

## Determination of Sex from Tooth Pulp Tissue

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**Abstract: Introduction:-**Forensic Odontology is an investigative aspect of dentistry that analyzes dental evidence for human identification. Forensic Odontology plays an important role in establishing sex, age, and race of victims. **Aim and Objectives:-** To determine the sex of an individual from tooth pulp tissue, using Fluorescent body test (F-Body test). **Materials and Method:-** The study sample consisted of **60 extracted teeth (maxillary and mandibular teeth combined)** which were collected from private dental clinics. Of these 60 teeth taken for study, 30 teeth were taken from Female patients and 30 teeth were taken from Male patients. The teeth were divided into 3 groups of 20 teeth each (each group consisting of 10 teeth each from both males and females), such that the 1<sup>st</sup> group consisted of teeth which has been extracted 1 day previously; the 2<sup>nd</sup> group consisted of teeth which has been extracted 30 days prior, 3<sup>rd</sup> group consisted of teeth which has been extracted 90 days prior. Each such group of 20 teeth was further divided into two **sub-groups A and B**, with 10 teeth in each sub-group (5 teeth from female patients and 5 teeth from male patients). All teeth in **sub-group A** were kept unpreserved in sterile containers at room temperature. All teeth in **sub-group B** were buried 10 cm. under soil. **Result-** Out of 60 samples, interpretation of sex was possible in 57 samples (95%) which consisted of 15 samples from male patients and 15 samples from female patients kept unpreserved in dry, sterile containers at room temperature and 13 samples from male patients and 14 from female patients buried 10cm. under soil. Interpretation in 3 samples (5%) was inconclusive which consisted of 2 samples from male patients and 1 from female patient buried 10cm. under soil.

**Keywords:** - Forensic Odontology, Y bodies.

Date of Submission: 03-08-2019

Date of acceptance: 19-08-2019

### I. Introduction

The word Forensic means 'debate'. It is derived from the Latin phrase meaning "before the forum" or where legal matters are discussed. Forensic Odontology is the application of the knowledge of dental sciences to the law, and encompasses areas such as identification of deceased and living, investigation of Orofacial trauma and bitemarks, and opinion on the dental issues. It involves the recognition, documentation, interpretation and presentation of evidence.

Forensic Odontology plays a small but significant role in this process. Human identification is the Forensic Odontologist's primary duty. By identifying the victims of crime and disaster through dental records, dentists assist those involved in investigation of the crime. Always a part of a bigger team, such personnel are dedicated to the common principles of all those involved in forensic casework: the rights of the dead and those who survive them.<sup>1</sup>

Determination of sex using skeletal remains presents a great problem for forensic experts especially when only fragments of the body are recovered. Forensic dentists can assist other experts in determining sex of the remains by using information of the dental and skeletal remains. Various features of teeth, like morphology,

crown size, root length etc, are characteristic for males and females. There are also differences in the skull pattern<sup>2</sup>.

Being composed of a homogenous mass of calcium apatite crystals, the tooth enamel is the hardest structure of the body, imparting resistance to fire, chemicals and toxins; hence damage to tooth is not an easy and immediate process. All the teeth possess the ability to resist to heat, chemicals, microbial attack and other environmental factors.<sup>3,4</sup>

Classification of Methods<sup>5</sup>:

- Visual method or clinical method
  - Microscopic methods
  - Advanced methods
1. Visual method or clinical methods:  
Differences between the sexes with respect to:
    - a. Tooth size
    - b. Root length and crown diameter
    - c. Using canine dimorphism
    - d. Tooth morphology
    - e. Dental index
    - f. Odontometric differences
  2. Microscopic methods
    - a. Sex determination using Barr bodies
    - b. Sex determination using F bodies (Fluorescent bodies)
  3. Advanced methods
    - a. Sex determination using Polymerase Chain Reaction (PCR)
    - b. Sex determination using enamel protein

The present study uses the tooth pulp tissue from extracted human teeth as the substratum for sexual characterization. The first reason to select the tooth pulp as the preferred tissue for this study is its sequestered location. Embedded in a chamber surrounded by intact highly mineralized dentin and enamel, the pulp is vulnerable to the external environment only at a small apical aperture. Tooth enamel is known to be virtually indestructible even when embedded in the ground from the evidence of the archeological sites (Mann & Wood,1970) and dentin, which is less mineralized, is also very tough, but does eventually become brittle over many millennia.<sup>6</sup> Sequestration of the pulp in these protective materials occasionally results in unusual pulpal preservation. For example, remnants of odontoblastic pulp cell processes in dentinal tubules have been reported in a histological study of Bronze age teeth(Falin,1961) and naturally mummified pulp tissue adhering to dentin in corners of the pulp cavity have been reported in the teeth from Belgium, circa seventh to ninth century A.D.<sup>7</sup> The second reason for the selection of the tooth pulp is its easy availability. Supplementing these two reasons, for selecting dental pulp, is the suitability of the majority of the cells of the pulp for gender characterization<sup>8,9,10</sup>. Thus, the present study is designed to expand current knowledge on the constraints pertaining to sex discrimination in pulp tissue, deposited in simulated forensic environments.

### **Aim**

To determine the sex of an individual from tooth pulp tissue, using Fluorescent body test (F-Body test).

### **Objectives**

1. To determine the sex of the subject from tooth pulp tissue.
2. To correlate the obtained sex of the subject with the known sex of the patient.
3. To evaluate the accuracy of sex determination based on Fluorescent Body (F-Body) examination.
4. To determine the reliability of accurate sex determination from tooth pulp tissue, keeping in view the effect of time in simulated forensic environments on pulp tissue, after extraction of teeth.

## **II. Materials And Methods**

It is a well known fact that sex can be determined from tooth pulp tissue in living as well as dead. Up to what postmortem interval it can be determined accurately, is still a matter of controversy. A prospective study for sex determination by tooth pulp tissue using Quinacrine nuclear staining was carried out in the Private clinics.

**Inclusion criteria:** Subjects/patients advised for prophylactic removal of asymptomatic tooth/teeth which were periodontally compromised, impacted or indicated for extraction as a part of orthodontic treatment needs, were considered for the study.

**Exclusion criteria:** subjects/patients with any clinical signs/symptoms of any obvious pathology associated with the concerned tooth/teeth were not considered for the study.

- ❖ The study sample consisted of **60 extracted teeth (maxillary and mandibular teeth combined)** which were collected from the private dental clinics.
- ❖ Sex and age of the patient, date of extraction, tooth specimen type and the reason for extraction was recorded.
- ❖ Of these 60 teeth taken for study, 30 teeth were taken from Female patients and 30 teeth were taken from Male patients.
- ❖ The teeth were divided into 3 groups of 20 teeth each (each group consisting of 10 teeth each from both males and females), such that the 1<sup>st</sup> group consisted of teeth which has been extracted 1 day previously; the 2<sup>nd</sup> group consisted of teeth which has been extracted 30 days prior, 3<sup>rd</sup> group consisted of teeth which has been extracted 90 days prior. Each such group of 20 teeth was further divided into two **sub-groups A and B**, with 10 teeth in each sub-group (5 teeth from female patients and 5 teeth from male patients).
- ❖ All teeth in **sub-group A** were kept unpreserved in sterile containers at room temperature.
- ❖ All teeth in **sub-group B** were buried 10 cm. under soil.
- ❖ Teeth were extracted according to method described by Kruger.
- ❖ Teeth were kept at different simulated forensic environments, without any preservation.
- ❖ Individual tooth pulp tissue was examined from the groups, at the intervals, as per schedule of the study.

#### **Sectioning of the tooth:**

- ❖ The tooth to be sectioned is embedded on modeling wax block.
- ❖ To free the pulp, the crown is separated longitudinally by using a carborundum disc at 30,000 rpm. Similarly the root is split for pulp removal.
- ❖ The whole of the pulp tissue is separated out of the pulp cavity, with the help of needle and forceps and transferred to a conical tube containing normal saline.
- ❖ It is then adequately washed in normal saline to remove any calcified bone or dentin particles or debris.

#### **Obtaining clear suspension of pulp cells :**

- ❖ The pulp tissue is transferred to a dry and clean conical centrifuge tubes and **0.5 ml of fixative (20% acetic acid)** is used to soften the dental pulp.
- ❖ It is then crushed / teased with the glass rod sufficiently to isolate the pulp cells.
- ❖ Again 2ml. of fixative (20% acetic acid) is added and the suspension is stirred well.
- ❖ A suspension thus obtained is centrifuged for 10 minutes at 1000 rpm.
- ❖ The supernatant is discarded, leaving behind the pellet in the centrifuge tube.
- ❖ 2ml. of fresh fixative is then added to re-suspend the pellet and the process is repeated till a clear suspension of the pulp cells is obtained.

#### **Preparation of smear :**

- ❖ Thin smears are prepared on a chilled fluorescence microscope glass slide by the air drying method i.e., by dropping 2-3 drops of the clear suspension of the pulp cells on the slide from a distance of few inches, to obtain a monolayer of cells.

#### **Fixing and Staining of the pulp cells :**

- ❖ After drying at room temperature, a few drops of **methanol** were added to fix the material.
- ❖ After natural evaporation of the methanol, the material is stained with **0.5% Quinacrine dihydrochloride** for 20 minutes.
- ❖ The slide is then washed with double distilled water and kept in **Mellvaine's buffer** (0.1M Citric acid, 0.2 M dibasic Sodium phosphate) with pH of 5.5 for 3 minutes.
- ❖ The slide is then washed with **0.4 g/L Magnesium chloride** for 10 minutes and then a drop of **Glycerol** is added.
- ❖ A cover slip is placed on top, avoiding trapping of any air bubbles.

#### **Specimen observation and Sex determination from Fluorescent-body ( F-Body) assessment:**

- ❖ The specimen is observed with Fluorescent microscope under oil immersion in dark field having Blue Glass as excitation filter and Orange Glass as barrier filter.

- ❖ Only those cells which contained the characteristic Y-chromatin i.e., a brightly fluorescent spot (F-Body) close to the nuclear membrane is counted as positive.
- ❖ **Sex is identified as Male :** if one/more fluorescent spot (F-Body) is observed in the nucleus of male with fluorescent Y-chromosome.
- ❖ **Sex is identified as Female :** if fluorescence of the whole nucleus (without F-body) is observed without any fluorescent spot.

### III. Results And Observations

- ❖ A prospective study for sex determination by tooth pulp tissue using Quinacrine nuclear staining was carried out in the private clinics.
- ❖ The present study sample consisted of **60 extracted teeth**; 30 teeth were taken from Female patients and 30 from Male patients.
- ❖ Presence/absence of F-bodies and the number of fluorescent cells in male and female specimens were recorded.
- ❖ Out of 60 samples, interpretation of sex was possible in 57 samples (95%) which consisted of 15 samples from male patients and 15 samples from female patients kept unpreserved in dry, sterile containers at room temperature and 13 samples from male patients and 14 from female patients buried 10cm. under soil. Interpretation in 3 samples (5%) was inconclusive which consisted of 2 samples from male patients and 1 from female patient buried 10cm. under soil. (**Table I**)
- ❖ Sex-wise distribution of fluorescent nucleus in different simulated forensic environments for Group 1 revealed that the mean value of fluorescent nuclei of male specimens kept unpreserved in sterile containers at room temperature was 57.0 and that of specimens buried under soil was 58.6 while the same for female specimens was 55.4 and 51.0 respectively, for Group 2 the mean value of fluorescent nuclei of male specimens kept unpreserved in sterile containers at room temperature was 40.4 and that of specimens buried under soil was 29.0 while the same for female specimens was 36.2 and 19.2 respectively, and for Group 3 the mean value of fluorescent nuclei of male specimens kept unpreserved in sterile containers at room temperature. was 16.6 and that of specimens buried under soil was 2.8 while the same for female specimens was 17.6 and 5.2 respectively(**Table II**)
- ❖ Group-wise distribution of statistical data for Subgroup A revealed a mean of 56.20 (Sd ±10.40) and a non-significant p value of 0.824 for Group 1; a mean of 38.30 (Sd ±6.88) and a non-significant p value of 0.635 for Group 2 and a mean of 17.10 (Sd ±3.54) and a non-significant p value of 0.682 for Group 3 (**Table III**)
- ❖ Group-wise distribution of statistical data for Subgroup B revealed a mean of 54.8 (Sd ±7.81) with a non-significant p value of 0.130 for Group 1; a mean of 24.1 (Sd ±6.80) with a non-significant p value of 0.258 for Group 2 and a mean of 4.0 (Sd ±3.19) with a significant p value of 0.011 for Group 3 (**Table IV**)
- ❖ One way **ANOVA** showed an overall statistically significant p value (<**0.0001**) between groups, establishing a credible reliability of the present study for sex determination.

**Table: I** Sex-wise distribution of the conclusive and inconclusive data between various subgroups

INTERPRETATION	MALE		FEMALE		GRAND TOTAL
	Subgroup A	Subgroup B	Subgroup A	Subgroup B	
CONCLUSIVE	15 (100%)	13 (86.66%)	15 (100%)	14 (93.33%)	57 (95%)
INCONCLUSIVE	0	2 (13.33%)	0	1 (6.66%)	3 (5%)
TOTAL SAMPLES	15	15	15	15	60

**Table: II** Sex-wise distribution of fluorescent nucleus (mean value) in different groups and subgroups

TEETH STORAGE PERIOD	SIMULATED FORENSIC ENVIRONMENT			
	Subgroup A		Subgroup B	
	MALE	FEMALE	MALE	FEMALE
GROUP 1	57.0	55.4	58.6	51.0
GROUP 2	40.4	36.2	29.0	19.2
GROUP 3	16.6	17.6	2.8	5.2

**Table: III** Group-wise distribution of statistical data for Subgroup A

GROUP	MEAN (fluorescent nucleus)	STANDARD DEVIATION	STANDARD ERROR	p VALUE
Group 1	56.20	±10.40	3.28	0.824 NS
Group 2	38.30	±6.88	2.17	0.635 NS
Group 3	17.10	±3.54	1.12	0.682 NS

**Table: VIII** Group-wise distribution of statistical data for Subgroup B

GROUP	MEAN (fluorescent nucleus)	STANDARD DEVIATION	STANDARD ERROR	p VALUE
Group 1	54.8	±7.81	2.47	0.130 NS
Group 2	24.1	±6.80	2.15	0.258 NS
Group 3	4.0	±3.19	1.01	0.011 S

#### IV. Discussion

Forensic medicine is an interdisciplinary science and makes use of scientific principles, facts and processes in order to arrive at a diagnosis that is required of it in the due course of law.<sup>11</sup> Determination of sex is an essential part of any forensic investigation. Sex determination is one of the most important means of establishing the identity of an individual. Establishment of identity brings about legal, social and emotional closure for the authorities and the deceased's family. Usually it is assessed macroscopically from soft tissue or skeletal morphology. In the case of adult skeletal material, when it is well preserved, there is about a 95-98% probability of correct sex determination from the combined metric and non-metric data of the skull and pelvis.<sup>12</sup>

In the present study a total of 60 samples of teeth, prophylactically removed from male and female patients, were grouped and processed at different time intervals of 1 day, 30 days and 90 days and were kept either unpreserved in clean, dry sterile containers at room temperature or buried 10 cm. under soil.

The study by Caspersson et al. (1968)<sup>13</sup> suggested that Quinacrine mustard and its derivatives bind with chromosomal DNA and demonstrate intense fluorescence. Similarly, Pearson et al. (1970)<sup>14</sup> reported the fluorescent staining property of Y-chromatin in the nuclei of buccal mucosal cells, lymphocytes and fibroblasts and they found that the distal portion of the long arm of Y-chromosome fluoresced so intensely that it was easily recognized in both interphase and mitotic nuclei as a distinctive fluorescent structure or F-body.

As per the above mentioned findings, Quinacrine dihydrochloride nuclear staining was selected for our study to determine sex from the tooth pulp tissue.

Out of 60 samples, interpretation of sex was possible in 57 samples (95%) over a period of 90 days. Out of 3 inconclusive samples, 2 samples were from male patients and 1 from female patient which were buried under soil. Our finding is in accordance with the results previously reported by Seno (1977) in teeth buried under soil, in which his sex diagnosis was also greatly reduced to 1 out of 7 teeth from males and 2 out of 10 teeth from females for the same time period as ours, presumably due to cellular decomposition.

In our study, we observed in males 46-67 (mean 57.0) fluorescent nuclei (with F-body) in samples 1 day after extraction, 33-48 (mean 40.4) fluorescent nuclei 30 days after extraction and 13-21 (mean 16.6) fluorescent nuclei 90 days after extraction, for samples kept at room temperature. By our observation it was revealed that the pulp tissue can be well preserved for a long time because of the natural drying.

In our study, we also observed in females 43-77 (mean 55.4) fluorescent nuclei (without F-body) in samples 1 day after extraction, 29-46 (mean 36.2) fluorescent nuclei 30 days after extraction and 14-24 (mean 17.6) fluorescent nuclei 90 days after extraction, for samples kept at room temperature.

Teeth stored at room temperature posed special problems for chromatin counts in our study. In any dehydrated tissue, pulp cells become firmly embedded in the fibrous pulp matrix. An attempt was made to separate the cells by the method outlined by Seno & Ishizu (1973), in which pulps were soaked in 20% acetic acid and crushed / teased with a glass rod sufficiently to isolate the pulp cells. This technique proved to have some disadvantages in terms of nuclear damage to cells, rendered fragile by post mortem decomposition, as well as the inclusion of a confusing assortment of fluorescent matrix debris impeding the examination of intact nuclei. The same problem was encountered by Whittaker (1975). The disparity between the data reported in our study and in the literature most likely reflects inter-observer error since sex chromatin evaluation involves subjectivity.

The pulp stability in outdoor environments (teeth buried 10 cm. under soil) for up to 90 days limit as recorded in our study, has also been documented. Yamamoto (1959) reported preservation of pulp cell fibroblasts buried for 15 days beneath 50cm. of sand. Similarly, Whittaker et al. (1975) included teeth which were allowed to putrefy in a humid atmosphere and found the reliability of this method for up to 10 weeks period. Similarly, Seno (1977) also, conducted F-body counts on pulp cells from teeth buried 1 month in mud at a depth of 25cm. Finally, Ionesiy (1980) made sex chromatin counts on pulps buried in soil for 20 days.

One way ANOVA showed an overall statistically significant p value (<0.0001) between groups, establishing a credible reliability of the present study for sex determination.

#### V. Conclusion

- ❖ F-body quantitation, as a means of sex determination was highly effective for teeth preserved at room temperature, irrespective of time duration.

- ❖ When samples are kept under soil for 90 days, determination of sex through this method was greatly reduced, as inconclusive results are seen in 2 out of 5 male samples and 1 out of 5 female samples, probably due to the complete bacterial decomposition of the pulp cells.
- ❖ There was a linear decrease of the frequency of fluorescent nuclei in both the sexes in our study as the storage time increased and this was more pronounced in frequency in samples buried under soil than in samples kept at room temperature for the same storage period.
- ❖ The sharp decline in the frequency of fluorescent nuclei in the samples buried under soil for the 90 day period can be explained by the onset of putrefactive processes leading to partial/complete decomposition of the pulpal cells.
- ❖ Group-wise distribution of statistical data showed non-significant p values for samples stored at room temperature for up to 90 days and for samples stored under soil for a period of 30 days while a significant p value (<0.011) was obtained for the samples buried under soil for 90 days period signifying a sharp decline in the frequency of fluorescent nuclei observed in the samples buried under soil for 90 days.
- ❖ One way ANOVA showed an overall statistically significant p value (<0.0001) between groups, establishing a credible reliability of the present study for sex determination.
- ❖ Decomposition rates vary geographically, correlating with macro environmental variables, like atmospheric temperature and humidity, climatic factors, wind speed and aridity. Micro environmental variables related to the locale also influence the result. These can be the soil type, depth of burial, amount of wilderness.
- ❖ So, we suggest the further analysis of fluorescence sex diagnostics in various situations of forensic importance such as time elapsed since death, special conditions of burial, temperature and calcinations to assess any changes in terms of diagnostic value.

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Dr. Tapan G. Modi" Determination of Sex from Tooth Pulp Tissue" IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 8, 2019, pp 61-66.