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**RESEARCH ARTICLE**

# "Blood group determination using DNA extracted from exfoliated primary teeth at various environmental conditions" –A PCR Study.

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## *Abstract*

Blood grouping plays a major role in forensic science in the field of person identification.

**Objectives:** To determine PCR based blood group on primary teeth pulp obtained from teeth stored under various environmental conditions like different pH, sea water and buried in soil for 6 months following extraction.

**Study design**: Dental pulp tissue was collected from 50 exfoliated primary teeth stored at varying pH (4, 7, and 10), sea water and buried in soil for 6 months and preserved at $4^{o}$C till DNA extraction was carried out. DNA was extracted using silica membrane based spin-column procedure of Q1Aamp DNA minikit from Bio-Rad. DNA was subjected to PCR amplification Monoplex allele – specific PCR primers for ABO genotyping.

**Results:** In our study overall 66 % samples showed a DNA yield. Cent percent results were obtained for samples studied at pH 4 and pH 7 whereas samples stored at pH 10 showed 10% result. Sea water and buried samples showed 80 % and 40 % results respectively.

**Conclusion:** PCR was found to be an effective method for blood group determination for teeth stored at various environmental conditions. Thus our study emphasizes that the blood grouping of tooth pulp by PCR method can be used for relative identification of individuals which will be of immense value to forensic dentistry.

## Introduction

Identification connotes determination or establishment of individuality of person- living or dead.[1, 2] The identification of an unknown individual has always has been of paramount importance to society. Identifiable information from oral structure is more than any other part of the body. [3]

Forensic dentistry plays a major role in the identification of those individuals who cannot be identified visually or by other means. An important feature of teeth is that they are the most indestructible part of the body and exhibit the least turnover. They not only survive death but also remain relatively unchanged thereafter for many thousands of years. Forensic Dentistry relies on this indestructibility.[4]

In the present day scenario where the number of crimes against children in the form of battering, physical/sexual abuse, kidnapping and abduction is on the rise, exfoliated primary teeth may be the only source of evidence available at the crime scene. 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. [5]

DNA analysis is an important method employed for forensic identification with a higher degree of certainty as compared to the other traditional methods. Teeth are a good source of DNA as they are well protected by the soft tissues of the oral cavity and their composition ensures a better resistance against extreme conditions of temperature, pH, water and aging and in many cases serves as the only tool available for victim identification.[6]

Pulp tissue is one of the most protected of oral tissues being surrounded on all the sides by dental hard tissues.[7]

Blood groups have been the corner stone's for identification of biological materials in forensic science and medicine. [8]The ABO system has been a major focus, since the record of this system is a very prevalent one and A, B and O (H) antigens on erythrocytes are also associated with other cells and tissues throughout the body and are known to be considerably stable to the various environmental conditions.[9]

The presence of ABO blood group antigens in the dental hard and soft tissues makes it possible to assist in identifying highly decomposed bodies where teeth and bone are the only significant tissues remaining. [6]

Blood group determination can be done from the pulp tissue by using methods like absorption elusion, Hem agglutination and Histochemical technique etc. Polymerase Chain Reaction stands above all the mentioned methods with high rate of sensitivity and specificity.[10]

Despite exposure of body to burial, mutilation, explosion or incineration it is usually possible to extract DNA from pulp tissue of sufficient quality and quantity to conduct a PCR based analysis.[11]

Hence this study was undertaken to determine the blood group from the exfoliated/ extracted primary teeth of children using PCR.

**OBJECTIVES**

- Blood group determination by slide agglutination on fresh blood collected from socket following the extraction of primary tooth.
- PCR based blood group determination on tooth pulp obtained from teeth stored at different pH (4, 7, and 10) for 6 months.
- PCR based blood group determination on tooth pulp obtained from teeth stored in sea water for 6 months.
- PCR based blood group determination on tooth pulp obtained from teeth buried in soil for 6 months
- To evaluate the application/usefulness of PCR as a tool for blood group determination from exfoliated/ extracted primary teeth in children.

## METHODOLOGY:

**Materials and Method**

**Sample collection** - 50 exfoliated/ extracted primary teeth for the study were collected from the children of age group 6-13 years reporting to the Department of Paedodontics and Preventive dentistry, after obtaining ethical clearance from the Yenepoya university ethical committee and written consent from parents.

**Exclusion criteria** – Root canal treated tooth, grossly decayed and infected teeth.

**Inclusion criteria** – Primary teeth with physiologic mobility or those indicated for serial extraction

Following routine tooth extraction procedure, blood was collected from the freshly extracted socket and blood group was determined was done by slide agglutination method and this was used as the control in the study (Figure 1).

**Grouping**

Samples were randomly divided into 3 groups.

Group A – 30 Teeth studied at different pH (5, 7, and 10) for 6 months, 10 samples in each pH.

Group B – 10 Teeth studied after burying in the soil for 6 months at a depth of 25cm.

Group C – 10 Teeth studied after storing in sea water (Figure 2 & 3).

**Collection and storage of dental pulp tissue** – Access opening was done on the collected teeth samples and dental pulp tissue was collected from each sample using a barbed broach which was then placed in vials with sterile normal saline and preserved at 4$^{o}$C till DNA extraction was carried out (Figure 4).

**DNA extraction**

Pulp tissue was initially digested by Proteinase K and DNA was extracted using silica membrane based spin-column procedure of Q1Aamp DNA minikit from Bio-Rad (Figure 5a, 5b).

Monoplex allele – specific PCR primers for ABO genotyping.

Following are the primers that were used.[12]

| Primer | Sequence (5 ¢ -3 ¢ ) |
|---|---|
| **ABO261d-F** | **AGGAAGGATGTCCTCGTGTTAC** |
| **ABO261-R** | **GTTCTGGAGCCTGAACTGCT** |
| **ABO526C-F** | **AGCTGTCAGTGCTGGAGATGC** |
| **ABO526-R** | **TCCACGCACACCAGGTAATC** |
| **ABO803G-R** | **CCGACCCCCCGAAGTACC** |
| **ABO803-F** | **GAGATCCTGACTCCGCTGTT** |

Each sample was tested separately for the presence of blood group A and B antigens. Absence of both the antigens in a sample indicated the blood group O. Presence of both blood group A and B antigens was seen in blood group AB. Amplification and analysis of amplified DNA products was performed using Bio-Rad CFX 96 Real time PCR detection system. After an initial denaturation at 95 $^{o}$C for 10 minutes, amplification was performed by using 35 cycles of 95 $^{o}$C for 5 seconds, annealing at 65 $^{o}$C for 10 seconds and extension at 72 $^{o}$C for 1 minute. Final reaction mixture of 25 microlitres per sample contained 15 microlitres of mastermix containing cyber green, 2 microlitres of primers and 8 microlitres of extracted DNA. The results were tabulated. The data was analyzed by comparison (based on percentage).

## RESULTS

In our study overall 66 % samples showed a DNA yield (Table 1). All the samples studied at pH 4 and pH 7 yielded DNA with cent percent result (Table 2a and 2b) , 10 % at pH 10(Table 2c) , 80 % in samples stored in sea water ( Table 3 ) and buried samples showed 40 % result which was amplified and blood group identified by using RT - PCR (Table 4) .

Results were considered positive for those samples whose results obtained by PCR coincided with the blood grouping performed by slide agglutination method on fresh blood from the tooth socket.

**Overall percentage of blood grouping for all samples (table 1)**

| | **Control group** | | **Study group** | |
|---|---|---|---|---|
| TOTAL SAMPLES | Positive* | Negative | Positive* | Negative |
| | 50 (100 %) | 0(0 %) | 33 (66 %) | 17 (34 %) |
| TOTAL POSITIVE | 50 (100%) | | 33 (66 %) | |

**Blood grouping of teeth stored for 6 months at pH 4 (acidic pH) (Table 2 a)**

| | **Control group** | | **Study group** | |
|---|---|---|---|---|
| Blood group | Positive* | Negative | Positive* | Negative |
| A | 4 (40%) | 0 (0%) | 4 (40%) | 0 (0%) |
| B | 4 (40 %) | 0 (0%) | 4 (40 %) | 0 (0%) |
| AB | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| O | 2 (20 %) | 0 (0%) | 2(20 %) | 0 (0%) |
| TOTAL | 10 (100%) | 0 (0%) | 10 (100%) | 0 (0%) |
| POSITIVE | 10 (100%) | | 10 (100%) | |

**Blood grouping of teeth stored for 6 months at pH 7 (neutral pH) ( Table 2 b)**

| | **Control group** | | **Study group** | |
|---|---|---|---|---|
| Blood group | Positive* | Negative | Positive* | Negative |
| A | 3(30%) | 0 (0%) | 3 (30%) | 0 (0%) |
| B | 3 (30 %) | 0 (0%) | 3 (30 %) | 0 (0%) |
| AB | 1 (10%) | 0 (0%) | 1 (10%) | 0 (0%) |
| O | 3(30 %) | 0 (0%) | 3 (30 %) | 0 (0%) |
| TOTAL | 10 (100%) | 0 (0%) | 10 (100%) | 0 (0%) |
| POSITIVE | 10 (100%) | | 10 (100%) | |

**Blood grouping of teeth stored for 6 months at pH 10 (Alkaline pH) (Table 2 c)**

| | **Control group** | | **Study group** | |
|---|---|---|---|---|
| Blood group | Positive* | Negative | Positive* | Negative |
| A | 2 (20%) | 0 (0%) | 0 (0%) | 2 (20%) |
| B | 4 (40 %) | 0 (0%) | 0 (0 %) | 4(40%) |
| AB | 0 (0%) | 0 (0%) | 0 (0 %) | 0 (0%) |
| O | 4 (40 %) | 0 (0%) | 1 (10 %) | 3 (30%) |
| TOTAL | 10 (100%) | 0 (0%) | 1 (10%) | 9 (90%) |
| POSITIVE | 10 (100%) | | 1 (10%) | |

**Blood grouping of teeth stored in sea water for 6 months (Table 3)**

| | **Control group** | | **Study group** | |
|---|---|---|---|---|
| Blood group | Positive* | Negative | Positive* | Negative |
| A | 3 (30%) | 0 (0%) | 3 (30%) | 0 (0%) |
| B | 2 (20 %) | 0 (0%) | 2 (20 %) | 0 (0%) |
| AB | 2 (20%) | 0 (0%) | 2 (20 %) | 0 (0%) |
| O | 3 (30 %) | 0 (0%) | 1 (10 %) | 2 (20%) |
| TOTAL | 10 (100%) | 0 (0%) | 8 (80%) | 2 (20%) |
| POSITIVE | 10 (100%) | | 8 (80%) | |

**Blood grouping of teeth buried in soil for 6 months (Table 4)**

| | **Control group** | | **Study group** | |
|---|---|---|---|---|
| Blood group | Positive* | Negative | Positive* | Negative |
| A | 4 (40%) | 0 (0%) | 2 (20%) | 2 (20%) |
| B | 3 (30 %) | 0 (0%) | 1 (10 %) | 2 (20%) |
| AB | 0 (0%) | 0 (0%) | 0 (0 %) | 0 (0%) |
| O | 3 (30 %) | 0 (0%) | 1 (10 %) | 2 (20%) |
| TOTAL | 10 (100%) | 0 (0%) | 4 (40%) | 6 (60%) |
| POSITIVE | 10 (100%) | | 4 (40%) | |


**Figure 1- ABO blood grouping by slide Agglutination method**

**Figure 2- Teeth studied after burying in the soil for 6 months at a depth of 25cm**

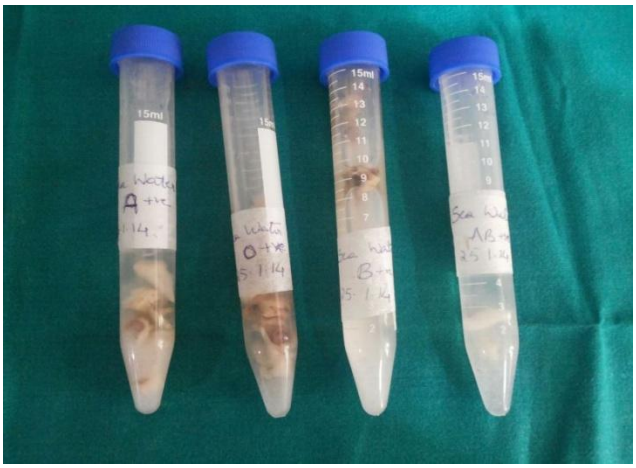


**Figure 3- Teeth studied after storing in sea water**


**Figure 4- Pulp tissue stored in saline**

**Figure 5a- DNA extraction**


**Figure 5b- BIO-RAD**

## DISCUSSION

The established importance of forensic dentistry for human identification, mainly when there is little remaining material to perform such identification (in fires, explosion, decomposing bodies or skeletonized bodies), has led dentists working with forensic investigation to become more familiar with the new molecular biology techniques. As teeth play an important role in forensic field, extracted DNA from the pulp tissue of the tooth can be used to discriminate one individual from another.

The use of blood group antigens in medico legal examination is based on the fact that once a blood group is established in an individual it remains unchanged throughout his/her life. The presence of blood group antigens in dental tissues makes it possible to assist in identifying highly decomposed bodies where teeth and bone are the only significant tissues remaining (Xingzi et al 1993).

Blood group determination from teeth using the PCR analysis can provide an important means of personal identification in the event of mass disaster such as air plane crash or fire or any other environmental conditions. Polymerase chain reaction is the most standardized technique in forensic field since the high rate of sensitivity and specificity have been noted in previous experiments using samples like saliva, blood, semen etc.[13]

The dental pulp undergoes degeneration, necrosis and putrefaction inside an exfoliated tooth which takes a period of weeks to months.[5] to check the usefulness of the pulpal remains during and after a period of time lapse at various environmental conditions was thus necessary. Hence in our study we used the teeth specimens exposed to different environmental conditions like varying pH (4, 7, 10), burying the teeth and sea water for a period of 6 months.

Determination of blood group was done using pulp and was correlated with the blood grouping of blood collected from the extraction socket of the same subject. In our study 33 teeth out of 50 showed positive results with 66 % success using pulp by PCR and control group showed cent percent results. The overall decrease in the success rate could be due to contamination of the tooth, time lapse for the procedure, variation in the pulp volume, loss of tissue during pulp extirpation and root resorption in the deciduous tooth. [14]

In the present study cent percent results were obtained when the teeth samples were stored in acidic (pH 4) and neutral (pH 7) solutions for 6 months whereas only 10% result was obtained when the teeth samples were stored in an alkaline (pH 10) solution for 6 months.

Schwartz TR, 1991 et al determined the effects of various environmental factors on the deoxyribonucleic acid (DNA) obtained from dental pulp. Extracted teeth were subjected to the following conditions: varying pH (3,7,10); temperature (4 degrees C, 25 degrees C, 37 degrees C, incineration); humidity (20%, 66%, 98%); various types of soil (sand, potting soil, garden soil); seawater; burying the teeth outdoors, and aging (one week to six months). It was determined that, aside from soil, the environmental conditions examined did not affect the ability to obtain high-molecular-weight human DNA from dental pulp.[15]

In the present study in teeth samples exposed to alkaline pH, it was possible to extract DNA from only 1 of 10 samples which could be due to denaturation of DNA structure. Change in conditions, can "denature" the DNA and cause the strands to separate. Adding strong bases, like NaOH, dramatically increases the pH, thus decreasing the hydrogen ion concentration of the solution and denaturing double-strandedDNA.[16,17]

Among the samples stored in sea water it was possible to extract DNA from 8 out of 10 samples.

According to Murakami et al, 2000 from the pulp of teeth stored in sea water, the sex could be determined in all 8 teeth immersed for 1 week and in 5 of 6 teeth immersed for 4 weeks.[18] Our result showed co-relation with Murakami et al [in] which they were able to determine the sex from teeth samples immersed in sea water for 4 weeks. This suggests that wet condition does not cause autolytic degradation or decay of the DNA content inside the teeth. Murakami et al also stated that from the teeth stored in soil, the sex could be determined accurately in all 8 teeth buried for 1 week, 7 of 8 teeth buried for 4 weeks, and in all 6 teeth buried for 8 weeks. [18]

In the present study out of 10 samples buried in soil for 6 months, DNA could be extracted from 4 samples.

Alvarez Garcia A et al observed the results of DNA typing on teeth subjected to aging, different temperatures and various environmental factors and it was found that teeth exposed to outdoor conditions provided better results than teeth buried in sand or soil. [19]

In buried teeth therefore, decay due to bacteria was considered to have progressed markedly by infiltration of microbial growth in to the pulp cavity and DNA of pulp tissue was considered to have been degraded to such a degree that amplification was not possible.

Evaluation of the PCR assay in comparison with slide agglutination test has enabled us to assess the accuracy and usefulness of this test with a good success rate. The results of the present study indicate that blood group determination of teeth by means of PCR is considered to be extremely useful for identification of markedly decayed or skeletonized bodies, which has been difficult using the conventional morphological methods contributing in identification of victims of various incidents and disasters including child abuse.

## CONCLUSION

Blood group determination is an important step in personal identification in forensic sciences. Surrounding environment has a definite role in preservation of pulp tissue. Primary teeth can be regarded as an effective tool in preservation of pulp tissue which can provide DNA for blood group determination and other DNA analysis procedures. Once this technique is standardized using a larger sample size and various other environmental conditions in determining the blood group from pulp tissue, these benefits can be offered to the society when need arises.

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## REFERENCES

1. Rothwell BR. Principles of dental identification. Dent Clin North Am 2001; 45: 2253-70.
2. Woolridge ED, Jr. Forensic Dentistry. In: Eckert WG, editor. Introduction to Forensic science. 2nd ed. The C.V. Mosby Company, St. Louis.1980.p 114-7.
3. Inamdar Prabhawati P.I, Inamdar Praveen Kumar I, Rai Kavitha, Mirajkar AM, Venkatesh Sangeetha .Teeth – Hidden treasure of blood group. Indian Journal of Forensic Medicine and Pathology. Jul - Sept 2011: 4(3); 113 -118.
4. Pretty I, Sweet D. A look at forensic dentistry – Part 1: The role of the teeth in the determination of human identity. Br. Dent. J. 2001; 190(7): 359-366.
5. Manju Gopakumar, Amitha M Hegde. Sex identification from exfoliated primary teeth – A PCR study. J Clin Pediatr Dent 2005: 30(1); 19-22.
6. Linjen I, Williams G. DNA research in forensic dentistry. Methods Find Exp Clin Pharmacol. 2001; 23:1-7.
7. Vij K. Identification. In: Textbook of Forensic Medicine and Toxicology Principles and Practice. 3rd edn. New Delhi; Elsevier India (P) Limited; 2005:50-51, 83.
8. Xingzhi X, Ji L, Hao F, Ming L, Zhuyao L. ABO blood grouping on dental tissue. J Forensic Sci 1993; 38(4): 956 -960.
9. Nishi K. ABO blood group typing in forensic autopsies. Nihon Hoigaku Zasshi 2005; 59(2): 111 – 7.
10. Woolridge ED, Jr. Forensic Dentistry. In: Eckert WG, editor. Introduction to Forensic science. 2nd ed. The C.V. Mosby Company, St. Louis.1980.p 114-7.
11. Sweet D, Hilderand DP. Recovery of DNA from human teeth by cryogenic grinding. J. Forensic. Sci. 1998; 43:1199.
12. Lee et al. Rapid direct PCR for ABO Blood Typing. Journal of forensic science 2011; 56(1) :179 -182.
13. Woolridge ED, Jr. Forensic Dentistry. In: Eckert WG, editor. Introduction to Forensic science. 2nd ed. The C.V. Mosby Company, St. Louis.1980.p 114-7.
14. Haertig A, Krainic K, Vaillant JM, Derobert L. Medico legal identification: teeth and blood groups. Rev Stomatol Chir Maxillofac. 1980; 81 (6): 361 – 3.
15. Schwartz TR[1], Schwartz EA, Mieszerski L, McNally L, Kobilinsky L. Characterization DNA obtained from teeth subjected to various environmental conditions. J Forensic Sci. 1991 Jul; 36(4):979-90.

16 M. Ageno, E. Dore, and C. Frontali. Alkaline denaturation of DNA. Biophysical Journal 1969; Volume 9:1281-1311.

17 Biochemistry 3017. The physical and chemical properties of nucleic acids. Memorial University.

18. Murakami H, Yamamoto Y, Yoshitome K, Ono T, Okamoto O, Shigeta Y, Doi Y, Miyaishi S, Ishizu H. Forensic study of sex determination using PCR on teeth samples. Acta Med Okayama. 2000 Feb; 54(1):21-32.

19. Alvarez Garcia A[1], Muñoz I, Pestoni C, Lareu MV, Rodríguez-Calvo MS, Carracedo A. Effect of environmental factors on PCR-DNA analysis from dental pulp. Int J Legal Med. 1996; 109(3):125-9.