Sex identification from exfoliated primary teeth - a PCR study

Manju Gopa Kumar* / Amitha M. Hegde**

Downloaded from http://meridian.allenpress.com/jcpd/article-pdf/30/1/19/1747501/jcpd_30_1_c7u44gx111411328.pdf by Bharati Vidyapeeth Dental College & Hospital user on 25 June 2022

Teeth endure postmortem degradation and extreme changes in ambient temperature and pressure better than most human tissues. In the present day scenario the growing number of crime against children in the form of battering, physical/sexual abuse, abduction and kidnapping, the use of exfoliated primary teeth, become many times the only evidence available at the crime scene. Despite exposure of the body to burial, mutilation, explosion or incineration, it is usually possible to extract DNA from pulp tissue of tooth with sufficient quality and quantity. Hence the present study was undertaken to find out the sex of a child from exfoliated/extracted deciduous teeth using a Polymerase Chain reaction(PCR) based analysis. Tooth samples were stored in room temperature after double coding for various periods. Dental pulp tissue was collected from each sample and DNA was isolated by proteinase-k digestion and phenol chloroform extraction methods. PCR amplification was done with two sets of oligonucleiotide primers. Amplification of X (131bp) and Y-specific sequences (172bp) in males and that of the X-specific sequence in females was observed and compared with the template DNA showing male and female controls. Determination of sexes of all freshly collected samples within 24 hours and after 1 month of extraction respectively gave 100% result. However, PCR was not found to be an effective method for sex determination after 6 months post extraction.

J Clin Pediatr Dent 30(1): 19-22, 2005

INTRODUCTION

Sex determination is an important element in the analysis of biological evidence submitted to forensic laboratories.[1] Sex determination from teeth can provide an important means of personal identification in the event of mass disaster such as an air plane crash or fire.[2]

Modern Forensic Dentistry encompasses a wide range of subjects that include dental identification of both living and deceased, assessment of age, investigation of bite marks, the comparison and identification of lip prints and the examination and evaluation of child abuse cases. Today members of the forensic and legal profession, law and enforcement personnel and a vast number of insurance companies are much more conscious of the values of dental evidence.[3]

The enamel and dentin of human teeth act like an armour coating to protect the DNA rich inner aspect of the tooth from various environmental conditions. Despite exposure of the body to burial, mutilation, explosion or incineration, it is usually possible to extract DNA from pulp tissue of tooth with sufficient quality and quantity.[4]

The dental pulp available from a primary tooth, even though in minor quantity can prove to be extremely useful if standardized and advanced methods of DNA analysis are used.[10]

In the recent years with the development of DNA analysis techniques like Polymerase Chain Reaction (PCR) the genetic typing of samples with very little or partially degraded DNA is possible.[2] Hence, the present study was undertaken to find out whether we could determine the sex of a child from exfoliated primary teeth using a PCR based analysis.

MATERIALS AND METHODS

Tooth samples for the study were collected from children who reported to the department of pedodontics and preventive children dentistry for the extraction of their exfoliating primary teeth. Sex of all the children from whom the teeth were collected was recorded

Extracted teeth were stored in room temperature for various periods. Coding and double coding of the samples were done in a double blind manner and were

* Manju Gopa Kumar, Postgraduate student, Department of Pedodontics and Preventive Dentistry, A.B. Shetty Memorial Institute of Dental Sciences

** Dr. Amitha M. Hegde, Professor and Head, Department of Pedodontics and Preventive Dentistry, A.B. Shetty Memorial Institute of Dental Sciences

Send all correspndence to Dr. Amitha M. Hedge, Professor and Head, Department of Pedodontics and Preventive Dentistry, A.B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore - 575018, Karnataka, India.

Phone: 0824-2224440
E-mail: amipedo@yahoo.co.in

randomly divided into 3 groups of 5 teeth each and studied at intervals as follows.

A. Group I. – Less than 24hrs
B. Group II – After one month
C. Group III - After 6months

Dental pulp tissue was collected from each sample by longitudinal sectioning of each tooth sample using carborandom disc and kept in normal saline. DNA was isolated from intact dental pulp tissue obtained from extracted teeth by proteinase-k digestion and phenol chloroform extraction methods.[5] Isolated DNA was then diluted with tris-ethylenediaminetetraacetic acid (TE) buffer and concentration was assessed by measurement of optical density of the solution at a wavelength of 260 nanometers before PCR amplification.

The isolated DNA was utilized for PCR amplification in Amplitaq DNA polymerase buffer for various thermal cycles. The thermal cycle profiles using the DNA thermal cycler were 36 cycles at 94° C for 1 min, 55° C for 1 min, and 72° C for 2 min, followed by preheating at 94° C for 5 min, then extension of the last cycle to 7 min. The overall time taken for PCR technique per sample was 2 to 3 hrs.

COMPARISON OF PCR PRODUCTS

The obtained PCR reactions products were analyzed by ultraviolet visualization of ethidium bromide stained and silver stained polyacrylamide gel after electrophoresis in Tris-borate-EDTA (TBE) buffer. Amplification of X (131bp) and Y-specific sequences (172bp) in males and that of the X-specific sequence in females were observed on the EB-stained gel and the silver stained gel in all dental pulp tissue samples and compared with the template DNA showing male and female controls. (Figs 1,2)

RESULTS

- The analyzed sex from all the 5 samples after 24 hours and after one month of extraction/exfoliation by PCR correlated with the actual sex of the child from where the respective specimens were collected.
- Out of the 5 samples only 2 correlated with the recorded sex of the child after 6 months of extraction/exfoliation by PCR.Two samples showed false positive results and one sample showed false negative result.

DISCUSSION

In the present day scenario the growing number of crime against children in the form of battering, physical/sexual abuse, abduction and kidnapping more often than not the dental surgeon/forensic dentists are presented with the remains of a child during the primary or mixed dentition stages. Thus in our study pulp tissue was collected from exfoliated/extracted primary teeth for sex determination.

Sex determination from pulp material can be done by different methods like fluorescence Y chromosome test, southern blot hybridization etc. PCR stands above all mentioned methods since the high rate of sensitivity and specificity have been noted in previous experiments.[6,7]

According to Witt& Erickson PCR is a rapid and reliable mean for sex determination because Alphoid Satelite Family (ASF) is located in the pericentromeric regions of human chromosomes and has a higher repeat organization.[8] This fact justified our choice of PCR as our technique for sex determination. The dental pulp undergoes degeneration, necrosis and putrefaction inside an exfoliated/extracted tooth. This sequential phenomenon takes a period of weeks to months. To check the usefulness of the pulpal remains during and after a period of time lapse was thus necessary. So we did use teeth specimens stored for various time intervals for the purpose of PCR study.

In the present study, sex could be determined with 100% accuracy up to 1 month after extraction by PCR. Only 40% positive result was observed after 6 months.

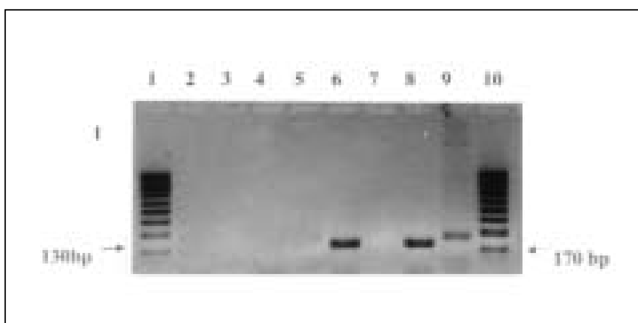

Figure 1. Template DNA Showing Male and Female Controls—Lanes 1 & 10 show the 100 bp(base pair) DNA ladder.
Lanes 6 & 7 shows Female control that is, lane 6 shows a band of 130bp(X specific sequence) and lane 7 is empty indicating the abasence of & specific sequence. Lanes 8 & 9 shows male control that is lane 8 shows a band of 130 bp(X specific sequence) and lane 9 shows a band of 170bp (Y specific sequence).

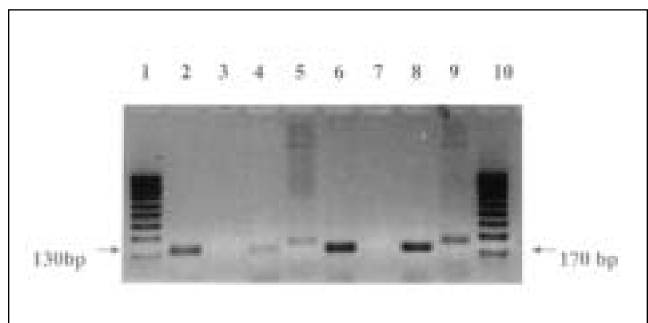

Figure 2. Results of the PCR Amplification of Alphoid Repeats of Human X & Y Chromosomes—Lanes 1 & 10 show the 100 bp (base pair) DNA ladder. Lanes 4 & 5 shows a male sample with both X specific (130bp) and Y specific (170bp) sequences. Lanes 2&3 shows a female sample with only X specific sequencee on lane 2.

Downloaded from http://meridian.allenpress.com/jcpd/article-pdf/30/1/19/1747501/jcpd_30_1_c7u44gx1t1411328.pdf by Bharati Vidyapeeth Dental College & Hospital user on 25 June 2022

Pillay in 1997 determined the sex from the pulp of freshly extracted human teeth utilizing the polymerise chain reaction. Identification of sex of the individual was 100 percent accurate in each case and this was in agreement with the present study.[9]

Murakami in 2000 evaluated the sex determination using PCR on tooth samples and sex could be determined by PCR of DNA extracted from the pulp of 16 freshly extracted permanent teeth and dentin including the surface of the pulp cavity of 6 freshly extracted primary teeth. Sex could be determined using the pulp in all 20 teeth provided at room temperature for 22 yrs.[10] In our present study only freshly extracted tooth samples up to one month showed an accuracy of 100% and deteriorated with time. This might be due to the putrefaction of the specimen as time proceeded or due to local conditions.

False positive results were seen in two samples after six months of extraction by PCR. A False positive result by PCR based techniques may be generated by human errors like mixing of specimens or mislabeling, unspecific amplification/detection and contamination. Unspecific amplification /detection can occur if temperature for annealing is too low, long primers or high salt concentration in reaction buffer.[11]

One sample showed false negative result after six months, which may be due to technical problems, low target concentration in a clinical specimen and the target not being recognized because of mutations and inhibition.[11]

Many communities have the habit of keeping the exfoliated primary teeth of their children as of sentimental value. This can always be of great value in case of disasters like fire, explosions or airplane crash where only dental remains are available and act as antemortem record and help in identifying a person.

From our study and from literature it was observed that high standard asepsis and favorable temperature need to be maintained while conducting PCR based analysis to avoid false positive and false negative results. A strict quality assurance and quality-testing program is absolutely mandatory. Keeping this scientific guideline, larger sample size and stringent aseptic measures may fetch us accurate results, which will be of immense value to Forensic Dentistry.

CONCLUSION

Our studies indicate that the co-amplification of both x and y sequences in a unique reaction mixture by PCR is a fast, human specific, sensitive and reliable method providing sex determination from pulp of limited amount up to one month of post extraction/exfoliation.

REFERENCES

1. Saiki R.H. Primer-directed enzymatic amplification of DNA with a thermo stable DNA polymerase. Science 239: 487–491, 1988.
2. Hanaoka Y &Kiyoshi Minaguchi. Sex determination from blood and teeth by PCR amplification of the Alphoid satellite family. J Forensic. Sci 41(5): 855–858, 1996.
3. Mody R.N. Forensic Odontology in dental practice. Dental Dialogue, IDA MSB 2:1993.
4. Sweet D& Hilderand DP. Recovery of DNA from human teeth by cryogenic grinding. J. Forensic. Sci 43: 1199, 1998.
5. Saiki R.H. Primer-directed enzymatic amplification of DNA with a thermo stable DNA polymerase. Science 239: 487–491, 1988.
6. Neeser B.S& Sabina Liechti-Gallati: Sex determination of forensic samples by simultaneous PCR amplification of alpha – satellite DNA from both the X and Y chromosomes. J. Forensic. Sci 40(2): 239–241, 1995.
7. Jiang B & Guo JY: Determination of sex using PCR of the X-Y homologous gene amelogenin. Fa Yi Xue Za Zhi 13(2): 68–70, 1997. (PMID 10322986)
8. Witt M& Erickson, R.D. A rapid method for detection of Y – chromosome DNA from dried blood specimens by the polymerase chain reaction. Hum. Genetics 82: 271–4, 1989.
9. Pillay U; Kramer B. A simple method for the determination of sex from the pulp of freshly extracted human teeth utilizing the polymerase chain reaction. J. Dent. Assoc. S. Africa 52(11): 673–7, 1997.
10. Murakami H, Yamamoto Y, Yoshitome K,Ono T, Okamoto O, Shigeta Y et al. Forensic study of sex determination using PCR on tooth samples. Acta Med-Okayama 54(1): 21–32, 2000. (PMID 10709619)
11. Hans – Joachin Burkardt . Standardization and Quality Control of PCR Analysis'' – Review. Clin. Chem. Lab. Med 38 (2): 87–1, 2000.

Downloaded from http://meridian.allenpress.com/jcpd/article-pdf/30/1/19/1747501/jcpd_30_1_c7u44gx1t1411328.pdf by Bharati Vidyapeeth Dental College & Hospital user on 25 June 2022

Downloaded from http://meridian.allenpress.com/jcpd/article-pdf/30/1/19/1747501/jcpd_30_1_c7u44gx1t1411328.pdf by Bharati Vidyapeeth Dental College & Hospital user on 25 June 2022