

To Evaluate the Sensitivity of ABO Antigen Determination from Dental Pulp at Various Temperatures over a Duration of 3 Months - A Diagnostic Study

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Abstract

Introduction: Every individual either living or dead has a unique identity as per the theory of uniqueness. Medico-legal examination recognizes the blood group as a unique indicator as it remains unchanged, forensic study uses teeth as the unique biological indicator, as they are the least destructible part of the body. The present study was conducted to evaluate the efficacy in retrieving ABO blood group antigens from pulp tissue at various temperatures like freezing temperature, 100°C and 200°C with room temperature being the baseline for the study and at various time intervals (same day, 14th, 30th and 90th day) post extraction by adsorption–elution method. **Materials and Methods:** A diagnostic study of 80 cases was included which involved extraction procedures under local anesthesia following the aseptic protocol. Sterile cotton/gauze was compressed in the socket and later the blood collected from it served as a control group for the study. The extracted teeth were washed and kept, stored in numbered bottles for different time periods after being subjected to different degrees of temperatures i.e. at room temperature, freezing temperature, 100°C and 200°C for 60 mins respectively. **Results:** Goodman-Kruskal Gamma test was used for statistical analysis to obtain the correlation between blood groups at different temperatures. After analyzing the ABO antigen for sensitivity at various degrees of temperature over different time intervals it was found that at freezing temperature and room temperature, antigens can be assessed, while at higher temperatures it is difficult to appreciate. **Conclusion:** Adsorption-Elution technique can be used as a reliable tool for determination of ABO blood group from the dental pulp of a deceased person.

Keywords: ABO Blood Grouping, Dental Pulp, Different Temperatures, Human Identification

Introduction

Identification of forensic evidence includes a multidisciplinary team which relies on methodologies

which are either presumptive or exclusionary in nature¹. Dr. Oscar Amoedo was considered as the father of forensic odontology². According to the Federation Dentaire Internationale in the year 1940, forensic dentistry was

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defined as “that branch of forensics which is in interest of justice deals with proper handling and examination of dental evidence with proper evaluation and presentation of dental findings”³. Human identification is significant not only to identify the deceased individual but also to play a major role in certain issues like, criminal investigations, insurance settlements etc.⁴. Rh blood factor antigen was discovered by Landsteiner and Alexander S. Wiener in the year 1940^{5,6}. Blood grouping among the individual once established remains unchanged throughout the life. Blood group of an individual can be used as a biological material in the field of forensics⁷.

Teeth are a major source of evidence in the field of forensics for identification purpose. Teeth are considered to be the hardest substance in the human body and can bear a temperature up to 1600°C without significant loss of microstructure⁷. In conditions like mass disaster, adverse environmental grounds, teeth form a vital source of personal identification, while other means of identification like facial and dermatoglyphic features, tattoos, marks etc. fail due to mutilation, decomposition and charring⁸.

Pulp is soft connective tissue present along the central hollow part of tooth. Pulp surrounded by dental hard tissues is considered to be the most protected oral tissue⁹. Thus in blood grouping, dental pulp tissues could be considered as a hall mark for identifying biological materials in forensic studies.

India is a country of paradoxes. On one hand, we have sophisticated laboratories in the big metropolitan cities and on the other hand in rural areas there is absence of even basic laboratory facilities to handle the routine procedures. In such case scenarios, DNA analysis for forensic purposes may not be possible or it may take a long time^{8,9}.

Hence ABO blood group analysis for preliminary investigations may be essential in forensic odontology. Adsorption-Elution method is one of the commonly used techniques to determine ABO blood group from dental pulp tissues^{10,13,14}. This technique was devised by Siracusa in 1923 and has been refined by Kind^{11,12}. In highly decomposed bodies where teeth and the bones are the only source remaining, the presence of ABO blood group antigen in the dental soft tissue helps in the process of identification^{10,12}. ABO blood grouping has been done by many investigators at room temperatures but in spite of extensive literature there were no studies on ABO blood group analysis where the teeth are exposed to high

temperatures like fire victims, electrical burns, aircraft fire accidents, victims of volcanic eruptions etc. or low temperature like in snow slide accidents.

The present study was done to evaluate the efficacy of ABO blood group antigens retrieval from pulp tissue at various temperatures like freezing temperature, extreme temperature 100°C and 200°C with room temperature being the baseline of the study and at various time intervals (same day, 14th, 30th and 90th day) post extraction.

Materials and Methods

A diagnostic study of 80 cases were included of age group 13 years and above with both male and female. The cases were obtained after surgical extraction of the teeth from the concerned department along with permission and written consent of the patient and bystander. The blood soaked cotton/gauze was used to determine the ABO blood group to avoid bias and was used as control. The extracted teeth were exposed to various temperatures, i.e. room temperature, freezing temperature (Remi Deep Freezer), 100°C and 200°C (Innovative incubator) and was stored for ABO antigen determination on same day, 14th, 30th and 90th day post extraction. The Inclusion criteria were male and female patients of age 13 years and above, permanent teeth, teeth extracted for orthodontic purpose and periodontal problems. The exclusion criteria were carious teeth, endodontically treated teeth, grossly decayed teeth, grossly destructed with exposed pulp cavities and calcified root canals and deciduous teeth.

Dental extraction procedures were performed under local anesthesia following aseptic measures. After extraction, the sockets were compressed with sterile cotton/gauze and blood was collected. Normal saline and anticoagulant were added, later diluted to this blood in a sterile test tube. Three glass slides were arranged and to each slide single drop of diluted blood was placed, followed by a drop of anti-serum A, B and D. Presence of agglutination after addition of antiserum indicated that the particular blood group was present and if agglutination occurred in a slide with antiserum D, it indicated its positivity. This was considered control for the study.

Running water was used to wash the extracted teeth followed by removal of debris with the help of the probe. Later the teeth were wiped with gauze and were stored in bottles, labelled with numbers. The tooth was

subjected to different degree temperatures i.e. at room temperature, freezing temperature (Figure 1) 100°C and 200°C for 60 minutes respectively and stored for different time intervals. The extracted teeth were assessed for ABO blood group by the method of Adsorption–Elution test using dental pulp, which was later compared to the control blood group.

The tooth was embedded on a block shaped modelling wax. Vertical split was placed parallel to the long axis of the tooth (Figure 2) with the help of carborundum disc. Later the dental pulp was scooped out with a sterile spoon excavator and was placed in a test tube containing a drop of saline. To each test tube 3 drops of anti A and anti B serum was added and left at room temperature for three hours. The test tube was centrifuged and the supernatant (antiserum) was pipetted out. Later each sample was washed 3 times with cold saline. Two drops of fresh saline was added to the sample, later the test tube was heated in a water bath at a temperature of 50-55°C for ten minutes to elute the antibodies. A drop of 0.5% A or B group red cell suspension was added and incubated for 30 min, later it was centrifuged at 1500-2000 RPM for 1 min. (flow chart Figure 3). Gentle shaking of the test tube was done, indicating the presence (Figure 4) or absence (Figure 5) of red blood cell agglutination, which was seen under the bright field light microscope at a magnification of 100x. (OLYMPUS BX40).

Results

The ABO blood group distribution of total 80 cases was tabulated according to temperature and time interval



Figure 1. Tooth exposed to lower temperature in freezer



Figure 2. Sectioned tooth

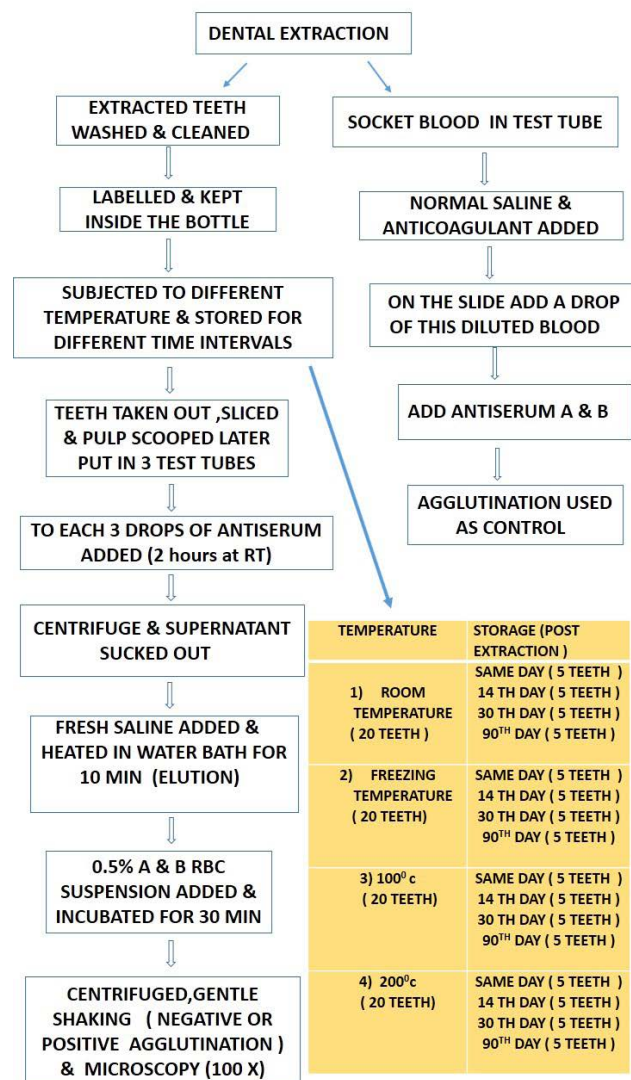


Figure 3. Flow chart of methodology

from the day of storing. In each temperature 20 samples were examined and among these 20 samples, 5 samples

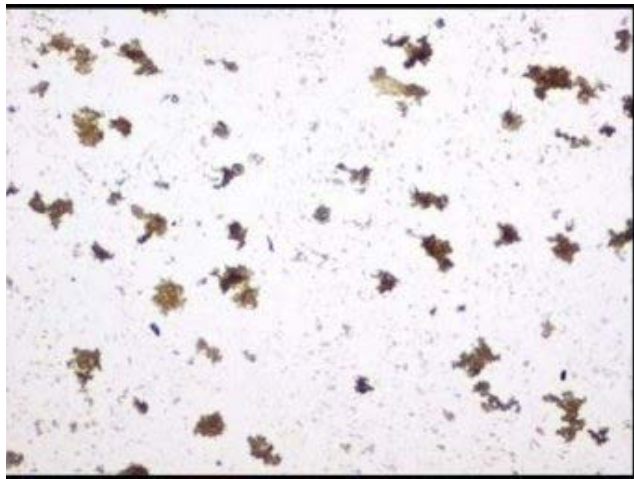


Figure 4. Photomicrograph of section under bright field microscope (100 x) showing presence of agglutination.

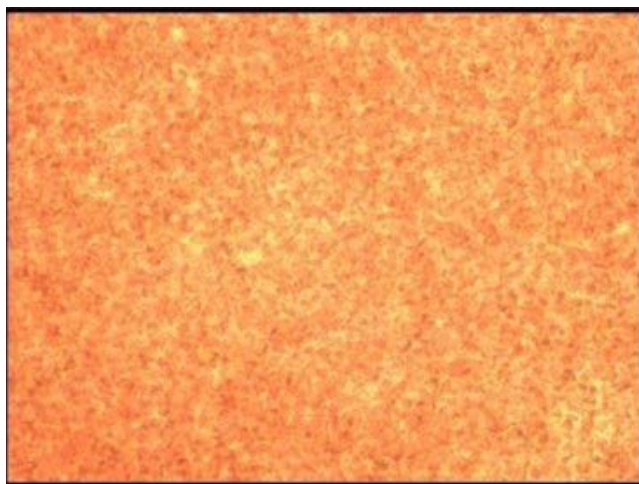


Figure 5. Photomicrograph of section under bright field microscope (100 x) showing no agglutination.

were made for same day, 14th day, 30th day and 90th day of post extraction and the results were tabulated and subjected to Goodman Kruskal Gamma test. Goodman Kruskal Gamma test was done for analysis to obtain the correlation between blood groups at different temperatures. The following formula was used:

$$\text{Goodman Kruskal Gamma} = \frac{Nc - Nd}{Nc + Nd}$$

Nc - stands for number of concordant pairs and Nd - stands for number of discordant pairs.

The correlation between the study and control groups of Room and Freezing Temperatures at various storage intervals were in good correlation (statistically significant) (Tables no 1 to 8). However the correlation between the study and control groups of high temperatures i.e. 100°C and 200°C at various storage intervals had negative correlation (statistically insignificant) (Tables no 9 to 16).

The sensitivity of pulp of various blood groups (ABO antigen) revealed that at room and freezing temperatures at various time storage intervals (the sensitivity was almost 80 to 100%) i.e. antigens can be calculated, while at higher temperatures (sensitivity was hardly 20%) it was difficult to detect the ABO antigens. (Figure 6/Graph)

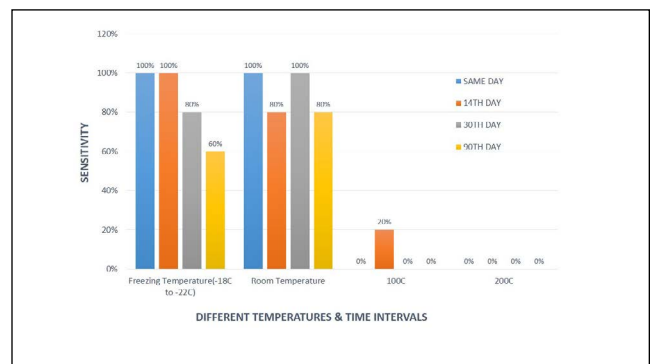


Figure 6 (Graph). Sensitivity of pulp for ABO antigen at various temperatures and at different time intervals.

Table 1. Blood grouping and sensitivity of dental pulp at room temperature on the same Day of Extraction

Blood Grouping at Room Temperature on Same Day of Extraction							
Blood Group	Control Group		Study Group		P Value	Results	Goodman Kruskal Gamma
	Positive	Negative	Positive	Negative			
A	2(40%)	0(0%)	2(40%)	0(0%)	>0.5	True Positive	Very Good Correlation
AB	0(0%)	0(0%)	0(0%)	0(0%)	-	True Positive	
B	3(60 %)	0(0%)	3(60 %)	(0%)	>0.5	True Positive	
O	0(0%)	0(0%)	0(0%)	0(0%)	-	True Positive	
OVERALL	5(100%)	0(0%)	5(100%)	0(0%)	>0.5		

Table 2. Blood grouping and sensitivity of dental pulp at room temperature on the 14th day of extraction

Blood Grouping at Room Temperature on 14 th Day of Extraction								
Blood Group	Control Group		Study Group		P Value	Results	Goodman Kruskal Gamma	
	Positive	Negative	Positive	Negative				
A	2(40%)	0(0%)	2(50%)	0(0%)	>0.5	True Positive	1	Good Correlation
AB	1(20%)	0(0%)	1(25%)	0(0%)	>0.5	True Positive	1	
B	1(20 %)	0(0%)	0(0 %)	(0%)	-	NIL	-1	
O	1(20%)	0(0%)	1(25%)	0(0%)	0.5	True Positive	1	
Overall	5(100%)	0(0%)	5(100%)	0(0%)	>0.5		0.6	

Table 3. Blood grouping and sensitivity of dental pulp at room temperature on the 30th day of extraction

Blood Grouping At Room Temperature On 30 th Day of Extraction								
Blood Group	Control Group		Study Group		P Value	Results	Goodman Kruskal Gamma	
	Positive	Negative	Positive	Negative				
A	2(40%)	0(0%)	2(40%)	0(0%)	>0.5	True Positive	1	Very Good Correlation
AB	1(20%)	0(0%)	1(20%)	0(0%)	>0.5	True Positive	1	
B	2(40%)	0(0%)	2(40 %)	(0%)	>0.5	True Positive	1	
O	0(0%)	0(0%)	0(0%)	0(0%)	-	True Positive	1	
Overall	5(100%)	0(0%)	5(100%)	0(0%)	>0.5		1	

Table 4. Blood grouping and sensitivity of dental pulp at room temperature on the 90th day of extraction

Blood Grouping at Room Temperature on 90 th Day of Extraction								
Blood Group	Control Group		Study Group		P Value	Results	Goodman Kruskal Gamma	
	Positive	Negative	Positive	Negative				
A	2(40%)	0(0%)	1(25%)	1(25%)	>0.5	True Positive/False Negative	0	Good Correlation
AB	0(0%)	0(0%)	0(0%)	0(0%)	-	True Positive	1	
B	2(40 %)	0(0%)	2(50 %)	(0%)	>0.5	True Positive	1	
O	1(20%)	0(0%)	1(25%)	0(0%)	.0.5	True Positive	1	
Overall	5(100%)	0(0%)	5(100%)	0(0%)	>0.5		0.6	

Table 5. Blood grouping and sensitivity of dental pulp at freezing temperature on the same day of extraction

Blood Grouping at Freezing Temperature on Same Day of Extraction								
Blood Group	Control Group		Study Group		P Value	Results	Goodman Kruskal Gamma	
	Positive	Negative	Positive	Negative				
A	0(0%)	0(0%)	0(0%)	0(0%)	-	True Positive	0	Very good correlation
AB	0(0%)	0(0%)	0(0%)	0(0%)	-	True Positive	0	
B	3(60 %)	0(0%)	3(60%)	0(0%)	>0.5	True Positive	1	
O	2(40%)	0(0%)	2(40%)	0(0%)	>0.5	True Positive	1	
OVERALL	5(100%)	0(0%)	5(100%)	0(0%)	>0.5		1	

Discussion

Forensic odontology is that speciality of dentistry which deals with application of dental evidence to the

field of law^{35,36}. Blood groups have been a foundation for identification of biological materials in forensic medicine^{10,13}. In most of the adverse environmental conditions, the teeth serve as a vital biological trace

Table 6. Blood grouping and sensitivity of dental pulp at freezing temperature on the 14th Day of Extraction

Blood Grouping at Freezing Temperature on 14 th Day of Extraction								
Blood Group	Control Group		Study Group		P Value	Results	Goodman Kruskal Gamma	
	Positive	Negative	Positive	Negative				
A	1(20%)	0(0%)	1(20%)	0(0%)	>0.5	True Positive	1	Very good correlation
AB	1(20%)	0(0%)	1(20%)	0(0%)	>0.5	True Positive	1	
B	2(40 %)	0(0%)	2(40 %)	0(0%)	>0.5	True Positive	1	
O	1(20%)	0(0%)	1(20%)	0(0%)	>0.5	True Positive	1	
Overall	5(100%)	0(0%)	5(100%)	0(0%)	>0.5		1	

Table 7. Blood grouping and sensitivity of dental pulp at freezing temperature on the 30th Day of Extraction

Blood Grouping at Freezing Temperature on 30 th Day of Extraction								
Blood Group	Control Group		Study Group		P Value	Results	Goodman Kruskal Gamma	
	Positive	Negative	Positive	Negative				
A	1(20%)	0(0%)	1(20%)	0(0%)	>0.5	True Positive	1	Good correlation
AB	0(0%)	0(0%)	1(20%)	0(0%)	>0.5	True Positive	-1	
B	3(60 %)	0(0%)	2(40 %)	1(20%)	>0.5	True Positive/False Negative	0.33	
O	1(20%)	0(0%)	1(20%)	0(0%)	>0.5	True Positive	1	
OVERALL	5(100%)	0(0%)	5(100%)	0(0%)	>0.5		0.6	

Table 8. Blood grouping and sensitivity of dental pulp at freezing temperature on the 90th Day of Extraction

Blood Grouping Freezing Temperature on 90 th Day of Extraction								
Blood Group	Control Group		Study Group		P Value	Results	Goodman Kruskal Gamma	
	Positive	Negative	Positive	Negative				
A	1(20%)	0(0%)	0(0%)	0(0%)	-	Nil	1	No correlation.
AB	0(0%)	0(0%)	1(20%)	0(0%)	-	False Positive	-1	
B	3(60 %)	0(0%)	2(40 %)	1(20%)	>0.5	True POSITIVE/FALSE NEGATIVE	0.3	
O	1(20%)	0(0%)	1(20%)	0(0%)	>0.5	True Positive	1	
Overall	5(100%)	0(0%)	5(100%)	0(0%)	>0.5		0	

Table 9. Blood grouping and sensitivity of dental pulp at 100°C on the same Day of Extraction

Blood Grouping at 100°C on Same Day of Extraction								
Blood Group	Control Group		STUDY GROUP		P VALUE	RESULTS	GOODMAN KRUSKAL GAMMA	
	Positive	Negative	Positive	Negative				
A	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-	TRUE POSITIVE	1	Negative correlation
AB	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-	TRUE POSITIVE	1	
B	5 (100 %)	0 (0%)	0 (0 %)	(0%)	-	NIL	-1	
O	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-	TRUE POSITIVE	0	
OVERALL	5 (100%)	0 (0%)	5 (100%)	0 (0%)	>0.5		-1	

Table 10. Blood grouping and sensitivity of dental pulp at 100°C on the 14th Day of Extraction

BLOOD GROUPING AT 100° C ON 14 TH DAY OF EXTRACTION								
BLOOD GROUP	CONTROL GROUP		STUDY GROUP		P VALUE	RESULTS	GOODMAN KRUSKAL GAMMA	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE				
A	0(0%)	0(0%)	1(33.3%)	0(0%)	-	FALSE POSITIVE	-1	Negative correlation
AB	1(20%)	0(0%)	1(33.3%)	0(0%)	>0.5	TRUE POSITIVE	1	
B	4(80 %)	0(0%)	0(0 %)	(0%)	-	NIL	-1	
O	0(0%)	0(0%)	1(33.3%)	0(0%)	-	FALSE POSITIVE	-1	
OVERALL	5(100%)	0(0%)	3(100%)	0(0%)	>0.5		-0.66	

Table 11. Blood grouping and sensitivity of dental pulp at 100°C on the 30th Day of Extraction

BLOOD GROUPING AT 100° C ON 30 TH DAY OF EXTRACTION								
BLOOD GROUP	CONTROL GROUP		STUDY GROUP		P VALUE	RESULTS	GOODMAN KRUSKAL GAMMA	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE				
A	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1	Negative correlation
AB	0(0%)	0(0%)	0(0%)	0(0%)	-	NIL	1	
B	4(80 %)	0(0%)	0(0%)	(0%)	-	NIL	-1	
O	0(0%)	0(0%)	0(0%)	0(0%)	-	NIL	-1	
Overall	5(100%)	0(0%)	5(100%)	0(0%)	>0.5		-1	

Table 12. Blood grouping and sensitivity of dental pulp at 100°C on the 90th Day of Extraction

Blood Grouping at 100° C on 90 th Day of Extraction								
Blood Group	Control Group		Study Group		P value	Results	Goodman Kruskal Gamma	
	Positive	Negative	Positive	Negative				
A	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1	Negative correlation
AB	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1	
B	2(40 %)	0(0%)	0(0%)	(0%)	-	NIL	-1	
O	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1	
Overall	5(100%)	0(0%)	0(100%)	0(0%)	>0.5		-1	

Table 13. Blood grouping and sensitivity of dental pulp at 200°C on the same Day of Extraction

BLOOD GROUPING AT 200° C ON SAME DAY OF EXTRACTION:								
BLOOD GROUP	CONTROL GROUP		STUDY GROUP		P VALUE	RESULTS	GOODMAN KRUSKAL GAMMA	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE				
A	0(0%)	0(0%)	0(0%)	0(0%)	-	NIL	-1	Negative correlation
AB	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1	
B	3(60 %)	0(0%)	0(0%)	(0%)	-	NIL	-1	
O	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1	
OVERALL	5(100%)	0(0%)	0(100%)	0(0%)	-		-0.66	

Table 14. Blood grouping and sensitivity of dental pulp at 200°C on the 14th Day of Extraction

Blood Grouping at 200°C pn 14 th Day pf Extraction							
BLOOD GROUP	CONTROL GROUP		STUDY GROUP		P VALUE	RESULTS	GOODMAN KRUSKAL GAMMA
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE			
A	0(0%)	0(0%)	1(100%)	0(0%)	-	FALSE POSITIVE	-1
AB	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1
B	3(60 %)	0(0%)	0(0%)	(0%)	-	NIL	-1
O	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1
Overall	5(100%)	0(0%)	1(100%)	0(0%)	>0.5		-1

Negative correlation

Table 15. Blood grouping and sensitivity of dental pulp at 200°C on the 30th Day of Extraction

Blood Grouping at 200°C on 30 th Day of Extraction							
Blood Group	Control Group		Study Group		P value	Results	Goodman Kruskal Gamma
	Positive	Negative	Positive	Negative			
A	2(40%)	0(0%)	0(0%)	0(0%)	-	NIL	-1
AB	0(0%)	0(0%)	0(0%)	0(0%)	-	NIL	1
B	3(60 %)	0(0%)	0(0%)	(0%)	-	NIL	-1
O	0(0%)	0(0%)	0(0%)	0(0%)	-	NIL	-1
Overall	5(100%)	0(0%)	0(0%)	0(0%)	-		-1

Negative correlation

Table 16. Blood grouping and sensitivity of dental pulp at 200°C on the 90th Day of Extraction

BLOOD GROUPING AT 200 ° C ON 90 TH DAY OF EXTRACTION							
Blood Group	Control Group		Study Group		P Value	Results	Goodman Kruskal Gamma
	Positive	Negative	Positive	Negative			
A	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1
AB	0(0%)	0(0%)	0(0%)	0(0%)	-	NIL	1
B	3(60 %)	0(0%)	0(0%)	(0%)	-	NIL	-1
O	1(20%)	0(0%)	0(0%)	0(0%)	-	NIL	-1
OVERALL	5(100%)	0(0%)	0(0%)	0(0%)	-		-1

Negative correlation

material in the process of identification^{7,8,10,28,29}. ABO blood group antigens are present in dental tissues and thus makes its possible in identification of putrefied bodies in cases where only teeth and bone are the vital remains^{10,14,15,20,32}, According to Rothwell B.R, blood group substance used in forensic procedures are based on the point that once the blood group of an individual is established, it remains unaltered through the life. The dental pulp tissues used for blood grouping are considered authentic for identifying biological material in medico-legal procedures^{4,6,15,16,17,33}.

Forensic experts for several years have been exploring a definitive method for blood typing of teeth. Shimura et al employed conventional adsorption inhibition

technique for hard tissue but the problem was complicated pretreatments and the results were inadequate. Later after few years adsorption inhibition technique was replaced by adsorption elution method which was solely used in forensic laboratories^{10,18,19,26}. Although the procedure was described 50 years ago, it was not widely used for forensic serology until 1960. Later clarified the technique. Vittorio Siracusa developed the adsorption elution procedure and is now employed in all forensic laboratories due to its proven sensitivity, reliability and reproducibility in identifying blood group of deceased individuals^{6,35}.

Mahabalesh Shetty conducted a study on ABO blood grouping from tooth material where pulp and dentine were used for ABO blood grouping using adsorption

elution method⁸. Sushmini Ballal found that the correlation of blood grouping from dentine and dental pulp with the blood collected from the extraction socket of respective teeth can be determined²⁹. Success rate was better when ABO blood group was determined at room temperatures^{7,12,22}. Researchers have suggested that dental pulp tissue can be used for ABO blood typing of victims⁷.

However in spite of extensive search of literature, there were no studies on ABO blood group analysis exposing teeth to high temperatures like fire victims, electrical burns, aircraft fire accidents, victims of volcanic eruptions etc. or low temperature like in snow slide accidents. Therefore in present study, we determined ABO blood group from individuals to examine that if the blood group antigens on the pulp remains possible even after subjecting the teeth to various temperatures and storing for up to 3 months.

In the present study, ABO blood group antigens at room temperature at different time intervals i.e. 18 out of 20 teeth showed 100% sensitivity rates, while two cases showed false positive results.

On the same day and 30th day post extraction, the sensitivity rate was 100%, whereas on 14th and 90th day post extraction it was found as 80%. The results correlated with the study done by Inamdar et al⁷. Blood grouping of dental pulp carried out on 30th, 90th and 180th day of extraction showed only 80% success rates⁷. Similar findings have been reported by Takata and Smeets showed 86% success rate up to 21 months post extraction^{22,24}.

ABO blood grouping in dentine and pulp showed 97% sensitivity in pulp⁸ whereas in the study conducted by Sushmini³⁰ i.e. 27 out of 30 teeth showed positive results in pulp and revealed 90% sensitivity. Negative results for ABO blood group antigen on 14th day post extraction could be due to cross contamination with aerobic gram negative bacteria present in saliva/tooth or due to increased number of foreign antigen borne by bacteria in teeth^{8,27,28,29,30}. The negative results on 90th day of extraction for ABO blood group antigen could be attributed to autolysis, dehydration, loss of pulp antigens or even invasion of certain bacteria in damaged teeth which pass on their random antigenic activities to them^{8,29}. It was observed by many authors (Inamdar and Ramanayaka) that storing pulp tissue more than 90th day of extraction can result in decrease in sensitivity rates^{7,23,34}. According to Heartig et al. the decrease in the success rate of their study at room temperature could be due to cell lysis, tooth contamination or time lapse for the procedure^{7,16,17}. Thus

we can hypothesize that the false positive results in the samples could be due to the massive growth of aerobic gram negative bacteria that tends to obscure the pulpal blood group antigen and it can produce cross reaction.

Negative results could also be due to inadequate and fibrosed tissues in the pulp with increase in age and calcification^{26,28}. Negative results can also occur from the specimen of post mortem dental pulp due to haemolytic erythrocytes, putrefaction and mummification or skeletonised of the body during post mortem interval according to Nalini^{21,32}. This finding was also consistent with the study done by Yumi Nakayama and Yasuhiro Aoki²³.

In present study when considering the higher temperatures (100°C and 200°C) at 10 °C on the day of extraction, 30th day and 90th day of extraction the sensitivity was found to be 0%, whereas on 14th day of extraction it showed 20% sensitivity which means 1 out of 5 cases were true positive, whereas on 14th day of extraction it also showed 2 false positive results. Hence caution should be exercised while considering the ABO blood group result in teeth exposed to such high temperatures. While at 200°C on the day of extraction, 14th day, 30th day and 90th day of extraction, ABO blood grouping did not show any results and the sensitivity was found to be 0%. This is in accordance with Mondal et al. who mentions that heating at 200°C over destroys blood group substances^{25,31}. Therefore we hypothesize that this finding may be due to effect of high temperatures that cause heat denaturation of antigens resulting in lack of reaction. In denaturation of antigens proteins lose the quaternary, tertiary and secondary structure. The characteristic variability of the denatured proteins shows loss of solubility to communal aggregation of the hydrophobic proteins to come closer and form bonding between them, so as to reduce the total area exposed to water³⁶⁻³⁸.

When the ABO blood grouping was analyzed at freezing temperature, it was found that on the day of extraction and 14th day of extraction sensitivity was found 100% .While as time intervals progressed from 30th day to 90th day of extraction, the sensitivity was reduced from 80% to 60% respectively. Several investigators have found contradicting results to our findings, showing that at lower temperature there will be denaturation of antigens³⁶.

Cold denaturation can spontaneously unwind proteins. Therefore we hypothesize that at lower temperatures the structure and antigenicity may be preserved for limited period i.e. possibly till a fortnight after which there could

be loss or denaturation of antigens. The denaturation of proteins is temperature dependent as described by the Molar Gibbs free-energy change. The mechanism behind the temperature induced denaturation is not clear and it could be due to changes in the structure of water³⁹.

The equilibrium between enthalpic (amount of internal energy in a compound) and entropic (amount of intrinsic disorder in a compound) effects are responsible for the stability of the proteins. Heat and cold denaturation are mainly due to increased entropic effects and hydration of internal non polar groups. So in either temperature level the proteins get denatured³⁸. The heat and cold induced denaturation temperatures define the temperature at which Gibbs free energy of unfolding equals zero. This free energy contributed by both enthalpic and entropic, change with temperature and gives rise to heat denaturation and to some, cold denaturation. Therefore before forming any tight bonding, antigens and antibodies in a solution have to overcome this large entropic barriers³⁹⁻⁴¹.

From our results we infer that there is not much sensitivity deviation observed between freezing and room temperature cases on the same day of extraction and 14th day of extraction. But on 30th and 90th day of extraction sensitivity was found decreasing which could be due to cold denaturation or cross contamination. And at higher temperature, the lower sensitivity rate which could be due to heat denaturation. Therefore, we can say that, it might be tricky for us to identify ABO blood group of the deceased exposed to high temperatures that is 100 and 200 degrees. Hence temperature and time duration, to which the deceased individual is exposed to, has to be considered during ABO blood grouping. In present study the positive results of ABO blood group obtained from dental pulp can conclude that pulps possess high potential value for application at high and freezing temperatures in forensic odontology.

Hence these results accentuates that the identification of individuals even after the span of 3 months of death by the virtue of Adsorption–Elution method can be relatively determined by blood typing of tooth pulp.

Conclusion

The present study depicts the ABO blood group at different temperatures and different time intervals reflect antigen sensitivity. The results of the study show significant reduction in sensitivity at higher temperatures

and with increasing the time of storage of pulp antigen. This indicates that ABO blood grouping can contribute in identification of deceased individual at room temperature, in lower temperatures for limited time, but it has restricted role at higher temperatures. However further diagnostic studies on larger series of cases are required to determine the practical use of ABO blood grouping in forensic odontology.

Conflict of Interests

The authors state no conflicts of interest.

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Ethics Approval

The study was certified by the institution. Verbal and written consent was obtained from the patient.

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