Original Research Article

Comparison of lipid profile in tobacco and non-tobacco abusers – A comparative study

Juveria Arshi¹, Mohammad Ali R Patel²,*, Mohammed Haneef³, Shakeel Ahmed⁴

¹Dept. of Oral Pathology, Al Badar Rural Dental College and Hospital, Kalburgi, Karnataka, India
²Dept. of Dentistry, KBN Teaching and General Hospital, Gulbarga, Karnataka, India
³Dept. of Maxillofacial and Oral Surgery, Khamis General Hospital, MOH, Asir, Saudi Arabia
⁴Dept. Oral & Maxillofacial Surgery, Al- Ameen Dental College and Hospital, Vijaypura, Karnataka, India

A R T I C L E I N F O

Article history:
Received 28-11-2020
Accepted 03-12-2020
Available online 18-02-2021

Keywords:
Tobacco
Saliva
Serum
Lipids

A B S T R A C T

Aims and Objectives: To evaluate and correlate the salivary and serum lipid profile in healthy individuals, tobacco chewers and smokers. Also, to compare the salivary lipid profile within each group statistically.

Materials and Methods: A total of 60 samples were taken, 20 in each group. Fasting blood and unstimulated saliva sample collected and the lipid analysis (Total Cholesterol - TCHL, Triglycerides - TGL, High density lipid cholesterol - HDL, Low density lipid cholesterol - LDL, very low-density lipid cholesterol - VLDL) were done on an autoanalyzer based on spectrophotometric principle.

Statistical analysis: Data was evaluated and statistical analysis was done using unpaired student “t” test and Karl Pearson’s correlation.

Results: A moderate correlation was found between salivary and serum lipid profile of the study group and control group with exception to LDL. Low lipid profile was observed in the study group in comparison with control group.

Conclusion: Saliva can be used as a non-invasive diagnostic tool for assessing lipid profile, however diagnostic value of saliva has to be determined in terms of sensitivity, specificity and reproducibility in larger samples and different disease setting.

© This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1. Introduction

Lipids are the biomolecules which are insoluble in water and soluble in solvents like chloroform and ether and these are heterogenous in nature and found in the cell membrane.¹,² Lipids are the main component of cell membrane and helps in the maintenance of cell integrity. It also helps in cell division, cell growth and DNA stabilization.¹,² The fatty acids in the diet are being converted into triacylglycerols by liver for fuel or as precursors. These triacylglycerols are then packaged with specific apolipoprotein into very low-density lipoprotein cholesterol (VLDL). These VLDL are transferred to muscles and adipose tissue through blood. Some VLDL gets converted into low density lipoprotein (LDL) by the loss of triacylglycerols. LDL helps in the transport of cholesterol to extra hepatic tissues through the specific plasma membrane receptors for LDL on these tissues. One more type of lipoprotein is High density lipoprotein cholesterol (HDL) helps in transfer of excess cholesterol back to the liver from the extrahepatic tissues.³

Cholesterol is considered to play an important etiological role in coronary heart disease.⁴ In literature it has been found to have inverse relationship of lipid profile to oral premalignant and malignant diseases.⁵,⁶ In literature, the oral squamous cell carcinoma is been reported as the most prevalent carcinoma of head and neck region.⁷ Oral premalignant lesions and conditions like leukoplakia, oral submucous fibrosis etc. are considered to have more potential to develop into oral malignancy.

* Corresponding author.
E-mail address: reach.draliomfs@gmail.com (M. A. R Patel).
These premalignant lesions and conditions significantly play important role in the pathogenesis of oral squamous cell carcinoma.8

The main etiology for oral premalignant and malignant diseases is tobacco consumption. The tobacco carcinogens cause oxidation/ peroxidation of polyunsaturated fatty acids by the production of free radicals and reactive oxygen species. The peroxidation of fatty acids in turn affects the constituents of cell membrane.9

For lipid profiling mostly serum is analyzed. But it is being reported saliva can also be used as an alternative to serum for diagnostic purpose.10 Saliva collection being non-invasive procedure makes it advantageous over serum.1

Therefore, present study is aimed to compare and correlate the serum and salivary lipid profile in healthy individual and tobacco abusers.

2. Materials and Methods

The cases for the study were selected and consent was taken from all the individuals. The cases are obtained from the Out-patient department of the institution. Total 60 cases are taken and divided into 3 groups A, B, C; 20 cases in each group. Group A comprises the healthy control individuals, Group B comprises the tobacco chewers, Group C comprises the tobacco smokers. Patient with chronic habits were included. But patient with any systemic diseases, other premalignant disorders and malignant disease were excluded.

Sample size estimation was done by using G Power software (version 3.0). A minimum total sample size of 60 was found to be sufficient for an alpha of 0.05, power of 95%, 0.36 as effect size (assessed from a similar study).

2.1. Serum sample

With all aseptic precautions, about 5 ml of venous blood was collected. The sample was then allowed to clot at room temperature. Later the sample was centrifuged (Figure 2) at 3000rpm for 10 mins to separate the serum. Immediately the serum was used for the estimation of lipid profile by autoanalyzer (Figure 3) using spectrophotometric principle.

2.2. Saliva sample

Before collecting the saliva sample (Figure 1) Patient is asked to rinse mouth thoroughly to avoid any contamination by debris and exfoliated cells. The patients were asked to pool the saliva in the floor of the mouth and to spit in sterile containers provided to them. Then unstimulated saliva was collected and centrifuged (Figure 2) at 10,000×g for 10 mins to avoid visible precipitates. After centrifugation the saliva sample was subjected to autoanalyzer (Figure 3) based on photometric principle.
2.3. Statistical analysis

The data obtained was subjected to statistical analysis. Descriptive statistics results was used to determine the mean and standard deviation. Comparison of all the parameters between the groups of serum and saliva was done by unpaired student “t” test. Correlation between serum and saliva of all the parameters to all the groups was done by Karl Pearson’s correlation.

3. Results

Mean TCHL, TGL, HDL, LDL and VLDL values in serum are given in (Table 1) for all three study groups viz healthy individuals, Tobacco chewers and among smokers whereas Mean TCHL, TGL, HDL, LDL and VLDL values in saliva are given in (Table 2) for all three study groups viz healthy individuals, tobacco chewers and among smokers. Significant differences were appreciated in TCHL, TGL level and VLDL level when compared in serum for Healthy individuals, tobacco chewers and smokers. Whereas for HDL level significant associations were seen between Healthy individuals and tobacco chewers only, and LDL significantly correlated between healthy individuals tobacco chewers and smokers as p <0.05 (Table 3). Significant differences were appreciated in TCHL, HDL and LDL level when compared in saliva for Healthy individuals vs tobacco chewers vs smokers as p <0.05 (Table 4). Moderate positive correlation between serum and saliva parameters were seen except for LDL level. R- values in (Table 5) shows moderate positive correlation between serum and saliva parameters except LDL.

4. Discussion

Cholesterol and triglycerides help in various physiological functions and are the important component of cell lipids. The main constituent of lipoproteins VLDL, LDL & HDL is cholesterol. LDL is the form in which plasma cholesterol is transported. Tobacco is the main etiological factors for various oral cavity lesion such as potentially malignant lesions, oral cancer etc.

Tobacco releases many carcinogens such as nicotine and nitrosamines which will cause peroxidation of poly unsaturated fatty acids which in results causes the release of free radicals. These free radicals cause tissue injury, damaging cellular DNA, proteins and lipids which will promote carcinogenesis/ tumorogenesis.

In our study we had found decrease in mean of serum HDL from control group to tobacco chewers & smokers (Table 1) which were in accordance to the results of Khurana et al (2000) who has also observed decrease in HDL from control group to tobacco chewers and smokers. We had also observed decrease in mean of serum TCHL & LDL from control group to tobacco chewers and smokers (Table 1) which was contrary to the results of Rao et al (2012) who had reported increase in TCHL, TG & LDL from control group to tobacco chewers & smokers.

VLDL in our study decreases from control group to tobacco chewers but increase in smokers from control group (Table 1) which was contradiction to Khurana et al (2000) study who observed increase in VLDL between control group and tobacco chewers & smokers.

We had observed statistically significant difference between control group and tobacco chewers in all the parameters (table 3) which was in consistent to the results of Rao et al (2012) who had also reported the same data. Whereas when control group was compared with the smokers, we observed statistically significant difference only in case of LDL, other parameters showed insignificant difference (Table 3) which was contrary to the results of Rao et al (2012) who observed statistically significant difference in all the parameters.

When the comparison was done in case of serum values of tobacco chewers and smokers TCHL, TG & VLDL shows significant difference (Table 3) which was in contrary to results of Khurana et al (2000) who had observed statistical insignificant difference. They concluded that tobacco abusing whether in chewing or smoking form has impact on lipid profile, thus can increase the susceptibility to cardiovascular diseases.

We have also evaluated salivary lipid profile in all three groups of all the five parameters. We had observed a moderate correlation between serum and saliva in control group, tobacco chewers and tobacco smokers of TCHL, VLDL, TG & HDL whereas low and negative correlation was found in case of serum and salivary LDL.

Our results were inconsistent with the study of Singh et al (2015) who had also observed moderate correlation between serum and salivary TCHL, TG, VLDL & HDL and low correlation between serum and salivary LDL. They suggested that some portion of plasma lipid gets filtered in saliva. They had stated that it could be possible due to several possible mechanisms. They concluded that saliva can be used as a diagnostic tool for lipid profiling.

Our results were contradicting the results of Karjalainen et al (1997) had performed a study to compare the salivary and serum cholesterol levels of healthy individuals. They found weak correlation between serum and salivary cholesterol correlation. They had concluded that saliva cholesterol levels reflect the serum cholesterol levels.

Our finding contradicts the findings of Chavan et al (2020) whose study found that There was no significant change in total cholesterol, LDL, VLDL, HDL in patients with tobacco addicts and tobacco non-addicts. Serum triglycerides are significantly decreased in tobacco addicts and in malignancy.
Table 1: Mean and standard deviation (SD) for three groups of all the parameters (serum)

|                | Healthy individuals | Tobacco chewers | Smokers   |
|----------------|---------------------|-----------------|-----------|
|                | Mean    | SD    | Mean    | SD    | Mean    | SD    |
| TCHL           | 214.8   | 43.46 | 147.5   | 5.27  | 172.9   | 17.46 |
| TGL            | 137     | 41.43 | 85.1    | 30.19 | 143.8   | 24.26 |
| HDL            | 50.1    | 14.74 | 34.9    | 4.3   | 39.5    | 4.88  |
| LDL            | 137.22  | 30.54 | 95.58   | 7.01  | 104.44  | 13.41 |
| VLDL           | 27.4    | 8.28  | 17.02   | 6.04  | 28.76   | 4.85  |

Table 2: Mean and standard deviation (SD) for three groups of all the parameters (saliva)

|                | Healthy individuals | Tobacco chewers | Smokers   |
|----------------|---------------------|-----------------|-----------|
|                | Mean    | SD    | Mean    | SD    | Mean    | SD    |
| TCHL           | 5.66    | 2.65  | 0.55    | 0.64  | 0.55    | 0.54  |
| TGL            | 3.9     | 2.19  | 2.23    | 2.15  | 6.11    | 4.45  |
| HDL            | 1.64    | 0.75  | 0.64    | 0.27  | 0.46    | 0.3   |
| LDL            | 3.26    | 2.48  | 0.93    | 0.48  | 1.13    | 0.79  |
| VLDL           | 0.78    | 0.43  | 0.44    | 0.43  | 1.22    | 0.89  |

Table 3: Comparison of all the parameters between the groups of serum by unpaired student “t” test

| Groups                              | TCHL | TGL | HDL | LDL | VLDL |
|-------------------------------------|------|-----|-----|-----|------|
| Healthy individuals and tobacco chewers | t value | p value | t value | p value | t value | p value | t value | p value | t value | p value |
| Healthy individuals and smokers     | 2.91  | 0.004 | 1.92  | 0.035 | 1.88   | 0.038 | 2.52   | 0.010 | 1.92   | 0.035 |
| Tobacco chewers and smokers         | 1.69  | 0.054 | 0.27  | 0.39  | 1.29   | 0.106 | 1.86   | 0.039 | 0.26   | 0.398 |

1) T- value in table 3 &4 < 1.73 for p=0.05 shows no significant difference.
2) T- value in table 3 &4 > 1.73 for p=0.05 shows significant difference.

Table 4: Comparison of all the parameters between the groups of saliva by unpaired student “t” test

| Groups                              | TCHL | TGL | HDL | LDL | VLDL |
|-------------------------------------|------|-----|-----|-----|------|
| Healthy individuals and tobacco chewers | t value | p value | t value | p value | t value | p value | t value | p value | t value | p value |
| Healthy individuals and smokers     | 3.55  | 0.001 | 1.02  | 0.16  | 2.35   | 0.015 | 1.73   | 0.05  | 1.02   | 0.16  |
| Tobacco chewers and smokers         | 3.58  | 0.001 | 0.85  | 0.2   | 2.73   | 0.006 | 1.54   | 0.07  | 0.85   | 0.2   |

1) T- value in Table 3 &4 < 1.73 for p=0.05 shows no significant difference.
2) T- value in Table 3 &4 > 1.73 for p=0.05 shows significant difference.

Table 5: Correlation between serum and saliva of all the parameters to all the groups by Karl Pearson’s correlation coefficient (r)

| Tests | Healthy individuals | Tobacco chewers | Smokers |
|-------|----------------------|-----------------|---------|
| TCHL  | 0.52                 | 0.52            | 0.64    |
| TGL   | 0.62                 | 0.58            | 0.56    |
| HDL   | 0.51                 | 0.63            | 0.51    |
| LDL   | 0.009                | -0.3            | 0.08    |
| VLDL  | 0.62                 | 0.58            | 0.56    |

R- values in the table shows moderate positive correlation between serum and saliva parameters except LDL.
5. Conclusion
Saliva can be used as diagnostic tool for the analysis of lipid profiling. It is advantageous because of non-invasive technique for its collection. However, diagnostic value of saliva has to be determined in terms of sensitivity, specificity and reproducibility in larger samples and different disease setting.

6. Source of Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

7. Conflict of Interest
None.

References
1. Chavan RP, Ingole SM, Jagtap VR, Desai WV, Kanchewad GS. Comparative Study of Serum Lipid Profile in Tobacco Addicts, Tobacco Non-addicts and Head–Neck Malignancy. *Indian J Otolaryngol Head Neck Surg*. 2020; doi:10.1007/s12070-020-01812-1.
2. Singh S, Ramesh V, Oza N, Balamurali PD, Prashad KV, Balakrishnan P. Evaluation of serum and salivary lipid profile: A correlative study. *J Oral Maxillofac Pathol*. 2014;18(1):4. doi:10.4103/0973-029X.131881.
3. Bailwad SA, Singh N, Jani DR, Patil P, Singh M, Deep G, et al. Alterations in serum lipid profile patterns in oral cancer: correlation with histological grading and tobacco abuse. *Oral Health Dent Manag*. 2014;13:573–9.
4. Shaheed AM, Zaidan TF, Mahmood RA. Low and high density lipoproteins in serum and saliva of ischemic heart disease patients. *J Bagh Coll Dent*. 2009;21(3):60–4.
5. Singh S, Ramesh V, Premalatha B, Prashad KV, Ramadoss K. Alterations in serum lipid profile patterns in oral cancer. *J Nat Sci, Biol Med*. 2013;4(2):374–8. doi:10.1142/S0120059113500061.
6. Lohe VK, Degwekar SS, Bhowate RR, Kadu RP, Dangore SB. Evaluation of correlation of serum lipid profile in patients with oral cancer and precancer and its association with tobacco abuse. *J Oral Pathol Med*. 2010;39(2):141–8. doi:10.1111/j.1600-0714.2009.00828.x.
7. Radhakrishna M, Idiculla JJ, Aiswarya CJ. Alterations in serum lipid profile patterns in patients with oral submucous fibrosis. *Health Sci*. 2014;1(3):1–12.
8. Sieroń A, Adamek M, Kawczyk-Krupka A, Mazur S, Ilewicz L. Photodynamic therapy (PDT) using topically applied δ-aminolevulinic acid (ALA) for the treatment of oral leukoplakia. *J Oral Pathol Med*. 2003;32(6):330–6. doi:10.1034/j.1600-0714.2003.00068.x.
9. Ames BN. Dietary Carcinogens and Anti-Carcinogens. *J Toxicol Clin Toxicol*. 1984;22(3):291–301. doi:10.3109/15563658408992561.
10. Kaufman E, Lamster IB. The diagnostic applications of saliva- A review. *Crit Rev Oral Biol Med*. 2002;13(2):197–212.
11. Khurana M, Sharma D, Khandelwal PD. Lipid profile in smokers and tobacco chewers—a comparative study. *J Assoc Physicians India*. 2000;48(9):895–902.
12. Rao S, Subash EY. The effect of Chronic Tobacco Smoking and Chewing on the Lipid Profile. *J Clin Diag Res*. 2013;7(1):31–4.
13. Karjalainen S, Sewön L, Soderling E, Larsson B, Johansson I, Simell O, et al. Salivary Cholesterol of Healthy Adults in Relation to Serum Cholesterol Concentration and Oral Health. *J Dent Res*. 1997;76(10):1637–43. doi:10.1177/00220345970760100401.

Author biography
Juveria Arshi, Assistant Professor
Mohammad Ali R Patel, Associate Professor
Mohammed Haneef, Registrar/Specialist
Shakeel Ahmed, Assistant Professor

Cite this article: Arshi J, R Patel MA, Haneef M, Ahmed S. Comparison of lipid profile in tobacco and non-tobacco abusers – A comparative study. *J Oral Med, Oral Surg, Oral Pathol, Oral Radiol*. 2021;7(1):37-41.