Comparison of Gender Determination from Dental Pulp and Dentin after Exposure to Various Environmental Conditions: A Polymerase Chain Reaction-based SRY Gene Study

Abstract

Purpose: Sex determination becomes the first priority by a forensic investigator in the process of identification of bodies mutilated beyond recognition due to mass disasters. Sex-determining region Y (SRY) gene is used as a sex-typing marker as the results are said to be 100% accurate. Therefore, an experimental study was conducted to rule out the possibility of the presence of SRY gene in tooth pulp and dentin after being exposed to various environments. Materials and Methods: Twenty extracted teeth of known gender were taken, of which ten were male and ten were female. The tooth samples were subjected to various environmental conditions for 60 days. Results: Among the total twenty samples, DNA isolation was done from all the samples of pulp and dentin. Gender determination using SRY gene was correct in all the samples of the pulp, and no interpretation of SRY was possible from the dentin though the DNA was isolated. Conclusion: The present study has found DNA retrieval can be done from human teeth irrespective of the storage time and environmental conditions for forensic caseworks and SRY gene to be a reliable biomarker for sex determination from the pulp tissue of teeth.

Keywords: DNA isolation, forensics, gender, polymerase chain reaction, sex-determining region Y gene

Introduction

Determination of sex using skeletal remains presents a great problem to forensic experts, especially when only fragments of the body are recovered.[1] Other parts of the body such as teeth that are resistant to all environmental conditions such as high temperature, cold, water and also in buried for longtime play an important role as they show particular differences in their tooth size like mesio-distal width and canine dimorphism between males and females.[2] Sex is also determined by observing the Barr bodies and F-bodies in X and Y chromosomes, respectively, under the microscope. All these methods have limitations, and the percentage of accuracy is not 100%. Advanced methods such as extraction of the DNA and polymerase chain reaction (PCR) amplification will assist accurately in determining the sex of the remains.[3]

There are various sex-typing markers used for the identification such as amelogenin, centromeric alphoid repeats, ZFX/ZFY zinc finger genes, DXYS156, and DYZ1, of which most frequently used in dental samples is amelogenin.[3] Several studies have observed deletion of this amelogenin gene on Y-chromosome, and the males have been wrongly identified as females. In these situations of unambiguous gender identifications, sex-determining region Y (SRY) gene is used for the identification of gender of amelogenin-deleted males.[4]

SRY stands for SRY gene. These gene codes for the SRY protein, which is responsible for further development as male. SRY is located on the short (p) arm of the Y-chromosomes at the position 11.3. The presence of SRY gene is very important for testis differentiation in the early embryo. If SRY gene is absent means, it develops ovaries. Therefore, SRY gene is used as a sex-typing marker, as the results are said to be 100% accurate.[1,3]

Therefore, the present study was conducted to rule out the possibility of the presence...
of SRY gene in tooth pulp and dentin after being exposed to various environments as SRY gene is least used as a sex-typing marker in the dental tissue samples with the presence of very little information in pulp and no information in dentin in the forensic dentistry.

Aim of the study

The study aimed to evaluate the comparison of gender determination using SRY gene from dental pulp and dentin after exposure of the tooth to various environmental conditions.

Objectives of the study

- To extract the DNA from dental pulp and dentin after exposure to various environmental conditions
- To determine the gender of a person from dental pulp tissue by amplifying the DNA sequences based on SRY gene
- To determine the gender of a person from dentin by amplifying the DNA sequences based on SRY gene.

Materials and Methods

Source of data

The study sample was calculated using a formula using the random sampling technique and twenty extracted teeth of known gender, of which 10 were male and 10 were female were taken into the study.

Inclusion criteria

1. Tooth samples of known age (18–40 years) and gender
2. Tooth samples immediately after extraction were taken, in which vital tooth was considered before extraction, but not vital pulp
3. Permanent tooth samples extracted for orthodontic treatment, impacted teeth or due to periodontal destruction.

Exclusion criteria

1. Tooth samples whose gender was not known
2. As the study was done exclusively on a permanent tooth, deciduous tooth samples were excluded from the study
3. As it was a new study, we wanted more quantity of pulp tissues rather than necrotic pulp and hence teeth with extensive decay involving pulp were excluded
4. Tooth with wear facets was excluded because most of the time pulp chamber and root canal will be calcified in the presence of wear facets
5. Restored and RC-treated teeth.

Methods

The present study was an experimental single-blinded study consisting of twenty extracted teeth and categorized into four groups consisting of five samples [Table 1] in each group based on the environmental conditions such as Group I (freshly extracted – 5 teeth); Group II (stored in seawater – 5 teeth); Group III (stored in garden soil – 5 teeth); and Group IV (stored in dry air at room temperature – 5 teeth) to which the teeth were exposed for 60 days. Further, the groups were subdivided into A and B subgroups. Subgroup A denotes pulpal tissue and B denotes dentin powder.

The tooth samples after being subjected to various environmental conditions such as stored in seawater, stored in garden soil, and stored in dry air at room temperature for 60 days, later it was taken out and [Figures 1-4] were washed in 5.2% sodium hypochlorite solution for around 30 s, then the teeth were cleaned and washed again with sterile distilled water for around 30 s.

Two investigators were involved in the extraction of pulp and dentin in this study. The tooth was wiped with cotton and by using hand trimmer with carborundum disc; each tooth was longitudinally sectioned into two halves [Figure 5]. The pulp tissue from each tooth was removed using an endodontic broach No. 21. The derived pulp sample was then put in a sterile Eppendorf tube containing a DNA extraction buffer (sodium dodecyl sulfate or sodium lauryl sulfate) [Figure 6]. The tubes were labeled accordingly and stored at normal room temperature.

Dentin powder was obtained using hand trimmer and straight fissure acrylic trimming bur. The powder was then collected into sterile Eppendorf tubes [Figure 7] and labeled accordingly and stored at normal room temperature.

Samples of both pulp and dentin were then sent to the department of molecular biology and immunology to the 2nd observer who do not know the gender of the patient for further procedure. In the next step, DNA extraction procedure was performed using modified proteinase K method for pulp and dentin. Later, PCR was done, in which the following set of PCR primers was used which are specific to SRY of the human Y chromosome that encodes a testes-determining factor which initiates male sex determination.

SRY F 5′-GAATATTCGGCTCTCCGGAG-3′
SRY R 5′-ACCTGTGTGCAGTTGCACT-3′

This set of primer amplifies a 418 base-pair fragment which is specific to Y-chromosome. Once the Y-chromosome is

| Table 1: Study groups |
|-----------------------|
| Groups                | Number of samples in each group | Sample numbers |
| Group I (freshly extracted) | 5 | 1-5 |
| Group II (stored in seawater) | 5 | 6-10 |
| Group III (stored in the garden soil) | 5 | 11-15 |
| Group IV (stored in dry air at room temperature) | 5 | 16-20 |

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amplified, then gel electrophoresis was performed at 25V for 2 h. The gel was taken into transilluminator for further analysis.

The gel was visualized under ultraviolet transilluminator. The target region from the DNA of each sample will be amplified and will be separated on the agarose gel depending on their size. Selection of primer set decides the target region to be amplified and the size of that amplified product. The primer set which was selected amplifies a 418 base-pair fragment which is specific to Y-chromosome. The DNA ladder was run simultaneously with each gel to obtain the bands of known sizes which was used in locating the band positions of test samples. Band at position 418 base pair is considered as positive or male sample and nonspecific bands or no bands are taken as negative or female samples.

**Results**

The present study was a randomized single-blinded study consisting of twenty extracted teeth and categorized into four groups consisting of five samples in each group based on the environmental conditions to which the teeth were exposed. The paired t-test was conducted for the comparison of concentration of DNA among pulp and
dentin samples. The sensitivity and specificity of SRY gene were observed in the present study.

Among the total twenty samples, DNA isolation was done from all the samples of pulp and dentin irrespective of their storage conditions, and concentration of DNA obtained from pulp and dentin in µg/ml is shown in Table 2. The paired t-test was conducted for the comparison of concentration of DNA among pulp and dentin samples. A total of twenty samples’ standard deviation of DNA from the pulp is 73.94952 and mean values is 207.9560. Standard deviation of DNA from dentin is 69.09232 and mean value is 139.4885. In the present study, $P < 0.001$ and it should be $<0.05$, and hence we can say much significant between pulp and dentin using the paired t-test which is shown in Table 3. Gender determination using SRY gene was correct in all the samples of the pulp from freshly extracted teeth shown in Table 4, pulp stored in seawater in Table 5, pulp stored in garden soil shown in Table 6, and stored in dry air at room temperature shown in Table 7. Interpretation of SRY gene was not possible from the dentin, though the DNA was isolated from dentin from various environmental conditions as shown in tables, respectively, from freshly extracted teeth shown in Table 8, pulp stored in seawater Table 9, pulp stored in garden soil shown in Table 10, and stored in dry air at room temperature shown in Table 11. Later, sensitivity and specificity of SRY gene were found to be 100% sensitive and 100% specific for gender determination from pulp for the present study using the following formulae.

Sensitivity is calculated by the formula: $\frac{a}{a + c} \times 100$.

Specificity is calculated by the formula: $\frac{d}{b + d} \times 100$.

### Table 2: Concentration of DNA obtained from pulp and dentin in (µg/ml)

| Sample number | Storage time (days) | Subgroup A (pulp) | Subgroup B (dentin) |
|---------------|---------------------|-------------------|---------------------|
| 1             | Nil                 | 312.1             | 124.3               |
| 2             | Nil                 | 231.9             | 114.2               |
| 3             | Nil                 | 323.7             | 97.1                |
| 4             | Nil                 | 318.4             | 154.6               |
| 5             | Nil                 | 219.4             | 121.1               |
| 6             | Nil                 | 113.8             | 113.8               |
| 7             | Nil                 | 91.42             | 129.3               |
| 8             | Nil                 | 118.3             | 94.2                |
| 9             | Nil                 | 219.4             | 211.1               |
| 10            | Nil                 | 218.9             | 134.8               |
| 11            | 60                  | 289.2             | 89.9                |
| 12            | 60                  | 320.1             | 121.4               |
| 13            | 60                  | 215.4             | 140.7               |
| 14            | 60                  | 131.4             | 189.4               |
| 15            | 60                  | 154.2             | 172.9               |
| 16            | 60                  | 212.3             | 191.3               |
| 17            | 60                  | 199.1             | 156.2               |
| 18            | 60                  | 156.3             | 212.38              |
| 19            | 60                  | 162.4             | 194.01              |
| 20            | 60                  | 151.4             | 118.06              |

DNA extraction was possible from all samples of pulp and dentin irrespective of their storage conditions.

\[
\text{Number of true negatives (d)} \times 100
\]

\[
\text{Number of true negatives (d) + Number of false positives (b)}
\]

Specificity = $\frac{10}{10 + 0} \times 100 = 100\%$.

Inference of the study was drawn by gel photo of genomic DNA loaded on 1% agarose gel [Figure 8] which represents DNA ladder of 1500 bp with five dentin samples on the left side, in which no inference of SRY gene was seen, and six pulp samples on the right side with bright bands at 418 bp suggestive of positivity to SRY gene or the male samples.
Comparison of concentration of DNA among pulp and dentin samples in a total of 20 samples SD of DNA from pulp is 73.94952 and mean values is 207.9560. SD of DNA from dentin is 69.09232 and mean value is 139.4885. In the present study, \( P<0 \) and it should be \(<0.05\), and hence we can say much significant difference between pulp and dentin using the paired \( t \)-test. SD: Standard deviation.

| Sample number | Actual gender | Inference by SRY gene |
|---------------|---------------|----------------------|
| 1A            | Male          | Male                 |
| 2A            | Female        | Female               |
| 3A            | Female        | Female               |
| 4A            | Male          | Male                 |
| 5A            | Female        | Female               |

All the pulp samples that were freshly extracted showed positive gender determination using SRY gene. SRY: Sex-determining region Y

| Sample number | Actual gender | Inference by SRY gene |
|---------------|---------------|----------------------|
| 6A            | Male          | Male                 |
| 7A            | Male          | Male                 |
| 8A            | Female        | Female               |
| 9A            | Male          | Male                 |
| 10A           | Female        | Female               |

All the pulp samples that were subjected to seawater for a duration of 60 days showed positive gender determination using SRY gene. SRY: Sex-determining region Y

| Sample number | Actual gender | Inference by SRY gene |
|---------------|---------------|----------------------|
| 11A           | Male          | Male                 |
| 12A           | Male          | Male                 |
| 13A           | Female        | Female               |
| 14A           | Male          | Male                 |
| 15A           | Female        | Female               |

All the pulp samples that were subjected to garden soil for a duration of 60 days showed positive gender determination using SRY gene. SRY: Sex-determining region Y

| Sample number | Actual gender | Inference by SRY gene |
|---------------|---------------|----------------------|
| 16A           | Male          | Male                 |
| 17A           | Female        | Female               |
| 18A           | Female        | Female               |
| 19A           | Male          | Male                 |
| 20A           | Female        | Female               |

All the pulp samples that were stored at room temperature for a duration of 60 days showed positive gender determination using SRY gene. SRY: Sex-determining region Y

| Sample number | Actual gender | Inference by SRY gene |
|---------------|---------------|----------------------|
| 1B            | Male          | -                    |
| 2B            | Female        | -                    |
| 3B            | Female        | -                    |
| 4B            | Male          | -                    |
| 5B            | Female        | -                    |

No interpretation was done in all the dentin samples regarding gender determination using SRY gene. SRY: Sex-determining region Y

| Sample number | Actual gender | Inference by SRY gene |
|---------------|---------------|----------------------|
| 6B            | Male          | -                    |
| 7B            | Male          | -                    |
| 8B            | Female        | -                    |
| 9B            | Male          | -                    |
| 10B           | Female        | -                    |

Interpretation could not be done in all the dentin samples that were subjected to seawater for a duration of 60 days regarding gender determination using SRY gene. SRY: Sex-determining region Y

| Sample number | Actual gender | Inference by SRY gene |
|---------------|---------------|----------------------|
| 11B           | Male          | -                    |
| 12B           | Male          | -                    |
| 13B           | Female        | -                    |
| 14B           | Male          | -                    |
| 15B           | Female        | -                    |

No interpretation was done in all the dentin samples that were subjected to garden soil for a duration of 60 days regarding gender determination using SRY gene. SRY: Sex-determining region Y

Determinination of gender of unknown human remains is one of the most important of the triad of dental profiling. When comparing a large amount of data for victim identification in mass calamities, gender identification gains the prime importance to establish the individual identity with accuracy. It will also categorize the groups of victims so that further identification procedures, including age assessment or personal identification procedures, can be effectively carried out without delay and in a cost-effective manner.[8]

SRY is a gene located on the short (p) arm just outside the pseudoautosomal region. It is the master switch that triggers
the events that convert the embryo from its “default” developmental pattern as a female into a developmental sequence that results in a male. If this gene is absent or inactivated, a mammal develops into a female, even if the Y-chromosome itself is present. Therefore, it is not the Y-chromosome that determines maleness or the presence of two X-chromosomes that determines femaleness; it is the presence or absence of the protein coded for by the SRY gene that determines gender in mammals.

SRY is uniquely important in mammalian biology, lying at the crossroads of male and female development in the embryo.

The present study includes twenty samples of extracted teeth which were stored in different environmental conditions and then sectioned to obtain pulp tissue and dentin powder from which the DNA was extracted for the purpose of sex determination using SRY gene.

Vemuri et al. subjected teeth to various environmental conditions such as incinerating teeth at 100°C, 200°C, 300°C, and 400°C for 15 min, immersing in seawater, and burying at 30-cm depth for 20–36 days.[9] Similarly, Alvarez García et al. subjected tooth to freeze at 4°C, 20°C, and 40°C for periods ranging from 2 weeks to 36 months, some teeth were immersed in sea and river water from 15 days to 6 months, some teeth were buried in a garden approximately 20 cm under the ground for periods of 2 weeks to 6 months, some teeth were exposed to open air for periods varying from 2 weeks to 6 months, and some teeth were incinerated in a dental ceramic furnace for 2 min at 75°C, 100°C, 200°C, 300°C, 400°C, and 500°C.[10] Murakami et al. also subjected teeth to different conditions such as immersion in seawater for 4 weeks, buried in soil for 4 weeks, some were kept at room temperature, and some teeth were subjected to incineration at temperatures of 100°C, 150°C, 200°C, and 250°C for 30 min.[11] Naik et al. stored teeth at room temperature for varying durations from 8 to 240 days.[12] Battepati and Shodan immersed some teeth in a bucket of drainage water, some were buried in sand taken from the seashore, some in sand taken from burial ground, and some in sand taken from desert and stored them for 2 months.[13] In all the above-mentioned studies after subjecting teeth to various environmental conditions, DNA was extracted from pulp tissue of all the samples.

Higgins et al. examined the availability of nuclear DNA in different areas of the dental hard tissues and explored the extent and nature of the variation within and between individuals and stated that nuclear DNA is available in widely variable quantities in dentin and cementum.[14] Zapico and Ubelaker extracted DNA from dentin to correctly identify the gender of an individual.[15] Hence, in the present study, an attempt was made to study and compare the potential of hard and soft tissues of teeth which were subjected to various environmental conditions after which the removal of pulp and dentin powder from the sections of teeth was done followed by the DNA extraction from both the hard and soft tissues of the tooth.[15]

In studies done by Vemuri et al. and Naik et al., the concentration of DNA obtained from pulp ranged from 16 to 34 µg/ml and 24.96–30.34 µg/ml, respectively.[9,12] The concentration of DNA obtained in this study from the pulp tissue ranged from 91.42 to 312.1 µg/ml. Zapico and Ubelaker obtained 1.15–28.2 µg/ml of DNA concentration from dentin,[15] whereas in this study, 0.78–220.1 µg/ml concentration of DNA was obtained from dentin.

So far, the most commonly used gene for the determination of gender is AMEL. Ajay et al. reported the use of AMEL gene from DNA extracted from dental pulp tissue.[16] Kashyap et al. reported 0.23% failure rate in amelogenin-based typing among the Indian population. According to them, a Y-specific locus should routinely be included along with an X-specific marker in forensic sex typing.[17] It has been found that sometimes AMEL assay may not be indicative of gender assignment due to its deletions.[18]

Kastelic et al. conducted a study using 115 male samples to determine the validation of SRY marker for forensic casework and found no gender discrepancy. The robustness was detected with the low amounts of male DNA among high concentration of female DNA which can be typed with the SRY male gender marker assay.[4] Naik et al. identified gender of all the twenty pulp samples by detecting the SRY gene or region with 100% accuracy.[12]

In accordance with the above two studies, sex determination in this study was performed using SRY gene, which is considered to be a signature gene to differentiate male and female.

In the present study which included twenty teeth exposed to different environmental conditions, the isolation of DNA was possible in all the samples of pulp and dentin irrespective of the environmental conditions in which the teeth were stored. Sex determination by using the SRY gene was possible in all the twenty samples of pulp, i.e., in all

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Table 11: Group IVB (stored in dry air at room temperature): Dentin

| Sample number | Actual gender | Inference by SRY gene |
|---------------|---------------|----------------------|
| 16B           | Male          | -                    |
| 17B           | Female        | -                    |
| 18B           | Female        | -                    |
| 19B           | Male          | -                    |
| 20B           | Female        | -                    |

No interpretation was done in all the dentin samples that were subjected to dry air at room temperature for a duration of 60 days regarding gender determination using SRY gene. SRY: Sex-determining region Y
the four groups of pulp, which were grouped as the freshly extracted teeth, teeth stored in seawater for 60 days, teeth stored in garden soil for 60 days, and teeth stored at room temperature for 60 days, but no interpretation was possible in the dentin samples from any of the groups using SRY gene probably denoting the absence of SRY in dentin. The SRY gene showed 100% sensitivity and 100% specificity in sex determination among twenty samples of pulp.

Summary of the Study

1. DNA was extracted from all the twenty samples of the pulp of different groups which were exposed to different environments
2. DNA was extracted from all the twenty samples of dentin of different groups which were exposed to different environments
3. Among the twenty samples of pulp tissue, all the twenty samples showed 100% accurate results for sex determination using SRY gene
4. Among the twenty samples of dentin tissue, no interpretation regarding sex determination was possible in any of the sample using SRY gene probably denoting the absence of SRY gene in dentin
5. Sensitivity of 100% and specificity of 100% were noticed with SRY gene in the pulp. Therefore, SRY gene in the DNA from a forensic sample of pulp tissue can be confirmatory to type the gender as male.

Conclusion

To conclude, the present study has found DNA retrieval can be done from human teeth irrespective of the storage time and environmental conditions for forensic caseworks and SRY gene to be a reliable biomarker for sex determination from pulp tissue of teeth. Furthermore, further studies with larger sample size, varied age groups, different DNA isolation methods, and various PCR protocols have to be carried out to rule out the appreciation of the SRY gene in dentin.

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Conflicts of interest

There are no conflicts of interest.

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