

Teeth-hidden treasure of blood group

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ABSTRACT

Purpose: To determine the ABO blood group from the dental pulp of extracted teeth at various time intervals by Absorption-Elution technique. **Method:** Extracted teeth (50 permanent and 50 deciduous) were collected and longitudinally sectioned. The pulp was scooped and used for blood grouping by Absorption-Elution technique. The blood grouping was done at intervals of, day one of extraction, day 14, day 30, day 90 & day 180 respectively. Blood from the freshly extracted socket was soaked with gauze which served as the control. **Results:** Overall, ABO blood groups could be identified from 88% of permanent teeth and 44% of deciduous teeth. As the time interval increased the number of positive results obtained decreased. **Conclusion:** The permanent teeth furnished higher percentage of positive results than the deciduous teeth, on the same day of the extraction and on the 14th day after the extraction respectively.

Keywords: Odontology, Human identification, Pulp, ABO blood grouping, Absorption - Elution technique.

INTRODUCTION

Forensic Odontology (dentistry) is an investigative aspect of dentistry that analyzes dental evidence for human identification.¹ Identifiable information from oral structure is more than any other part of the body. 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 [2].

Blood groups have been the corner stones for identification of biological materials in Forensic science and medicine [3]. The ABO system has

been a major focus, since the record of this blood 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 violent conditions as heating or drying [4].

The existence of blood group antigens in the dental tissues (i.e. enamel, dentin, and pulp) have been the subject of debate for a long time.^{5,6} 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 [3].

Characterizing body fluid stains by absorption-elution typing for ABO group was one of the most significant advances in forensic biology. Absorption-Elution technique was devised by Siracusa (1923) and refined by Kind, who employed it almost exclusively for blood typing of teeth in Forensic Science laboratory.⁷ Though adaptation of the more sensitive assays may increase the utilization of these tissues but of particular value would

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for blood group substances in teeth. The absorption-elution technique is a relatively easy and economical method. No sophisticated storage method is required and reexaminations of samples are undemanding.⁸ Thus we chose this method for our study.

Fresh or recent tooth specimen could be expected to provide a good source for the determination of blood group. However there is the possibility of loss of pulpal antigens due to autolysis and dehydration in long standing tooth remains.⁹ Therefore we thought it was justifiable to study the blood group antigens of the dental pulp at various time intervals i.e. on the day of extraction, 14th, 30th, 90th and 180th day after the extraction of permanent and deciduous teeth.

MATERIALS & METHODS

The study subjects were randomly selected from the department of Pedodontics and oral surgery, after obtaining informed consent.

Inclusion criteria

The permanent teeth indicated for extraction due to periodontal and orthodontic problems. The primary teeth with physiologic mobility or those indicated for serial extractions.

Exclusion criteria

Infected teeth, root canal treated teeth, teeth from individuals above 40 years of age. In correspondence to the time interval (i.e. on the day of extraction, 14th, 30th, 90th and 180th day of extraction.) we divided our samples into five groups (i.e. Group I-V) and planned a sample of 20 teeth (10 permanent and 10 deciduous teeth) in each group. In accordance, bottles were numbered into which the teeth were to be stored. The routine extraction procedure was carried out. The extracted teeth were washed in running water, wiped with gauze and randomly placed in their respective bottles. Blood-stained compresses from the extraction wound served as controls.

ABO grouping (agglutination method) was performed on blood-stained compresses from the extraction wound and the results were noted. Blood groups from the dental pulp were identified by Absorption-Elution technique

This method was carried out at stipulated time intervals and was then compared with that of the control. The teeth were embedded in the modeling wax block. Carborandum disc was used to longitudinally section the teeth embedded in wax blocks. The dental pulp was scooped with sterile spoon excavators, which was then placed in a test tube containing a drop of saline and sterile cotton thread. The test tubes were placed in the incubator at 56°C for 30 minutes so that the blood group antigens of dental pulp were absorbed by the sterile cotton thread.

Blood stained threads of 2 mm length were cut and placed in a drop of anti-A serum in a slide cavity. Similar pieces were placed in anti-B serum. The slides were then kept in moist chamber at 4°C for 2 hours for complete absorption. After absorption, the antiserum was pipetted off from the thread by capillary pipettes and then the thread was thoroughly washed 3 to 4 times in ice cold saline, for the complete removal of unreacted antibodies from it. Slides were again placed in moist chamber and placed in an incubator at 56°C for 30 minutes to break the antigen - antibody bond (Elution).

One drop of a 0.5% suspension of known RBC blood group was added and the samples were again placed in the humidified recipient and were incubated at 56° C for 15 minutes to enhance agglutination. The slides were then removed from the incubator to be kept at room temperature for 45 minutes to 1 hour and were observed under microscope at magnification 100x for agglutination i.e. ABO blood groups. The slides were agitated before reading agglutination.

The results were tabulated. The data was analyzed by comparison (based on percentage).

RESULTS

Cent percent positive results were obtained on the same day and on the 14th day after the extraction of permanent teeth (table1&2). But only 80% success rates were achieved on the same day of extraction of primary teeth and 70% on 14th day of extraction of primary teeth

Table 1. Blood grouping on the day of extraction (Group I)

Blood groups	Permanent teeth				Deciduous teeth			
	Control group		Study group		Control group		Study group	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
A	4(40%)	0(0%)	4(40%)	0(0%)	4(40%)	0(0%)	3(30%)	1(10%)
B	3(30%)	0(0%)	3(30%)	0(0%)	1(10%)	0(0%)	1(10%)	0(0%)
AB	1(10%)	0(0%)	1(10%)	0(0%)	1(10%)	0(0%)	1(10%)	0(0%)
O	2(20%)	0(0%)	2(20%)	0(0%)	4(40%)	0(0%)	3(30%)	1(10%)
Total positive	10(100%)	0(0%)	10(100%)	0(0%)	10(100%)	0(0%)	8(80%)	2(20%)
	10(100%)		10(100%)		10(100%)		8(80%)	

Table 2. Blood grouping on the 14th day of extraction (Group II)

Blood groups	Permanent teeth				Deciduous teeth			
	Control group		Study group		Control group		Study group	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
A	3(30%)	0(0%)	3(30%)	0(0%)	1(10%)	0(0%)	1(10%)	0(0%)
B	2(20%)	0(0%)	2(20%)	0(0%)	3(30%)	0(0%)	3(30%)	2(20%)
AB	1(10%)	0(0%)	1(10%)	0(0%)	2(20%)	0(0%)	2(20%)	1(10%)
O	4(40%)	0(0%)	4(40%)	0(0%)	4(40%)	0(0%)	4(40%)	0(0%)
Total positive	10(100%)	0(0%)	10(100%)	0(0%)	10(100%)	0(0%)	7(70%)	3(30%)
	10(100%)		10(100%)		10(100%)		7(70%)	

Table 3. Blood grouping on 30th day after extraction (Group III)

Blood groups	Permanent teeth				Deciduous teeth			
	Control group		Study group		Control group		Study group	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
A	2(20%)	0(0%)	2(20%)	0(0%)	1(10%)	0(0%)	1(10%)	0(0%)
B	4(40%)	0(0%)	3(30%)	1(10%)	4(40%)	0(0%)	2(20%)	2(20%)
AB	0(0%)	0(0%)	0(0%)	0(0%)	1(10%)	0(0%)	0(0%)	1(10%)
O	4(40%)	0(0%)	3(30%)	1(10%)	4(40%)	0(0%)	1(10%)	3(30%)
Total positive	10(100%)	0(0%)	8(80%)	2(20%)	10(100%)	0(0%)	4(40%)	6(60%)

Table 4. Blood grouping on 90th day after extraction (Group IV)

Blood groups	Permanent teeth				Deciduous teeth			
	Control group		Study group		Control group		Study group	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
A	2(20%)	0(0%)	1(10%)	1(10%)	1(10%)	0(0%)	0(0%)	1(10%)
B	5(50%)	0(0%)	4(40%)	1(10%)	4(40%)	0(0%)	1(10%)	3(30%)
AB	1(10%)	0(0%)	1(10%)	0(0%)	3(30%)	0(0%)	0(0%)	3(30%)
O	2(20%)	0(0%)	2(20%)	0(0%)	2(20%)	0(0%)	1(10%)	1(10%)
Total	10(100%)	0(0%)	8(80%)	2(20%)	10(100%)	0(0%)	2(20%)	8(80%)
positive	10(100%)		8(80%)		10(100%)		2(20%)	

Table 5. Blood grouping on 180th day after extraction (Group V)

Blood groups	Permanent teeth				Deciduous teeth			
	Control group		Study group		Control group		Study group	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
A	2(20%)	0(0%)	2(20%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
B	6(60%)	0(0%)	4(40%)	2(20%)	5(50%)	0(0%)	0(0%)	5(50%)
AB	0(0%)	0(0%)	0(0%)	0(0%)	2(20%)	0(0%)	0(0%)	2(20%)
O	2(20%)	0(0%)	2(20%)	0(0%)	3(30%)	0(0%)	0(0%)	3(30%)
Total	10(100%)	0(0%)	8(80%)	2(20%)	10(100%)	0(0%)	0(0%)	10(100%)
positive	10(100%)		8(80%)		10(100%)		0(0%)	

(Table 1, 2).

An Eighty percent success rate was achieved on the 30th, 90th and 180th day after the extraction of the permanent teeth (table 3, 4 & 5). But we could achieve only 40% on the 30th day, 20% on the 90th day and 0% on the 180th day after the extraction the deciduous teeth respectively (Table 3, 4 & 5).

DISCUSSION

The resilience of the teeth and its supporting tissues to pre- and post-mortem assaults provides a wealth of information for those interested in the identity of the deceased. Chemical attack, burning, burial, submersion, and even severe head and neck trauma are all withstood by the dentition to an extent where identification is possible [10].

The determination of ABLI(O) specific

antigens from finger toe nails, hair, teeth, bones and muscles can be used for the identification of victims [11]. Various authors have reported that the dental pulp tissue can be used for ABO blood typing of victims [5,9,12,13,14]. Several techniques like Mixed-Agglutination, two-dimensional Absorption-Inhibition (2-D-AI), Electrophoretic technique, Infusion-sedimentation phenomenon etc including Absorption-Elution technique have been used in Forensic Science for ABO blood group typing [3,9,12,13].

In the present study, the blood grouping of dental pulp from the permanent teeth carried out on the same day, and on the 14th day furnished 100% success rates but the blood grouping of dental pulp carried out on the 30th, 90th and 180th day furnished only 80% success rates. This is in contrast to the study done by Sharma and Chattopadhyaya [5]. Cent percent success rate was achieved up to the 24th month after the extraction, irrespective of the type of dentition. Similar findings have also been reported by Takata [15]. Whereas Smeets⁶ et al reported 86% success rate up to 21 months after the extraction of the permanent teeth.

In accordance to Heartig et al [9] the overall decrease in the success rate of our study could be due to cell lysis; contamination of the tooth or time lapse for the procedure. The lower success rate of deciduous teeth could be attributed to variation in the pulp volume, loss of the tissue during sectioning and root resorption in the deciduous teeth.

The dental tissue can get contaminated with aerobic gram negative bacteria (*e. coli* & *s. marcescens*). These microorganisms' process ABO blood group like antigens, simulating a B-type blood group [14]. This could be another reason for higher failure rates. As majority (i.e. 36) of our samples was of the blood group B type. More sensitive methods like multiplex single primer extension reaction, serologically active proteases could be used to control the moisture or heat sensitivity [17,18].

Thus our study emphasizes that the blood typing of tooth pulp by Absorption-Elution method can be used for relative identification of individuals.

CONCLUSION

In our study the permanent teeth exhibited higher percentage of positive results than the deciduous teeth. The blood group identified on the same day of the extraction and on the 14th day after the extraction demonstrated a higher percentage of positive results.

Based on our findings we could probably culminate that the absorption- elution technique is a reliable, economical and easy method for grouping blood samples from the dental pulp.

REFERENCES

1. Hemanth M, Shetty VM, Shetty N, Karkera BV. Sex determination using dental tissue. *Medico-Legal Update*. 2008; 8(2): 2008-07 - 2008-12.
2. 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.
3. Xingzhi X, Ji L, Hao F, Ming L, Zhuyao L. ABO blood grouping on Dental Tissue. *J Forensic Sci* 1993; 38(4): 956 - 960.
4. Nishi K. ABO blood group typing in forensic autopsies. *Nihon Hoigaku Zasshi*. 2005; 59(2): 111-7.
5. Sharma AK, Chattopadhyay PK. ABO blood grouping from the dental pulp. *Journal Forensic Sci Soc*. 1993; 33(1): 39 - 44.
6. Smeets B, van de Voorde H, Hooft P. ABO blood grouping on tooth material. *Forensic Sci Int*. 1991; 50(2): 277-84.
7. Kind SS. Absorption elution grouping of dried blood smears. *Nature*. 1960; 185: 397-398.
8. Nishi K., Rand S, Nakagawa T, Yamamoto A, Yamasaki S, Yamamoto Y et.al. ABO Blood Typing from Forensic Materials - Merits and demerits of detection methods utilized in our laboratories, and biological

- significance of the antigens Anil Aggrawal's Internet Journal of Forensic Medicine and Toxicology [serial online], : http://www.geradts.com/anil/ij/vol_006_no_002/papers/paper001.html; 2005; 6(2): (July-December2005). (Accessed on June 09, 2010).
9. Haertig A, Krainic K, Vaillant JM, Derobert L. Medicolegidentificatio: teeth and blood groups. *Rev Stomatol Chir Maxillofac*. 1980; 81(6): 361-3.
 10. Pretty IA. Forensic dentistry: 1. Identification of human remains. *Dental update* 2007; 34(10): 621-2, 624-6, 629-30.
 11. Garg RK. Determination of ABH (O) blood group substances from finger and toe nails. *Z Rechtsmed*. 1983; 19: 17-19.
 12. Mukherjee JB, Chattopadhyay PK. Blood grouping from teeth by absorption elution technique and its role in establishing identity. *Med Sci Law* 1976; 6(4): 32-40.
 13. Asylbaeva LB, Barsegiants LO, Galitskii FA. Efficacy of using different modifications of Absorption-Elution reaction for the detection of antigen P1 in blood stains. *Sud Med Ekspert*. 2004; 47(1): 15-6.
 14. Derobert K, Craninic A, Heartig, Surigon M. Identificatio du groupe sanuin ABO au niveau des dents humaines. *Acta Med Leg Soc. (Lieg)*. 1980; 2: 99-125.
 15. Takata H. Studies on blood groups of human teeth. Part 1. Identification of ABO blood groups from permanent and deciduous teeth by means of elution test. *Jpn J Legal Med*. 1973; 27: 46-54.
 16. Hooft P, van de Voorde H and Van Dijck P. Blood group simulating activity in aerobic gram negative oral bacteria cultured from fresh corps. *Forensic Science Int*. 1991; 50: 263-268.
 17. Asylbaeva LB, Barsegiants LO, Galitskii FA. Efficacy of using different modifications of Absorption-Elution reaction for the detection of antigen P1 in blood stains. *Sud Med Ekspert*. 2004; 47(1): 15-6.
 18. Sakharov RS, Fedulova MV, Gureeva NB, Nofal KhK, Droz AF, Shupik Iup. The use of proteases for enhancing the sensitivity of detecting ABO system antigens by an Absorption-Elution technique in blood stains. *Sud Med Ekspert*. 1998; 41(2): 40-4.