

## Forensic application of saliva : Evaluation of association of gender with the refractive index of saliva among the young adults of Western India

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**Abstract:-** Biological fluids have been playing a vital role in most of the cases involving the criminal investigation. They are found to be useful as markers of identification. Though there are plenty of studies available in blood, there are very less number of studies available on saliva. A study has been undertaken with saliva and has been analyzed without subjecting it to any chemical and biological examinations. But there was a physical examination done with the parameter of refractive index. In this study a total of 101 males and 101 females have been considered. From these subjects, the saliva has been collected and has been analyzed for refractive index. There was a significant difference noted among the females and males.

**Key words:** saliva, sex determination, refractive index

### INTRODUCTION

Saliva is one of the most complex biological fluids that has imperative role in forensic sciences. It is well known that there is always an exchange of minute particles when there is a contact. This has been noted by Edmond Locard. This is a popular principle of exchange<sup>(1)</sup>. In an offence against an individual such as sexual assault, child abuse, physical assault or attempted homicide cases, bite-marks are usually found. One of the least common evidences collected from such incidents is that of saliva. It may be obtained either from the victim or suspect. This is more commonly present in them. The Double swab technique is usually used to collect saliva from the skin and the collected saliva is subjected to a DNA analysis for identification purpose<sup>(2)</sup>. The physical properties of saliva are less concentrated as compared to other biological fluids. The work done by Reiss (1909) on the refractive index of human serum, shows the importance of physical properties of body fluids<sup>(3)</sup>. Without subjecting the saliva for any chemical or biochemical profiling, there is a scope of getting it done through the assessment of the physical properties such as a refractive index. This is one of the few parameters that is least explored till date. By applying the easier and least investigative procedures, there is a scope of assessing the primary characteristics. The refractive index value is directly proportional to the concentration of the fluid<sup>(4)</sup>. In this study, an evaluation has been made with the refractive index value of male and female saliva to identify if any significant differences are evident.

### MATERIALS AND METHODS

The informed consent has been obtained from all the subjects. This study includes non-stimulated salivary sample of 101 males and 101 females. The inclusion criteria are of the age group between 18 to 30 years. They are all of normal, both physically and psychologically as well. The influence of various pathological conditions such as the upper respiratory disorders and other ailments of the oropharynx is excluded. The subjects having the habits of smoking, alcoholism and paan chewing habits also have been excluded. Their oral hygiene has been screened and participants with chronic periodontitis, decay and other pathological diseases are also excluded. The materials required for the analysis of saliva sample are, an automatic refractometer (J357 Rudolf research), sterile cotton, sterile blood collecting plain tube (4ml), sterile disposable syringe (2.5ml), sterile masks and gloves, diethyl ether, distilled water and tissue paper. The saliva is collected by keeping sterile cotton on the parotid and submandibular region for 30 secs to 1 min (Fig 1.1). The cotton soaks due to the imbibition of saliva. (Fig 1.2) The saliva soaked cotton is placed in the sterile syringe and squeezed to collect the saliva in the sterile blood collecting plain tube (4ml) (Fig 1.3). This collected saliva is loaded on the automatic refractometer to find out the refractive index value (Fig 1.4). The automatic refractometer is standardized (calibrated) by distilled water for every 4 samples of saliva. Before loading, each sample it is cleaned properly with a sterile tissue paper. Between 4 samples, it is cleaned with diethyl ether and standardized with distilled water to check the calibration of the instrument.



Fig: 1.1



Fig: 1.2

Fig: 1.1 the saliva is collected by keeping sterile cotton on the submandibular region for 30 secs to 1 min.

Fig: 1.2 the cotton soaks due to the imbibition of saliva.



Fig: 1.3



Fig: 1.4

Fig: 1.3 the saliva soaked cotton is placed in the sterile syringe and squeezed to collect the saliva in the sterile blood collecting plain tube (4ml).

Fig: 1.4 This collected saliva is loaded on the automatic refractometer to find out the refractive index value.

#### **Result**

| <b>Group Statistics</b> |        |     |           |                |                 |
|-------------------------|--------|-----|-----------|----------------|-----------------|
|                         | Gender | N   | Mean      | Std. Deviation | Std. Error Mean |
| RI                      | Female | 101 | 1.3339979 | .00033759      | .00003359       |
|                         | Male   | 101 | 1.3341379 | .00050186      | .00004994       |

### Independent sample test

|        |                             | Levene's Test for Equality of Variances |      | t-test for Equality of Means |         |                 |                 |                       |   |            |
|--------|-----------------------------|---|------|------------------------------|---------|-----------------|-----------------|-----------------------|---|------------|
|        |                             | F                                       | Sig. | t                            | Df      | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference |            |
|        |                             |   |      |                              |         |                 |                 |                       | Lower                                     | Upper      |
| R<br>I | Equal variances assumed     | 3.404                                   | .067 | -2.325                       | 200     | .021            | -.00013993      | .00006018             | -.00025861                                | -.00002126 |
|        | Equal variances not assumed |   |      | -2.325                       | 175.118 | .021            | -.00013993      | .00006018             | -.00025871                                | -.00002115 |

T test was done to find out whether there is a difference between RI value of male and female group. Levene's test for equality of variance was done. As the significance level of equal variance is assumed to be 0.067(above 0.05), we continue to see the first line. The two tailed significance is 0.021(below 0.05), and it is proved that there is a significant difference in RI between males and females. (Null hypothesis is not accepted).

### Discussion

The secretion of saliva which includes the consistency and other physical parameters, are influenced by various factors such as the nutritional intake, seasonal variation, circadian rhythm, BMI and others(5). In our study, the sample was collected during the month of September when the temperature is usually around 30 to 35 degree Celsius in the northern part of Gujarat. The samples were collected during pre-lunch and pre- snacks time period. The refractive index value of saliva was found to be almost similar without any significant difference between the readings in the particular individuals. In all the samples, a same trend was noticed. This suggested that there is consistency uniformity in their RI value. There was a statistically significant difference found exist between male and female RI value. This variation in the RI value may be attributed to a number of factors. Prodan et al study states that there is difference between components of salivary secretion of males and females. In that study it has been mentioned about the total protein concentration, amylase, chitinase and secretory Iga quantity variation between male and female (6). We suspect that these changes in composition of male and female saliva reflected in the RI value.

### Conclusion

There is a significant difference found between male and female salivary refractive index value. This will be attributed to the presence of different protein concentrations, hormone levels, as well as Iga of males and females, which leads to changes in the density of saliva between both males and females. This change in density that influences the refractive index value of the saliva helps to do a sex determination. Since this study is purely done on the basis of assessment of refractive index, further studies are required to verify the particular components that are responsible for the change in density between male and female saliva which gives rise to changes in the refractive index value.

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