel P(2014**) was contrary to our study.[5,6,11]

IV. VLDL

VLDL is a type of lipoprotein which is synthesized in the liver, the function of these lipoprotein is to deliver energy rich triacylglycerolto cells. In Table 3 the mean VLDL of Control was the highest followed by Leukoplakia and OSCC the least (Control > Leukoplakia > OSCC). Hence as severity increases mean VLDL decreases. Comparing the mean VLDL of three groups, ANOVA showed significantly different VLDL among the groups (F=72.93, p<0.001) (Table 3). Further, comparing the mean difference in VLDL between the groups, Tukey test showed significantly different and lower VLDL in both Leukoplakia (p<0.001) and OSCC (p<0.001) as compared to Control (Table 7). Further, the mean VLDL also differ and lower (12.1%) significantly in OSCC as compared to Leukoplakia (p<0.001) (Table 7). These findings were similar to our study done by **Ashutosh K. (2015),Mehta R (2014), Kumar P (2012)** while the study done by **Goel P (2014**) was contrary to our study and also the study done by **Ganavi BS (2014)** observed that the alteration in VLDL is not significant in OSCC and in Leukoplakia patients. **Mahesh N (2014)** observed higher VLDL level in Leukoplakia than control that is not similar to our study.[5,6,7,11,12,13]

V. TG

The observed and the mean TG of Control was the highest followed by Leukoplakia and OSCC the least (Control > Leukoplakia > OSCC) (Table 3). Further, comparing the mean difference in TG between the groups, Tukey test showed significantly different and lower TG in both Leukoplakia (p<0.001) and OSCC (p<0.001) as compared to Control (Table 8). Further, the mean TG also differ and lower (12.1%) significantly in OSCC as compared to Leukoplakia (p<0.001) (Table 8). In other words, as severity increases mean TG decreases. Comparing the mean TG of three groups, ANOVA showed significantly different TG among the groups (F=72.74, p<0.001) (Table 3).The result of our study was similar to the study done by **Baduni A(2015), Mahesh N (2014)Mehta R (2014), Ganavi BS (2014), Bailwad SA(2014), Kumar P (2012).**[5,6,7,10,13,14]

Thus, to sum up as Lipids and cholesterol are transported through the bloodstream by lipoprotein particles, Hypolipidemia has been suggested as a risk factor for the development of cancer, even though no direct link has been shown yet. However,

some authors believe that hypolipidemia is the result rather than the cause of cancer.

Conclusion:

An inverse association between leukoplakia and OSCC and serum lipid profile has been concluded in our research. The change in plasma lipid levels may be used as a diagnostic or prognostic biochemical indicator for early diagnosis of oral premalignant and malignant conditions. However, a detailed study on large sample size and on role of cholesterol in neoplasia should be carried out for better understanding of this inverse relationship of serum lipid profiles and oral pre malignant and malignant conditions. One established causative factor for the development of head/neck cancer and oral precancerous disorders is tobacco use. Our research thus provides new opportunities for the use of serum lipid profile as a diagnostic marker to distinguish between precancerous and cancerous situations based only on serum lipid levels.

Financial Support and Sponsorship

Nil

Conflicts Of Interest

There are no conflicts of interest

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Table 1: Distribution of subjects and allocation of groups

Subjects/Patients	Group name	No of subjects (n=75) (%)
Normal healthy	Control	25 (33.3)
Leukoplakia	Leukoplakia	25 (33.3)
Oral squamous cell carcinoma	OSCC	25 (33.3)

Table 2: Basic characteristics of three groups

Basic characteristics	Control (n=25) (%)	Leukoplakia (n=25) (%)	OSCC (n=25) (%)	F/χ^2 value	p value
Age (yrs): Mean ± SE	49.48 ± 1.77	48.08 ± 2.07	52.68 ± 2.20	1.36	0.263
Gender : Male	21 (84.0)	21 (84.0)	23 (92.0)	0.92	0.630
Female	4 (16.0)	4 (16.0)	2 (8.0)		

Comparing the mean age of three groups, ANOVA showed similar age among the groups (F=1.36, p=0.263) i.e. did not differ significantly. Comparing the gender proportions (M/F) of three groups, $\chi 2$ test showed similar gender proportions among the groups ($\chi 2$=0.92, p=0.630) i.e. also not differd significantly.(Table 2)

Table: 3 MEAN TC, HDL, LDL, VLDL, TG (mg/dl) of three groups

1	GROUP	MEAN (± SE)	F VALUE	P VALUE
Total cholesterol TC (mg/dl) of three groups	CONTROL	177.08 ± 2.14	71.35	<0.001
	LEUKOPLAKIA	156.10 ± 3.26		
	OSCC	137.34 ± 1.18		
2	**GROUP**	**MEAN (± SE)**	**F VALUE**	**P VALUE**
HDL (mg/dl) of three groups	CONTROL	56.43 ± 1.30	23.01	<0.001
	LEUKOPLAKIA	51.61 ± 1.23		
	OSCC	45.30 ± 0.94		
3	**GROUP**	**MEAN (± SE)**	**F VALUE**	**P VALUE**
Low density lipoprotein LDL (mg/dl) of three groups	CONTROL	87.21 ± 2.85	13.05	<0.001
	LEUKOPLAKIA	77.34 ± 3.26		
	OSCC	68.17 ± 1.45		
4	**GROUP**	**MEAN (± SE)**	**F VALUE**	**P VALUE**

Very Low density lipoprotein (VLDL) (mg/dl)of three groups	CONTROL	33.44 ± 0.52	72.93	<0.001
	LEUKOPLAKIA	27.15 ± 0.34		
	OSCC	23.88 ± 0.77		
5	**GROUP**	**MEAN (± SE)**	**F VALUE**	**P VALUE**
Triglycerides (TG) (mg/dl) of three groups	CONTROL	167.20 ± 2.61	72.74	<0.001
	LEUKOPLAKIA	135.76 ± 1.70		
	OSCC	119.38 ± 3.83		

The mean TC of Control was the highest followed by Leukoplakia and OSCC the least (Control > Leukoplakia > OSCC)
The mean HDL of Control was the highest followed by Leukoplakia and OSCC the least (Control> Leukoplakia > OSCC).
The mean LDL of Control was the highest followed by Leukoplakia and OSCC the least (Control > Leukoplakia > OSCC).
The mean VLDL of Control was the highest followed by Leukoplakia and OSCC the least (Control > Leukoplakia > OSCC).
The mean TG of Control was the highest followed by Leukoplakia and OSCC the least (Control >Leukoplakia > OSCC).
(Table 3)

Table :4
Comparison of mean difference in TC between the groups by Tukey test

Comparison	Mean difference	% mean change	q value	p value	95% CI (mean difference)
Control vs. Leukoplakia	20.98	11.8	8.92	<0.001	13.02 to 28.94
Control vs. OSCC	39.74	22.4	16.89	<0.001	31.77 to 47.70
Leukoplakia vs. OSCC	18.76	12.0	7.97	0.001	10.79 to 26.72

The mean TC differ and is lower (12.0%) significantly in OSCC as compared to Leukoplakia in the above table.(156.10 ± 3.26 vs. 137.34 ± 1.18, q=7.97, p<0.001) (Table 4)

Table :5
Comparison of mean difference in HDL between the groups by Tukey test

Comparison	Mean difference	% mean change	q value	p value	95% CI (mean difference)
Control vs. Leukoplakia	4.82	8.5	4.14	0.013	0.88 to 8.76
Control vs. OSCC	11.13	19.7	9.57	<0.001	7.19 to 15.07
Leukoplakia vs. OSCC	6.31	12.2	5.42	0.001	2.37 to 10.25

The mean HDL differ and is lower (12.2%) significantly in OSCC as compared to Leukoplakia in the above table. (51.61 ± 1.23 vs. 45.30 ± 0.94, q=5.42, p=0.001).(Table 5)

Table : 6
Comparison of mean difference in LDL between the groups by Tukey test

Comparison	Mean difference	% mean change	q value	p value	95% CI (mean difference)
Control vs. Leukoplakia	9.87	11.3	3.75	0.027	0.95 to 18.79
Control vs. OSCC	19.04	21.8	7.22	<0.001	10.12 to 27.96
Leukoplakia vs. OSCC	9.17	11.9	3.48	0.043	0.25 to 18.09

The mean LDL differ and is lower (11.9%) significantly in OSCC as compared to Leukoplakia in the above table.(77.34 ± 3.26 vs. 68.17 ± 1.45, q=3.48, p=0.043).(Table 6)

Table :7
Comparison of mean difference in VLDL between the groups by Tukey test

Comparison	Mean difference	% mean change	q value	p value	95% CI (mean difference)
Control vs. Leukoplakia	6.29	18.8	11.05	<0.001	4.37 to 8.22
Control vs. OSCC	9.57	28.6	16.80	<0.001	7.64 to 11.50
Leukoplakia vs. OSCC	3.28	12.1	5.75	<0.001	1.35 to 5.20

The mean VLDL differ and is lower (12.1%) significantly in OSCC as compared to Leukoplakia (27.15 ± 0.34 vs. 23.88 ± 0.77, q=5.75, p<0.001) in the above table. (Table7).

Table :8
Comparison of mean difference in TG between the groups by Tukey test

Comparison	Mean difference	% mean change	q value	p value	95% CI (mean difference)
Control vs. Leukoplakia	31.44	18.8	11.03	<0.001	21.80 to 41.09
Control vs. OSCC	47.82	28.6	16.78	<0.001	38.18 to 57.46
Leukoplakia vs. OSCC	16.38	12.1	5.75	<0.001	6.73 to 26.02

The mean TG differ and is lower (12.1%) significantly in OSCC as compared to Leukoplakia (135.76 ± 1.70 vs. 119.38 ± 3.83, q=5.75, p<0.001) in the above table (Table 8).

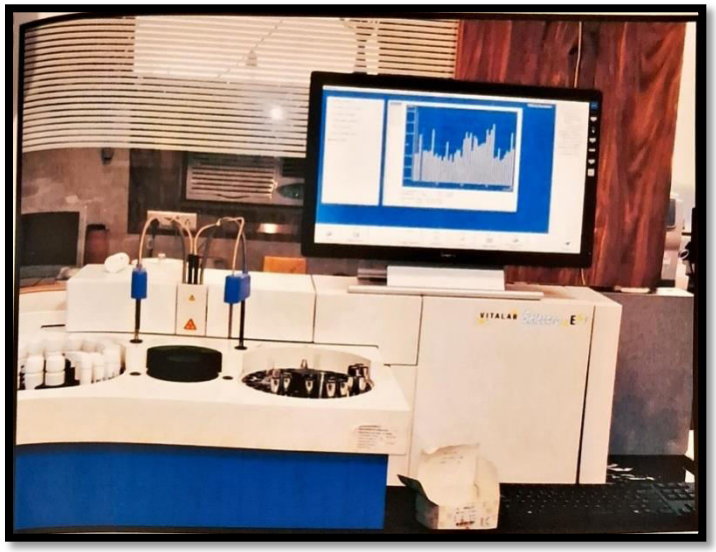

Figure 1 – Fully automatic Bio-chemistry analyser (vitalabselectra ex)

Figure 2 –Centrifugator (Leo-Lab)

Figure3- Serum Vial