ABSTRACT:

Background: Lipids are major cell membrane components essential for various biological functions. They are utilized during the process of lipid peroxidation which plays an important role in carcinogenesis by release of free radicals. Since saliva reflects the chemical constitution of blood to a certain extent, the salivary lipid levels may mirror the changes demonstrated in plasma lipid levels. Aim: To evaluate and compare plasma and salivary lipid profile levels in patients with potentially malignant lesions. Material and methods: This study included total 150 individuals (males of age group 20-60 years) divided into three groups. Group I: 50 individuals without the habit of tobacco consumption and without any oral mucosal lesions, taken as control, Group II: 50 individuals with the habit of tobacco consumption in any form but without any oral mucosal lesions and Group III: 50 individuals with the habit of tobacco consumption in any form and with the presence of oral potentially malignant lesions. Lipids including total Cholesterol, LDL, HDL, VLDL and triglycerides were analyzed in plasma and saliva. Results: Highly significant decrease in plasma lipid profiles, except VLDL and triglycerides, in individuals with potentially malignant lesions. Salivary values reflected the plasma lipid profile values except HDL which showed a significant increase in individuals with potentially malignant lesions. Conclusion: Tobacco consumption can cause a significant change in plasma lipid profile only in the presence of oral potentially malignant lesions. Further, saliva may be used as a fluid to assess the lipid profiles in lieu of plasma as it mimics the plasma lipid profile changes.

Keywords: Potentially malignant lesions, Lipid profile, Tobacco, Free radicals, Carcinogenesis.

I. INTRODUCTION

Lipids are major cell membrane components essential for various biological functions including cell growth and division of normal and malignant tissues. Tobacco consumption in different forms is highly prevalent in the society. Tobacco contains many carcinogens like nicotine and nitrosamines which induce generation of free radicals and reactive oxygen species, which are responsible for high rate of peroxidation of polyunsaturated fatty acids which further release peroxide radicals thus affecting essential constituents of cell membrane and may be involved in carcinogenesis. Because of lipid peroxidation, there is a greater utilization of lipids including total...
Cholesterol, lipoproteins and triglycerides for new membrane biogenesis. Cells fulfill these requirements either from circulation or by synthesis through metabolism. The most commonly used laboratory procedures for assessing lipid profile involve the analysis of the blood. Other biologic fluids have also been used for similar biochemical tests and saliva offers some distinct advantages. Lipids are also secreted in saliva. Saliva can be collected from individuals by non-invasive techniques therefore, lipid profile can be also evaluated in saliva. Lipid profile levels in whole saliva particularly; triglycerides alone or in conjunction with have been used to monitor patients at increased risk of ischemic stroke.

The significance of variations in tissues/ blood cholesterol levels in diagnosis and treatment of various diseases has been studied by several workers. Because of the association of tobacco carcinogens and cellular proliferation, serum lipids may be altered in oral potentially malignant lesions.

This study thus aimed to evaluate and compare the plasma and salivary lipid profile levels in patients with potentially malignant lesions through the following objectives: (1) To compare plasma lipid profile levels between patients with potentially malignant lesions and control groups; (2) To compare salivary lipid profile levels between patients with potentially malignant lesions and control groups; and (3) to compare salivary and plasma lipid profiles levels in patients with potentially malignant lesions and control groups.

II. MATERIALS AND METHODS

The study population included 150 individuals in total (all males) between the age group of 20-60 years and was divided into three groups; group I, II and III of 50 subjects each. Individuals with contributory medical history like cardiovascular diseases, hypertension and diabetes mellitus which can alter lipid profiles were excluded from the study. In order to eliminate the effect of drugs on lipid profiles like metformin, the individuals who had history of any drug usage were also excluded from the study. The study was approved by communication of decision of the ethics committee (Project no. IEC 11) and a written informed consent was taken from all the individuals in the total study population.

Accordingly, the subjects for the study group were grouped as follows:

Group I – 50 healthy individuals without the habit of tobacco consumption and without any oral mucosal lesions were taken as control.

Group II – 50 individuals with the habit of tobacco consumption in any form but without any oral mucosal lesions were included in this group.

Group III – 50 individuals with the habit of tobacco consumption in any form and with the presence of oral potentially malignant lesions were included in this group. An incisional biopsy was taken for all the 50 individuals and oral potentially malignant lesions were confirmed by histopathological examination using Haematoxylin and Eosin staining.

Subjects were clinically examined to exclude the possibility of any other oral or systemic disease. Subjects were asked to fast for 12 hours before their morning appointment. 2ml of blood was drawn from antecubital vein with minimal trauma under aseptic conditions. The plasma was separated by centrifugation and the supernatant separated and analyzed. Saliva samples were then collected from patients in restful and quiet circumstances after flushing of mouth with tap water. The whole saliva was collected for 5 minutes by the subject leaning forward and spitting saliva into test tubes that were kept in crushed ice and immediately after collection; the samples were centrifuged, supernatant separated and analyzed. Lipids profile levels were estimated using ERBA kits and an automated biochemical analyzer (MEDLAB 2300 V4). Plasma and salivary cholesterol and triglycerides concentration were measured by enzymatic methods. The concentration HDL was measured by the method of Burstein et al. The concentration of LDL-cholesterol was calculated from the concentration of cholesterol, HDL and triglycerides by Friedwald formula. VLDL concentration was estimated directly by dividing triglyceride value on 5.

Data was further examined for statistical analysis (p value) using one way anova, bonferroni test and pearson correlation. p value < 0.05 was considered as statistically significant; while p value < 0.001 was considered as
statistically highly significant. Correlation between the groups was calculated using Pearson correlation, and was considered significant at the level of $> 0.3$.

III. RESULTS

Table 1 shows that the mean plasma cholesterol, HDL, LDL, triglyceride and VLDL levels decrease from Group I to Group II with a further decrease in Group III. Non significant decrease in the mean plasma cholesterol ($p$ value 0.809), HDL ($p$ value 1.00), LDL ($p$ value 0.336), triglyceride ($p$ value 0.249) and VLDL levels ($p$ value 0.511) were seen from individuals with no tobacco consumption to individuals with tobacco consumption but without any potentially malignant lesions. Highly significant decrease in the mean plasma cholesterol, HDL and LDL levels were seen in individuals with potentially malignant lesions when compared to individuals with tobacco consumption but without any potentially malignant lesions ($p$ value 0.00) and to individuals with no tobacco consumption ($p$ value 0.00). Non significant decrease in the plasma triglyceride and VLDL levels were seen in individuals with potentially malignant lesions when compared to individuals with tobacco consumption but without any potentially malignant lesions ($p$ value 1.00). Significant decrease in the plasma triglyceride ($p$ value 0.043) and a non significant decrease in the VLDL levels ($p$ value 0.461) were seen in individuals with potentially malignant lesions from individuals with no tobacco consumption.

Table 2 shows that the mean salivary cholesterol, LDL, triglyceride and VLDL levels decrease from Group I to Group II with a further decrease in Group III the mean salivary HDL decrease from Group I to Group II with a increase in Group III. Salivary lipid values followed those of plasma lipid values in every except the HDL which showed a significant increase ($p$ value 0.019) in individuals with potentially malignant lesions when compared to individuals with tobacco consumption but without any potentially malignant lesions and a non significant increase ($p$ value 0.074) when compared to individuals with no tobacco consumption.

Table 3 shows a significant positive correlation of plasma and salivary cholesterol (Pearson value 0.311), triglycerides (Pearson value 0.726) and VLDL (Pearson value 0.720) when compared in all groups in the study population and a non significant correlation was seen for HDL (Pearson value $-0.015$) and LDL (Pearson value 0.135) when compared in three Groups.

<table>
<thead>
<tr>
<th>LIPID PROFILE</th>
<th>TOBACCO CONSUMERS</th>
<th>TOBACCO CONSUMERS WITH POTENTIALLY MALIGNANT LESIONS</th>
<th>TOBACCO NON CONSUMERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOLESTEROL</td>
<td>17.22 ± 5.45</td>
<td>14.87 ± 4.70</td>
<td>206.61 ± 30.54</td>
</tr>
<tr>
<td>HDL</td>
<td>2.57 ± 1.03</td>
<td>2.42 ± 1.32</td>
<td>44.50 ± 13.47</td>
</tr>
<tr>
<td>LDL</td>
<td>8.38 ± 4.55</td>
<td>6.94 ± 3.33</td>
<td>135.46 ± 21.12</td>
</tr>
<tr>
<td>TRIGLYCERIDES</td>
<td>31.61 ± 16.19</td>
<td>27.60 ± 11.27</td>
<td>133.29 ± 30.02</td>
</tr>
<tr>
<td>VLDL</td>
<td>6.33 ± 3.24</td>
<td>5.52 ± 2.25</td>
<td>26.64 ± 6.01</td>
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</table>

TABLE 2: Comparison of salivary lipid profile in various groups.

<table>
<thead>
<tr>
<th>LIPID PROFILE</th>
<th>TOBACCO CONSUMERS</th>
<th>TOBACCO CONSUMERS WITH POTENTIALLY MALIGNANT LESIONS GROUP I (MEAN± SD)</th>
<th>TOBACCO NON CONSUMERS GROUP II (MEAN± SD)</th>
<th>TOBACCO CONSUMERS WITH POTENTIALLY MALIGNANT LESIONS GROUP III (MEAN± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOLESTEROL</td>
<td>17.22 ± 5.45</td>
<td>14.87 ± 4.70</td>
<td>206.61 ± 30.54</td>
<td>170.65 ± 25.73</td>
</tr>
<tr>
<td>HDL</td>
<td>2.57 ± 1.03</td>
<td>2.42 ± 1.32</td>
<td>44.50 ± 13.47</td>
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<td>133.29 ± 30.02</td>
<td>129.23 ± 27.80</td>
</tr>
<tr>
<td>VLDL</td>
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<td>5.52 ± 2.25</td>
<td>26.64 ± 6.01</td>
<td>25.78 ± 5.55</td>
</tr>
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TABLE 3: Correlations between and salivary lipid profile in various groups using Pearson correlation.
Because of peroxide radicals. This affects activity of.

Comparing the plasma lipid profile levels in various groups; a non significant decrease in the all the lipid parameters was found in individuals with tobacco consumption but without any potentially malignant lesions, when compared to the individuals without tobacco habits.

These results were consistent with earlier studies done by Patel P et al (2004) but were inconsistent with studies done by Tuckler LA (1989), Adam B (1999), M Khurana et al (2000) who found a significant increase in total triglycerides, total cholesterol, LDL, VLDL and a significant decrease in total HDL in smokers as compared to non-smokers. The results of the present study showed that the tobacco carcinogens may have only a negligible effect on the lipid utilization.

Highly significant decrease in the plasma cholesterol, HDL and LDL levels was seen in individuals with potentially malignant lesions when compared to individuals with tobacco consumption but without any potentially malignant lesions (p value 0.00) and to individuals with no tobacco consumption (p value 0.00). Similarly, Patel P et al (2004), Lohe VK et al (2010) and Nayak P et al (2010) suggested a significant decrease in plasma total cholesterol and high density lipoprotein in patients with cancers as well as in patients with oral precancerous conditions as compared to controls. Nayak P et al (2010), who conducted a study on alteration in plasma lipid profile in precancerous conditions, also found that LDL levels significantly decreased in oral potentially malignant lesions.

Cellular uptake and regulation of cholesterol is mediated by lipoprotein receptors especially located on the surface of the cells. For transport in plasma, cholesterol is packaged into lipoproteins, which are then taken up and degraded by cells to fulfill demands for cellular functions. In some malignant diseases, blood cholesterol undergoes early and significant decrease. Low levels of cholesterol in the proliferating tissues and in blood compartments could be due to the process of carcinogenesis. Secondly, the excessive use of tobacco products has been associated with various lesions in the oral cavity. It is believed that tobacco carcinogens induce generation of free radicals and reactive oxygen species, which are responsible for high rate of oxidation/ per oxidation of polyunsaturated fatty acids. This peroxidation further releases peroxide radicals. This affects essential constituents of cell membrane and might be involved in carcinogenesis / tumorigenesis. Cholesterol plays a key role in the maintenance of cell integrity and is the major constituent of cell membrane. Because of lipid peroxidation, there is a greater utilization of cholesterol suggesting that the decrease in plasma cholesterol, HDL and LDL levels in oral potentially malignant lesions in comparison with normal oral mucosa can be explained on the basis of the underlying disease process and also because of tobacco carcinogens associated with tobacco consumption. Thirdly, cholesterol is an essential constituent of lipoprotein fractions like LDL, HDL and VLDL. 75% of the plasma cholesterol is transported in the form of LDL cholesterol. Body cells sequester cholesterol from LDL fraction of lipoproteins. LDL receptors are necessary for metabolizing circulating LDL cholesterol levels and nearly 80% of the plasma LDL cholesterol is cleared by LDL receptors. High activity of LDL receptors attributes for lowering the plasma cholesterol levels. Some malignant cells have been shown to have increased LDL-receptor activity. This could be the reason for the significant decrease in plasma cholesterol, HDL and LDL levels in individuals with potentially malignant lesions.

Non-significant decrease in the mean plasma triglyceride levels and mean plasma VLDL levels were seen in individuals with potentially malignant lesions when compared to individuals with tobacco consumption but without any potentially malignant lesions (p value 1.00). Similar results have also been reported by Patel P et al (2004) and Lohe VK et al (2010).

Significant decrease in the triglyceride (p value 0.043) and a non significant decrease in the VLDL levels (p value 0.461) were seen in individuals with potentially malignant lesions from individuals with no tobacco consumption. In contrast to our results, Patel P et al (2004), Lohe VK et al (2010) and Nayak P et al (2010) in their studies

<table>
<thead>
<tr>
<th>SUBJEC TS</th>
<th>0.311</th>
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<tbody>
<tr>
<td>HDL</td>
<td>0.203</td>
<td>0.203</td>
</tr>
<tr>
<td>LDL</td>
<td>0.181</td>
<td>0.181</td>
</tr>
<tr>
<td>TRIGLYCERIDES</td>
<td>0.741</td>
<td>0.741</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.739</td>
<td>0.739</td>
</tr>
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Correlation is significant at the level > 0.3
showed a non significant difference in the plasma triglyceride levels between same groups but their results showed variations in VLDL that were similar to our results.

This significant decrease seen in triglycerides in the present study can be explained on the basis that some decrease will be there when there is increased utilization of lipid particles due to new membrane formation during the process of carcinogenesis and also due to lipid peroxidation of cell membrane because of tobacco carcinogens.

The non significant decrease in VLDL levels could be explained on the basis that some decrease can take place when there is increased utilization of lipid particles due to new membrane formation during the process of carcinogenesis and also due lipid peroxidation of cell membrane due to tobacco carcinogens but these cannot cause an overall significant decrease in VLDL. \[13,13\]

Significant positive correlation has been found between plasma and salivary total cholesterol, triglycerides and VLDL levels and a non significant correlation has been found between salivary and plasma HDL and LDL levels. This means some amount of plasma lipid components are filtered into the saliva in proportion to the plasma lipid levels. Similar comparisons have been done by Natheer Al Rawi (2010,2011), who did two different studies and compared plasma and salivary lipid profile in individuals with ischemic heart stroke and the diabetes mellitus, and suggested that lipid fractions particularly triglycerides can be assessed in saliva and may be used alone or in combination with other lipid parameters. \[14,15\]

Total salivary lipid values followed those of plasma lipid values in every except the HDL which showed a significant increase in individuals with potentially malignant lesions. Thus, saliva may act as a filtrate of plasma and the salivary values are found to be reflecting the plasma values but in case of HDL due to smallest particle size as compared to other lipid molecules there are chances of more filtration of plasma HDL levels through the salivary ducts into the saliva in the potentially malignant lesions.

Thus, this study shows an inverse significant correlation between plasma lipid profile and presence of oral potentially malignant lesions therefore, showing the effect of tobacco consumption and utilization of lipids in oral potentially malignant lesions. Tobacco consumption alone cannot cause significant change in plasma lipid profile. Further, saliva may be used as a fluid to assess the lipid profiles in lieu of plasma as it mimics the plasma lipid profile changes.

REFERENCES

15. Rawi NAL. Salivary lipid per oxidation and lipid profile levels in patients with recent ischemic stroke. Journal of International Dental and Medical Research 2010;3(2):67-64.