ORIGINAL RESEARCH

Determination of ABO Blood Grouping from Dentin and Pulp from Freshly Extracted Teeth and Teeth Stored in Sea Water Using Absorption Elution and Absorption Inhibition Method

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Abstract

Introduction: Forensic Odontology plays a role in discerning a deceased individual in any disaster condition. In highly necrotised bodies, ABO blood group antigens can be found from teeth.

Aim: We studied ABO blood grouping from dentin and pulp in freshly extracted teeth and also from the teeth stored in sea water.

Materials and Methods: A total of 60 samples were selected & divided into 3 groups with 20 samples each. Group I analyzed within period of a week without any storage medium, group II, III were analyzed after 1 and 2 month of storage in sea water.

Results: Statistical analysis was done using chi square test. By Absorption –Elution, pulp in group I, II, III, gave 90%,75%,75% of positivity. In dentin, Group I, II, III showed 55%, 45%, 20% of positivity. By Absorption -Inhibition method pulp in group I, II, III showed 45%, 20%, 0% of positivity. In dentin, group I, II, III showed 20%,5%,5% of positivity.

Conclusion: This study concluded that pulp is most reliable than dentin even in sea water storage and absorption elution is most effective method in blood group identification in teeth than absorption inhibition method.

Keywords: Absorption elution, Absorption inhibition, Blood grouping, Dentin, Pulp, Sea water

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Introduction

Human identification is a backbone for the reorganization of an unknown individual and is of importance to society.[1] Forensic odontology plays a role in discerning a deceased individual in a decomposed state, burned, or at any crime or disaster condition.[2]

The habitat and the meteorological conditions like temperature, moisture, and accessibility to insects play a critical role in mortification.[3] When the carcass remains are found in underwater or underground, it often takes a longer time to find the skeletal remains after death because of the impact of the environment.[4]

For medico-legal investigations, blood group determination plays a vital role in identifying the victim and criminals.[5] Blood group is distinctive for each and every person. Apart from the blood, blood group antigens are produced in various body secretions like semen, sweat, amniotic fluid and saliva. In addition to all the above, ABO antigens are also present in the teeth.[6] Since teeth being the hardest of all body parts, it can withstand onslaught of harshness of environment for a longer time. It remains intact even when it is exposed to 500°C of heat.[5] In highly necrotized bodies, ABO blood group antigens can be found from teeth where they are the only significant tissue remaining.[7] Soft and hard tissues in teeth consist of blood group substances, genetic markers, as enzymes that help identify highly decomposed or chattered bodies.[8] Absorption –Elution (A-E), Absorption- Inhibition (A-I), Mixed agglutination methods, PCR technique (Polymerase chain reaction) are used to determine blood group antigens in mass disaster. A-E and A-I are commonly used methods.[9]

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Various studies have determined blood grouping from freshly extracted teeth and teeth stored in different mediums like soil, water, and saline or in various climatic conditions for many years to duplicate the natural calamities and experimented post-mortem changes in teeth.[10]

No other previous studies have used seawater as a storage medium for blood group determination. This study focused on certain environmental calamities and air crash accidents happening over or in the mid-sea where identifying an individual in that situation is very worse as the bodies will be in a decomposed state, so during that time, teeth will be the only identification tool to find the bodies.In this study, we aimed to determine blood group typing in teeth stored in seawater by Absorption –Elution (A-E) and Absorption- Inhibition (A-I) techniques. Our objective was to determine the reliability of blood grouping from pulp and dentin and also to compare the efficiency of Absorption elution and absorption inhibition techniques from samples without storage medium and in samples stored in seawater for 1 and 2 month time period.

Materials and Methods

In the present study, the samples were collected from our institution and also from private dental clinics (Institutional ethical committee- 213/KSRIDSR/EC/2018). 60 samples were selected and divided into 3 groups with 20 samples in each group. In group I, 20 teeth were analyzed within period of a week without any storage medium. Group II, III samples were analyzed after 1 month, 2 months of storage in sea water. Permanent teeth which were extracted for orthodontic purpose or poor periodontal status were included. Carious teeth, grossly decayed teeth, root canal treated teeth and deciduous teeth were excluded. Control blood group was determined from socket blood by slide agglutination method. The collected teeth were stored in seawater. Tooth was split into two halves and pulp was extirpated and remaining tooth containing dentin was pulverized, and then subjected to absorption elution and absorption inhibition technique. For absorption elution technique, extirpated pulp and powdered dentin were taken in three test tubes and antiserum A, B and D was added and kept

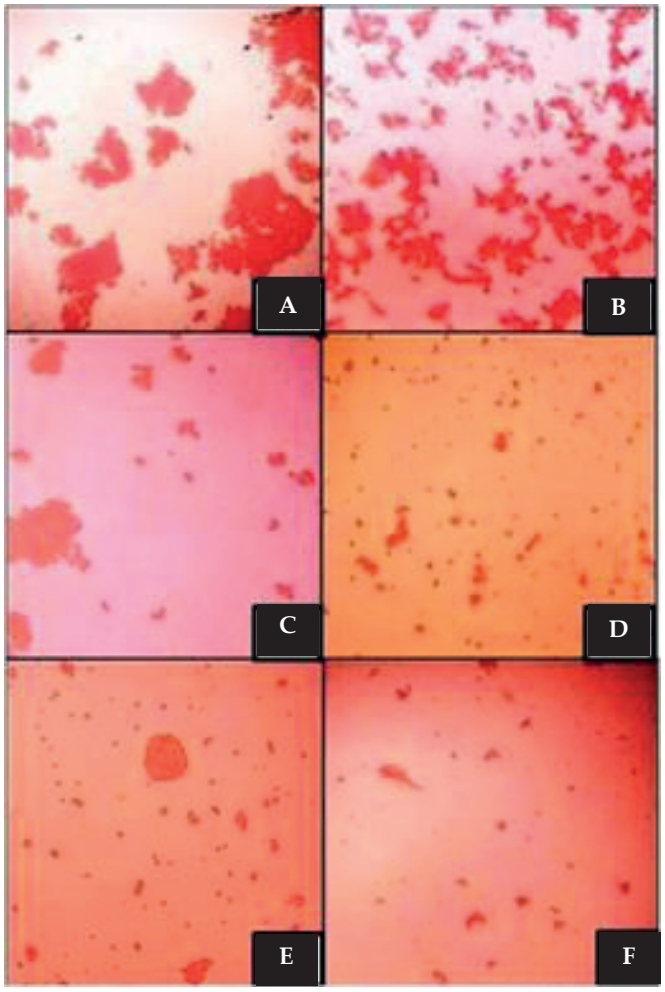

Fig. 1: Presence of agglutination in pulp **(A)** dentin **(B)** in Group I, Pulp **(C)** and dentin **(D)** in Group II, Pulp **(E)** and dentin **(F)** in Group III

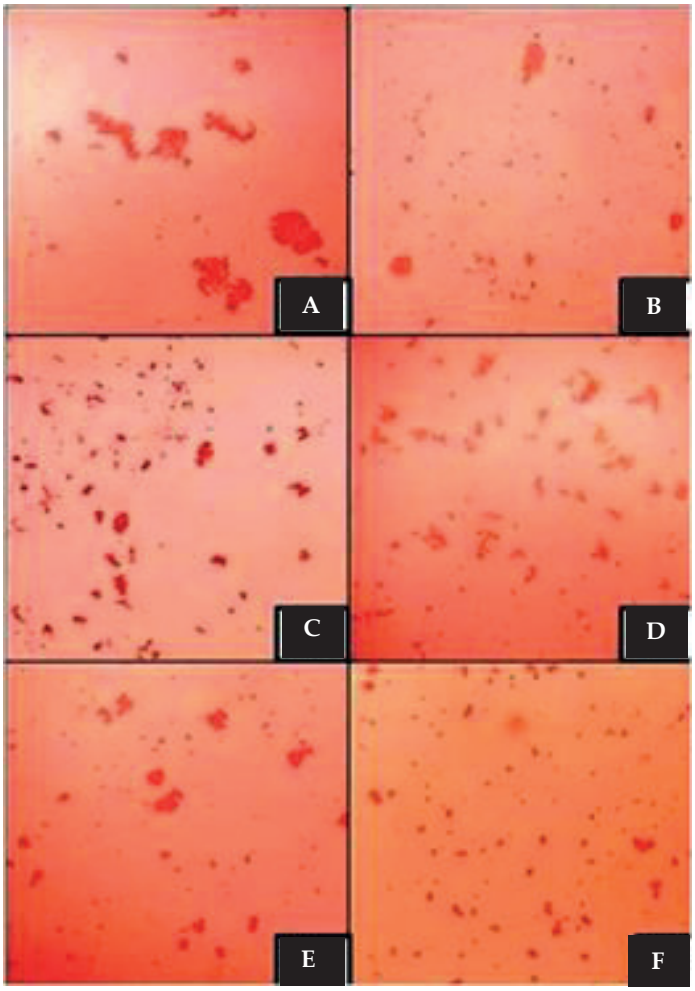

Fig. 2: Presence of agglutination indicating negative correlation with control in pulp **(A)** and dentin **(B)** in Group I, Pulp **(C)** and dentin **(D)** in Group II, Pulp **(E)** and dentin **(F)** in Group III

overnight in refrigerator at 4 degree Celsius. The samples were washed with cold saline and 3 drops of cold saline is added and centrifuged at 3000 rpm (Rotation Per Minute).Test tubes were heated in the water bath for 10 minutes for 56 -degree Celcius and freshly prepared 0.5% of RBCs was added and incubated for 30-degree Celcius for 30 minutes and centrifuged at 2000 rpm for 10 minutes. For Absorption –Inhibition technique, extirpated pulp and powdered dentin were taken in three test tubes, soaked in 1:10 dilution of Antiserum A, B, D for 10 minutes. Freshly prepared 0.5% of RBCs was added and incubated for 30 minutes. On microscopic examination for absorption elution technique if the sample shows the presence of agglutination, it is considered as positive, if no agglutination occurs, it is considered as negative. For absorption inhibition technique, if the sample shows absence of agglutination it is considered as positive, if agglutination occurs then it is interpreted as negative (Figure 1 and 2). Presence of agglutination indicating negative correlation with control in pulp (2E) and dentin (2f) from tooth examined after 2 month of storage in sea water.

Results

This study was designed to determine the blood group from teeth that were stored in seawater. All the data collected were tabulated and assessed for statistical analysis using Statistical Package for Social Science (SPSS) software version 16. Pearson's chi-square test was done to compare the blood group typing in pulp and dentin and to compare the efficiency of A-E and A-I techniques. The level of significance ($p< 0.05$) was employed in all statistical comparisons.

In pulp, by using the A-E method, group I showed 90% of positive results and 75% positive results in group II and group III. In dentin, group I showed 55% of positive results, and in group II and III, it showed 45% and 20% of positive results. By using the A-I technique, pulp showed 45% of positive results in group I, 20% of positive results in group II, and 0% positive results in group III with the control group. In dentin, 20% of positive results in group I, 5% of positive results in group II and III with the control group. Statistical analysis was done

Table 1: Comparison of Blood Group Typing in Pulp and Dentin Between 2 Methods

(n=20)		A-E						A-I						Pearson Chi Square Test
		GROUPII		GROUP I		GROUPIII		GROUPI		GROUPII		GROUPIII		
		n	%	N	%	n	%	n	%	n	%	n	%	
Pulp	POSITIVE	18	90	15	75	15	75	9	45	4	20	0	0	P < 0.05
	NEGATIVE	2	10	5	25	5	25	11	55	16	80	20	100	
Dentin	POSITIVE	11	55	9	45	4	20	4	20	1	5	1	5	
	NEGATIVE	9	45	11	55	16	80	16	80	19	95	19	95	

Table 2: Comparison of 2 Methods in Blood Group Typing in Pulp

METHOD (n=20)	GROUP I						GROUP II						GROUP III						Pearson Chi Square
	positive		negative		False positive		Positive		negative		False positive		positive		negative		False positive		
	n	%	n	%	n	%	n	%	n	%	n	%	N	%	n	%	n	%	P < 0.05
AE	18	90	2	10	0	0	15	75	4	20	1	5	15	75	4	20	1	5	
AI	9	45	8	40	3	15	4	20	11	55	5	25	0	0	13	65	7	35	

Table 3: Comparison Of 2 Methods in Blood Group Typing in Dentin

METHOD (n=20)	GROUP I						GROUP II						GROUP III						Pearson Chi Square
	Positive		Negative		False positive		positive		negative		False positive		positive		negative		False positive		
	n	%	n	%	n	%	n	%	N	%	n	%	N	%	n	%	n	%	P < 0.05
AE	11	55	8	40	1	5	9	45	7	35	4	20	4	20	11	55	5	25	
AI	4	20	11	55	5	25	1	5	13	65	6	30	1	5	13	65	6	30	

using the Pearson chi-square test, and the p-value was found to less than 0.05, which indicates a statistical significance between pulp and dentin. Overall, the percentage of positivity was observed more in pulp when compared to dentin (Table 1).

When comparing the two methods (A-E and A-I) in pulp, by A-E method, group I gave 90 % of positive results, and in group II & III, it showed 75% showed positive results. In A-I method, group I showed 45 % positive results, and group II showed 20% of positive results and group III showed 0% of positive results. Statistical analysis was done using Pearson chi-square test, and the p-value was found to less than 0.05, which indicates a statistical significance among the two methods in pulp (A- E & A-I) (Table 2).

When comparing the two methods (A-E and A-I) in dentin, by using A-E method group I, gave 55 % of positive results, and group II showed 9% showed positive results, and group III showed 4% positive results. Using the A-I method, group I showed 20 % positive results, group II showed 5% positive results, and group III showed 5% positive results. Statistical analysis was done using Pearson chi-square test, and the p-value was found to less than 0.05, which indicates a statistical significance among the two methods in dentin (A- E & A-I) (Table 3).

Discussion

Forensic odontology is an important part of forensic science because the peculiarities of the dental tissues were preserved even when exposed to extreme heat and cold temperatures or in water or subjected to dirt and fire.[11]

The role of blood group antigens in forensic identification is based on the fact that the blood group of an individual is well established once in a lifespan, and it remains constant throughout their life. Even though many improvements were being done in "DNA" technology, "Blood group" determination is still considered to be an important tool in forensic identification.[12] An Austrian-American physician Dr. Karl Land Steiner in 1900 described ABO blood grouping. ABO type depends upon the presence of A and B genes which are present on chromosome 9.[10] There are three standard methods for determining blood grouping in the forensic investigation which includes Absorption elution, Absorption inhibition and Mixed agglutination.

In this study, sixty teeth were collected, and samples were divided into three groups containing 20 samples in each and blood grouping was determined by Absorption elution and Absorption inhibition techniques. In group I, samples are examined without any storage medium; in group II, samples are stored for one month in seawater and in group III, samples are stored for two months in seawater.

Many have studied blood group determination by Absorption elution technique in pulp & dentin in freshly extracted teeth or from teeth stored in a different medium with varying time periods. By searching search engines like Pubmed and Google, no other studies were available to which evaluated blood group antigen from dentin and pulp by using absorption elution technique from teeth stored in seawater to compare the present study results. Therefore, our results pertaining to fresh teeth without storage medium (Group I) can be compared directly, but the other two groups (II & III) (one month and two-month storage in seawater) can be compared indirectly only with those studies which had a storage medium whose propery mimics similar to sea water like saline. In our study, by using the Absorption Elution method, pulp showed 90% of positivity in group I (without storage medium). Present study results were similar to the original studies done by Aswanth et al (2012),[13] Iftikhar F et al,[14] Dil F et al (2018),[15] Prabhawati I et al (2011),[7] Kumar PV et al (2016)[16] which showed positive result from 80% to 100 % in pulp from freshly extracted teeth by using A-E technique in freshly extracted teeth.

By using the Absorption Elution method, dentin showed 55% of positivity in group I (without storage medium) in the present study. Our results were similar to the results of the original studies done by Sunitha. S et al (2017)[5] and Vala et al (2017),[17] which showed 60% to 70% of positive results in dentin. However, our results were not in accordance with the study done by Kumar PV et al (2016),[16] which showed 100% of positive results. The difference may be because of a high degree of mineralisation which may be difficult to obtain blood group antigens in dentin.

In this present study by using A-E method, pulp showed 75% of positivity for group II (1-month storage in seawater) and group III (2 months storage in seawater). In a study done by Nayar et al (2017),[18] Chandan D. N et al (2018),[19] blood group was determined from the pulp by the A-E method. They stored teeth in saline water. Pulp showed 74% to 65% of positivity in teeth stored in saline water. Their results are similar to the present study, which gave 75% of positivity in teeth stored for 1 and 2 months in seawater.

In this present study, by using the A-E method, dentin showed 45% of positivity for group II (1-month storage in seawater), and group III showed 20% positive results. Using the Keywords: Blood group antigen, dentin, absorption elution technique, seawater in Google and Pubmed search, no published study was available to evaluate blood group antigen from dentin by using absorption elution technique from teeth stored in seawater to compare the results of the present study.

In this present study by using Absorption- Inhibition, pulp showed 45% positive results in group I (without storage medium), 40 % of positive results in group II (1 month in seawater), 15% of positive results in group III (2 months in seawater storage). Dentin showed 20% of positivity in group I, 5% of positivity in group II and 5% positivity in group III. No published study was available to evaluate blood group antigen from dentin and pulp by using absorption inhibition technique from teeth stored in seawater to compare the results of the present study.

In the present study comparison of two techniques were done in teeth with seawater as a storage medium. The A-E method showed 80% of positive results in the pulp, but A –I method showed 21.7% of positive results. The A-E method showed 47% of positive result in dentin, and A-I method showed a 5% positive result and it shows that the absorption elution method is better for blood group determination in both pulp and dentin when compared to absorption inhibition. By searching the search engines in PubMed and Google, no other studies were available to correlate with the present results related to determining blood grouping from both absorption elution and absorption inhibition technique in pulp and dentin with seawater as a storage medium.

Conclusion

From the observations of this study, pulp is more reliable than dentin even in sea water. The percentage of positivity decreases as the time period in storage medium increases in both pulp and dentin and Absorption elution is most effective method than Absorption inhibition in blood group identification in teeth. Limitation of the study includes smaller sample size and awaits corroboration from similar studies in near future.

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