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Original Research Paper

Dentistry

"SEX CHROMATIN IN DENTAL PULP AS AN INDICATOR IN GENDER IDENTITY"

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ABSTRACT

Background and Objectives: Identification means determination of individuality of a person. Identification is important for legal, humanitarian reasons, in solving criminal cases, problems of inheritance, funeral rites. The estimation of sex is one of the pillars of forensic identification. The tooth organ is the hardest organ in the human body, with a loose connective tissue of dental pulp situated within a rigid encasement of mineralized surrounding tissues. Sex can be determined by the study of X&Y chromosomes in the cells which are not undergoing active division. X chromatin in its inactivated form is present as a mass against the nuclear membrane in females is known as Barr body. Presence or absence of X chromosome can be studied from buccal smears, skin biopsy, blood, cartilage, hair root sheath, and tooth pulp. The present study was undertaken to identify and evaluate the presence of X chromatin in dental pulp as an indicator in gender identity. **Materials and Methods:** The study was performed on healthy premolars, from a total of 80 subjects inclusive of 40 teeth extracted from males and 40 teeth extracted from females after informed consent. The collected tooth specimens were divided in to two groups. Group I consisted of 20 teeth from males, 20 teeth from females. Group II consisted of 20 teeth from males, 20 teeth from females. Group I premolars were immediately preserved in 10% formalin solution for upto 7 days. Group II premolars were buried in mud at room temperature for 4 months. The dental pulp tissues were conventionally extirpated and stained with hematoxylin & eosin stain. All the blinded sections of the dental pulp were observed for X chromatin/Barr bodies systematically using an Olympus BX53 microscope with a 100x magnification. **Results:** In group I, Dental pulp was positive for X chromatin in 100% females for up to 7days with 100% accuracy. In group II, Dental pulp was positive for X chromatin in 45% of females after 4 months with 45% accuracy. The accuracy of identifying X chromatin in dental pulp diminished over a period of time. Dental pulp was negative for X chromatin in teeth of 100% males both at 7days (Group I) & 4 months (Group II). **Conclusion:** The identification of X chromatin in dental pulp as a gender determinant can be ascertained with high reliability within 7 days of time. Whereas the reliability of identifying X chromatin in dental pulp as a gender determinant decreased in the course of time. Consequently, we can recommend identification of X chromatin in dental pulp as an indicator in gender identity within a short period of time.

KEYWORDS : Gender determination, Dental pulp, X chromatin.

INTRODUCTION

Identification means determination of individuality of a person[1]. The concept of "identity" is a set of physical characteristics, functional or psychic, and normal or pathological that defines an individual[2].Human identification is a mainstay of civilization and identification of unknown individuals has always been of paramount importance to society[3]. Identification of an individual living or dead is based on the theory that all individuals are unique[16]. The main attributes of biological identity are sex, age, stature, and ethnic background of the individual which are also called the 'Big four' in forensic context[4]. Sex determination is the first step in forensic identification[5].

Dental identification has long been considered a reliable method when other methods fail because of critical body conditions or unavailability of body parts[16]. Teeth are known to be unique organs made of the most enduring mineralized tissues in the human body. They are tissues characterized by structures of extraordinary resistance to putrefaction and effects of external agents that cause destruction of soft tissues of the body[6]. Tooth pulp is embedded in a hard tissue casting that protects it from the detrimental effects of impact, trauma, and heat. Sex can be determined by the study of X & Y chromosomes in the cells which are not undergoing active division. Presence or absence of X chromosome can be studied from buccal smears, skin biopsy, blood, cartilage, hair root sheath, and tooth pulp. The Barr body is a condensation of X chromatin present at the nucleus of cells in female individuals which was first discovered by Barr and Bertram in 1949[7]. With this background this study was undertaken to identify and evaluate the presence of sex chromatin in dental pulp as an indicator in gender identity.

The present study was designed to identify and evaluate the accuracy of the X chromatin in dental pulp over a period of time and to ascertain the reliability of X chromatin in the dental pulp as an indicator in gender identity.

MATERIALS AND METHODS:

Subjects were selected from patients reporting to the Department of Oral Medicine and Radiology and Oral and Maxillofacial Surgery, M. R. Ambedkar Dental College and Hospital, Bangalore. The study was performed on healthy premolars, from a total of 80 subjects inclusive of 40 teeth extracted from males and 40 teeth extracted from females after informed consent. Subjects belonged to the age group of 15 to 40 years.

The present study was approved by the ethical review board of M. R. Ambedkar Dental College and Hospital, Bangalore. A detailed case history was recorded to ensure the selection of ideal subjects. The subjects with the following criteria were included in the study:

- Teeth extracted for orthodontic purposes.
- Teeth free from dental caries, pulp and periapical diseases & periodontal diseases.

Exclusion criteria:

- Teeth having dental disorders.
- Subjects with chromosomal abnormalities.

The teeth were extracted intact by conventional technique under LA, washed with sterile water to remove residual blood. The collected tooth specimens were divided in to two groups. Group I consisted of 20 teeth from males, 20 teeth from females. Group II consisted of 20 teeth from males, 20 teeth

from females. Group I premolars were immediately preserved in 10% formalin solution for upto 7 days. The dental pulp tissues were then conventionally extirpated through an access cavity on the occlusal surface of the teeth using standardized K-files. The tissues were stored immediately after excision in 10% formalin solution for 24 hrs for fixation. After this time, the tissues were stained with hematoxylin & eosin stain and serial sections of 5μm thickness made.

Group II premolars were buried in mud at room temperature for 4 months. After 4 months teeth were removed from the mud. The dental pulp tissues were then conventionally extirpated through an access cavity on the occlusal surface of the teeth using standardized K-files. The tissues were stored immediately after excision in 10% formalin solution for 24 hrs for fixation. After this time, the tissues were stained with hematoxylin & eosin stain and serial sections of 5μm thickness made.

All the blinded sections of the dental pulp were observed for X chromatin/Barr bodies in fibroblasts systematically using an Olympus BX53 microscope with a 100x magnification.

RESULTS:

Table I: Time Span

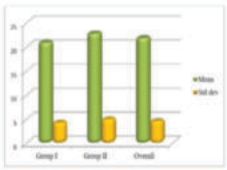
Subjects	Time span
Group I	7 days
Group II	4 months

Teeth from subjects were divided into two groups based on time span. Group I teeth were placed in 10% formalin for upto 7 days & Group II teeth were buried in mud for 4 months. (Table I)

AGE: Table II: Mean Age of the Study Groups

Age (yrs)	Mean	Std dev
Group I	20.97	4.29
Group II	22.85	4.98
Overall	21.91	4.63

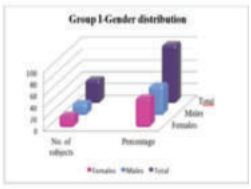
AGE: GRAPH I: Mean Age of the Study Groups



The mean age of Group I subjects was 20.97 years ± 4.29SD (standard deviation) and that of Group II was 22.85 years ± 4.98 SD. (Table II)

GENDER: Table IIIa: Group I-Gender distribution

Gender	No. of subjects	Percentage
Females	20	50
Males	20	50
Total	40	100



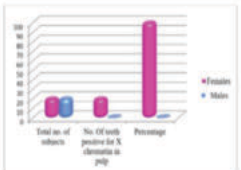
Graph II

In group I out of 40 (100%) subjects 20 (50%) subjects were males and 20 (50%) were females. (Table IIIa)

Table IVa: Group I –Dental pulp positive for X chromatin

Gender	Total no. of subjects	No. Of teeth positive for X chromatin in pulp	Percentage
Females	20	20	100
Males	20	0	0

Graph IV: Group I –Dental pulp positive for X chromatin



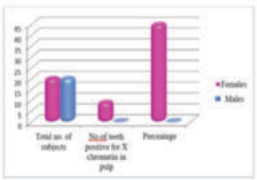
In group II out of 40 (100%) subjects 20(50%) subjects were males and 20 (50%) were females. (Table IIIb)

In group I out of 20(100%) teeth of females, dental pulp of all 20(100%) were positive for X chromatin & out of 20(100%) teeth of males, dental pulp of all 20(100%) were negative for X chromatin. (Table IVa)

Table IVb: Group II –Dental pulp positive for X chromatin

Gender	Total no. of subjects	No. of teeth positive for X chromatin in pulp	Percentage
Females	20	9	45
Males	20	0	0

Graph V: Group II –Dental pulp positive for X chromatin



In group II out of 20(100%) teeth of females, dental pulp of 9 (45%) were positive for X chromatin & out of 20(100%) teeth of males, dental pulp of all 20(100%) were negative for X chromatin. (Table IVb)

DISCUSSION:

Time Span

In the present study subjects were divided into two groups based on time span. Group I teeth were placed in 10% formalin for upto 7 days & Group II teeth were buried in mud for 4 months. Environmental conditions such as burial conditions and soil environment have a critical role in the decomposition rate which considerably effects forensic identification[8]. In this study an attempt has been made to find out upto what extent of time after death sex can be determined by identifying X chromatin in dental pulp keeping in view the effect of environment as in burial in soil.

Age

The present study included subjects of age between 15 to 40years. The mean age of Group I subjects was 20.97 years ± 4.29SD (standard deviation) that of Group II was 22.85 years ± 4.98 SD. This is the most common age for orthodontic intervention. In orthodontic treatment premolars are the most commonly extracted teeth, could be easily sourced and were therefore selected for the study. Manish et al found that the age of the subject did not show any significant effect on the percentage of fluorescent body observed in male dental pulp and Barr body observed in female dental pulp. They found that sex chromatin could be efficiently recognized and counted even in teeth with acute and chronic inflammatory processes, and also in those with the regressive alterations as in attrition and abrasion[9].

Gender

In the present study, in group I out of 40 (100%) subjects 20 (50%) subjects were males and 20 (50%) were females. In group II out of 40 (100%) subjects 20(50%) subjects were males and 20 (50%) were females. Barr body is found only in those cases in which more than one X chromosome is present and thus it is not found in male cells. In normal men, no Barr body are reported, and in 46XX women, one Barr body in cell nuclei is observed[9].

Group I –Dental pulp positive for X chromatin

In group I out of 20(100%) teeth of females, dental pulp of all 20(100%) were positive for X chromatin & out of 20(100%) teeth of males, dental pulp of all 20(100%) were negative for X chromatin. We identified X chromatin in dental pulp of 100% of females. This group of teeth were immediately preserved in 10% formalin for up to 7 days.

The findings of our study are in agreement with the findings of the study carried out by Suazo et al. They conducted a study on healthy premolars. The teeth were healthy, extracted by orthodontic indication, and immediately preserved in 5% formalin solution for an average of 7 days. They evaluated the diagnostic performance of the test. The performance of the test was 100%; of the 50 cells observed per plate, mean Barr bodies positive cells was 20.4 (SD 0.44) in female samples.

There was no positive cell in preparations of male subjects. The diagnostic test observation of sex chromatin in dental pulp is a reliable test for sex determination. This study provides a gold standard for performance assessment in which the teeth have suffered extreme physical conditions, such as incineration and dumping[5].

Some studies have reported that pulp begins to deteriorate in the course of time. Duffy JB et al (1991)conducted a study based on seven experiments shows that, in Northwest coast outdoor environments in both summer (three experiments) and winter (three experiments), the stability of dental pulp nuclei ranges from 4 days to 2 weeks. The seventh experiment serves to describe the morphological sequence observed in nuclear putrefaction[10]. Marko Vavpotic (2009) et al conducted a study on human corpses. During the autopsy, the jaws were resected to keep teeth in situ, and every day one tooth was extracted. They found that the number of odontoblasts drops during the time after death, and no odontoblasts remain in the pulp after 5 days[11]. Monica Mehendiratta(2015) et al found that Dental pulp buried in a coastal environment goes through a specific series of morphological and histological changes which can be interpreted up to 144 h from burial, after which pulp ceases to exist[12].

Group II –Dental pulp positive for X chromatin

In group II out of 20(100%) teeth of females, dental pulp of 9 (45%) were positive for X chromatin & out of 20(100%) teeth of males, dental pulp of all 20(100%) were negative for X chromatin. This group of teeth were buried in mud at room temperature for 4 months. We identified x chromatin in dental pulp in 45% of females.

The present study is in concordance with other studies which have also shown results where there is decrease in identification of X chromatin in dental pulp. Following are studies which have been conducted over different periods of time under several diverse conditions and have found varying accuracy of identification of X chromatin in dental pulp.

Seno(1977) et al. conducted a study wherein they found that, in 25cm teeth buried in mud and sand in the open, sex can be determined in 100% of cases after one month; however, this is reduced by 20% after 3 months because of cellular decomposition[13]. Duffy(1991) et al. found the possibility of determining the sexual dimorphism by barr body in dental pulp after one year of dehydration or being subjected to 1000 c for 1 hour[10]. Das(2004) et al. conducted a study wherein they were able to differentiate sex with certainty upto three weeks and with decreased accuracy they could determine up to 10 weeks[14]. Suazo I (2011) et al found the positive results for females in the tooth subjected to temperature until 4000c,whereas tooth subjected to further high temperature it was not possible to find any viable tissue for analysis[5]. Sandoval (2014) et al. analyzed the sample considered 47 dental pulps, taken from teeth under burial conditions during a period of a month[8]. They found that identifying X chromatin positive cells showed a general accuracy of the diagnostic test for the overall samples subjected to burial conditions in a 98.9%, and an overall sensitivity of 97.5% for men and 100% for women. Manisha M. Khorate (2014) et al found that gender determination from human pulp is possible up to 7 weeks. The percentage of F body and Barr body decrease gradually as the time interval increases[9].

The accuracy of identification of Barr body reduced over a period of time due to cellular decomposition of pulp tissue by bacterial action. The release of intracellular hydrolytic enzymes initiates the process of cellular disintegration. The rate of decomposition increases as the microorganisms invade these tissues[12].

Limitations of this method are because of the alterations at the chromosomal level, patients with abnormalities can yield false positive or false negative results. Sauzo et al in their study found that in control men, no Barr bodies were reported, and in 46XX women, one Barr body in cell nuclei was observed. However, in men and women with aneuploidy, 47XXY men also have a Barr body, and 47XXX women have two[5].

CONCLUSION:

In the present study we identified X chromatin in dental pulp of 100% of females for up to 7days with 100% accuracy whereas we identified X chromatin in dental pulp in 45% of females after 4 months with 45% accuracy. The accuracy of identifying X chromatin in dental pulp diminished over a period of time. We can therefore conclude that the identification of X chromatin in dental pulp as a gender determinant can be ascertained with high reliability within 7 days of time. Whereas the reliability of identifying X chromatin in dental pulp as a gender determinant decreased in the course of time. Consequently, we can recommend identification of X chromatin in dental pulp as an indicator in gender identity within a short period of time. In view of the fact that dental pulp is well protected, preserved within highly imperishable teeth and is easily obtainable, this method of identifying X chromatin in dental pulp for gender determination is simple, quick, cost effective and can positively play an important part in forensic investigations.

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