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ABO BLOOD GROUPING FROM DENTIN AND PULP OF FRESH AND AGED TEETH BY MODIFIED ABSORPTION–ELUTION TECHNIQUE

Shimaa M. Motawei*, Rehab Rizk El-Zehary** *and* Ahmed Shteiwi ***

ABSTRACT

Background : Teeth are one of the hardest, most stable and most durable structures in the body. They are also easily accessible for examination. Therefore, teeth are a first-rate material for forensic investigations.

Objectives: This study was carried out to test the possibility of detection of ABO blood groups through examination of the pulp of teeth as a soft tissue and the dentin as a hard tissue, and also to evaluate the reliability of teeth stored for a relatively long period of time as a source for blood group identification by absorption–elution technique with some modifications.

Materials & Methods: Fifty-two sound human teeth were examined for the ABO blood groups in both dentin and pulp tissues for both fresh and aged teeth, and results were compared with the blood groups obtained by testing a blood sample of the same individual using the simple slide test. Teeth were split using a new *Hand-held pulp Isolator* instrument.

Results: The teeth pulp showed a very highly positive correlation in both fresh and aged teeth though it decreased slightly in the latter. Dentin showed a positive correlation in both fresh and aged teeth groups indicating that the dentin as the hard tissue of the teeth is quite reliable to detect blood groups. Effects of the age, sex, and jaw distribution on the blood grouping from the teeth were also carried out.

Conclusion: The hard and soft tissues of teeth are reliable sources for blood group determination and help in human identification. Sensitivity of the ABO antigens detection in dental material is higher in the dental pulp than the dental hard tissue, and it decreases with the lapse of time. Modified absorption elution method is accurate in that respect. Introduction of the new Hand-held pulp Isolator instrument provides more teeth material to be tested, hence more accurate.

KEY WORDS: Blood grouping, Teeth, Forensic Identification, Absorption–elution, Dentin, Pulp.

* Lecturer of Forensic Medicine & Clinical Toxicology, Faculty of Medicine, Mansoura University, Egypt.
** Assistant Professor of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt.
*** Dentist in the Ministry of Health and Population, Egypt.

INTRODUCTION

Blood grouping is a main step in identification of biological materials. The term "Blood groups" refers to inherited antigens on the red blood cell surface detected by specific antibodies. The use of blood grouping in medico-legal investigations is based on the fact that once the blood group and the Rhesus factor are established; they remain unchanged throughout life [1]. The ABO blood groups system was first described by Karl Landsteiner in 1900 and remained the mainstay of forensic blood groups investigations, being the primary, common, obvious, and easily detectable marker [2].

Although advances have been made in Deoxyribonucleic acid (DNA) analysis, fingerprinting, etc., blood grouping still has a major role in the forensic practice in the field of personal identification, paternity disputes, and other scenarios. ABO blood grouping is examined in suspects of criminal cases and paternity disputes before DNA profiling. This is attributed to the fact that genetic and antigenic constituents of an individual remain not affected by environmental conditions [3].

Blood group antigens are detected in many body fluids including saliva. Blood grouping from dried stains by elution procedures was described more than 70 years ago but was not employed widely in Forensic Serology until 1960, when Kind refined the technique. The absorption elution (AE) procedure; originally described by Siracusa, is now used by all forensic laboratories because it has proved to be more sensitive, reliable and reproducible than the elution procedures alone [4].

Forensic Odontology has established itself as a major and indispensible science in medicolegal practice. Teeth have well-known forensic importance because they are the hardest of all human tissues, they are stable chemically and their characteristics are maintained for a long time after death even in the most adverse environmental conditions. The presence of blood group substances and other genetic markers such as enzymes in soft and hard dental tissues makes teeth a possible tool to help in the identification of highly decomposed bodies [5].

Dentin is the part of the tooth situated beneath the enamel and the cementum and surrounds the pulp cavity. Dentin is harder than bone and forms the major portion of the tooth. Dentin has higher ratio of cells to matrix than bone and it is easier to obtain blood elements from it than from bone. It is assumed that blood group substances in dentin are located in dentinal tubules. The existence of blood group antigens in the tooth dentin and enamel has been substantiated by infusion and sedimentation phenomena combined with inherently present antigens. This theory describes the infusion of water-soluble antigens from saliva into the tooth tissue [6].

The pulp tissue is one of the most protected tissues being surrounded from all sides by dental hard tissues. The pulp contains numerous blood vessels; hence blood group antigens are certainly present in teeth pulp [7].

The pulp tissue being contained within the dental hard tissues makes it protected and post-mortem degradation is seen very late in it. Therefore, it can be examined postmortem even in adverse environmental conditions [8].

Regarding the dentin, the diffusion of ABO substances from the pulpal cavity wall to the dentin edge and to the enamel gradually decreases with age due to increased calcification and less diffusion of antigens from the blood and saliva to the dental hard material [6].

The possibility of loss of antigens secondary to autolysis and dehydration or the presence of foreign antigens secondary to bacterial action in carious teeth warrants the accuracy of teeth as a tool for blood grouping in forensic practice [6].

This study was carried out to test the accuracy of detection of ABO blood groups from the pulp and the dentin of fresh and aged teeth, using a new non-

heat instrument for teeth splitting *'The Hand-held pulp Isolator'* and a modified absorption elution technique for blood groups antigens detection.

MATERIALS AND METHODS

Fifty-two sound human permanent teeth were collected from patients who underwent teeth extraction for the purpose of orthodontic treatment or due to poor periodontal status. The age range of patients was 14-60 years old. Twenty-six teeth were tested fresh and the other 26 teeth were preserved for six to twelve months and then examined.

Carious teeth were excluded because of the possibility of showing false positive results. Also, grossly abraded teeth were excluded. The age, sex and jaw distribution of the teeth included in the study are shown in table (1).

Written consents, history of a dental treatment and relevant medical history were obtained from patients selected for the study. The patients were examined under artificial illumination and extraction procedures were done under local anesthesia and complete aseptic conditions.

After the extraction, the socket was compressed with sterile gauze piece and 0.5 ml of blood was collected from the site under aseptic condition, stored in labeled Ethylene Diamine Tetra Acetic Acid (EDTA) bottles and tested for ABO blood grouping using the forward grouping slide method at room temperature. The determined blood groups were tabulated and used as the gold standard. Extracted teeth were washed under running water to remove debris, blood and saliva, and then they were dried with gauze and placed in labeled plastic containers until used in a dry state.

The collected teeth were divided into two equal groups; each of 26 teeth. Group I in which teeth were analyzed fresh, and Group II where teeth were stored in labeled containers for a period of 6-12 months and then analyzed.

From each tooth, the pulp was extirpated and the remaining tooth structure was pulverized. The tooth was split longitudinally by the *Hand-held Pulp Isolator* (*Shteiwi, 2015. A device, patent pending No. PCT/ EG2015 / 000026*) designed to split teeth without heat generation) (Figure 1).

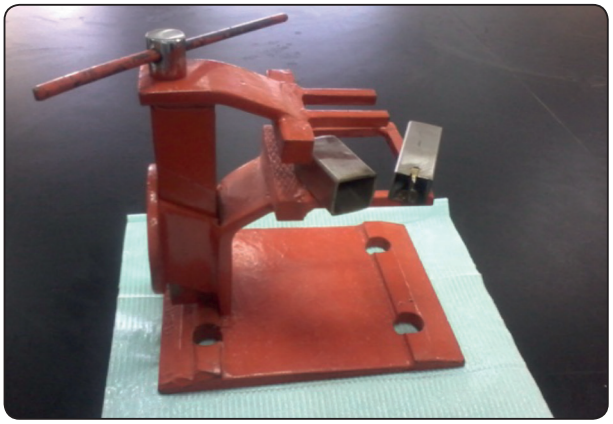

Fig. (1): Three-Dimensional Design showing the Hand-Held Pulp Isolator "first edition" (Shteiwi. A device, patent pending No. PCT/ EG2015 / 000026).

This part of the work was done in the MERC (Mansoura Experimental Research Center) in Mansoura Faculty of Medicine. A drop of saline was put into the pulp chamber to wet the pulp, which was extirpated using dental excavator (Figure 2).

The remaining part of the tooth was pulverized

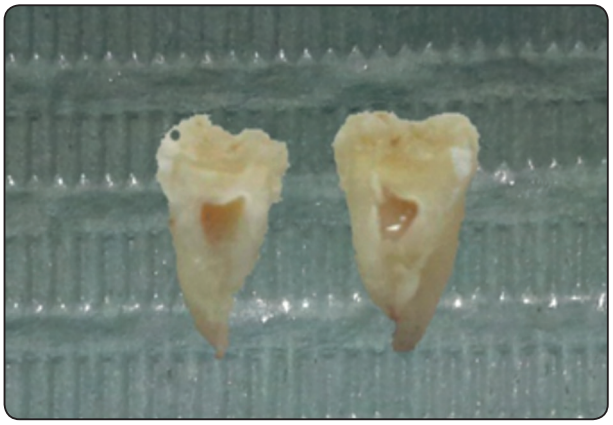

Fig. (2): A tooth splitted longitudinally and wetted by adding saline into the pulp chamber.

with special mortar and pestle to get a very fine dentin powder (Figure 3). The extirpated pulp and dentin powder were stored in sterile labeled recipients. In a blind study, pulp and pulverized dentin powder were then subjected to AE test.

The pulp and the pulverized tooth powder were divided each into two equal amounts, and were put in sterile labeled test tubes, to each of these test tubes; 3 drops of anti-sera A and B were added. The test tubes were plugged with cotton and were placed at 4°C overnight to allow absorption of anti-sera.

After washing away of unbound serum by washing each sample for 6 times with 10 ml ice cold saline solution, the samples were placed at 4°C, then washed at the same temperature to increase agglutination intensity [8].

Then, the samples were centrifuged for 5 min. at 4000 rpm to remove the excess saline. The combined antibody with the specific antigen is eluted at 56° by placing the test samples in a pre-heated water bath at 56°C for 10 min. The elute was then tested with the indicator red blood cells of the appropriate group by mixing the supernatant of the centrifuged samples at 1500- 2000 rpm for 1- 2 min., with a drop of 0.5% A and B red blood cell suspensions. The mix was gently shaken and incubated at 37°C for 30 min. to enhance agglutination. After that, one drop of the solution was placed on a microscopic slide, covered with a cover slip, and agglutination was observed under the microscope at a magnification of ×10 and ×40 (Figure 4).

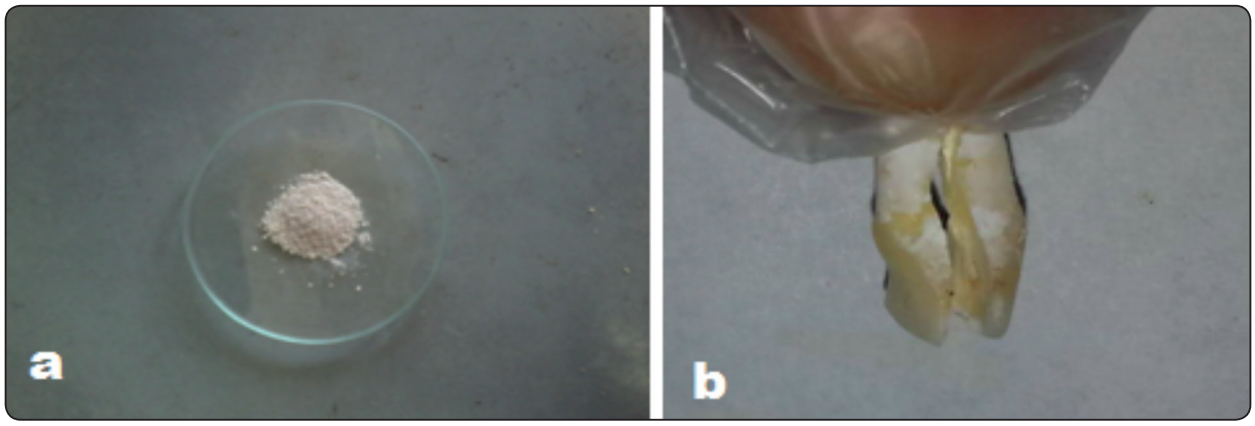

Fig. (3): Pulverized dentin (a), Dried pulp tissue (b).

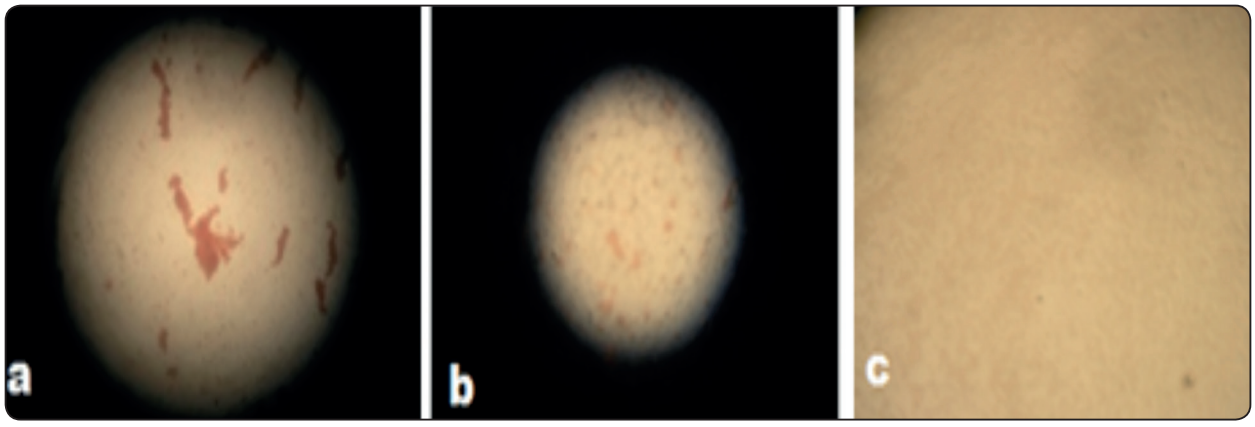

Fig. (4): Photomicrograph of the tooth extract (×10) (a) & (×40) (b) showing agglutination of RBCs (Red blood cells) & (×40) (c) showing non-agglutination of RBCs.

The data obtained were tabulated, coded, and then analyzed using the computer program SPSS (Statistical Package for Social Sciences) version 20.0 for Windows® (SPSS Inc., Chicago, IL, USA). Quantitative data was presented as numbers and percents. Comparison between groups was done using Chi-square test. Correlation between variables was tested using Spearman correlation coefficient.

RESULTS

Table (1) shows the age, sex and jaw distribution of the 52 teeth used in the study. Tables (2, 3, 4 & 5) show the performance of dentin and pulp as compared to the reference blood groups and in relation to the different factors of the age, the sex, and the jaw distribution of the teeth in the two studied groups. The teeth pulp showed 100% correlation to the reference blood groups in the age ranges 14-20 and 21-30 years in the group I where teeth were analyzed fresh, and lower accuracies in older age groups, which were 80% in 31-40 years subjects and 66.67% in 41-50 years subjects. However, dentin showed little lower accuracies to the reference blood groups. It was 87.5% accuracy in the age range 14-20 years, 90% accuracy in 21-30 years old age group, 80% and 66.67% in the age groups of 31-40 and 41-50 years, respectively. Aged teeth showed less accuracy to the reference blood groups in both the pulp and the dentin. The pulp performance in different age groups showed a significant difference as compared to the reference blood groups in groups I (P= 0.03) and non-significant difference in group II (P= 0.19). Regarding dentin performance in different age groups, it showed non-significant differences in group I and significant differences in group II (0.60 and 0.03, respectively).

There was no significant differences between males and females in the accuracy of both the pulp and dentin as compared to the reference blood groups in both groups of fresh and aged teeth (P= 0.90, 0.21, 0.91, 0.97 respectively).

Also, there were no significant differences in performance of the pulp and dentin in relation to the jaw distribution of teeth in both groups (Table 5).

There was found no mistyping, hence, a strong positive correlation between the pulp, the dentin and the whole teeth blood groups versus the reference blood groups in both fresh and aged teeth (Table 6).

TABLE (1) Age, Sex, and Jaw Distribution of Teeth of the two groups

	Age in years					Sex Distribution		Jaw Distribution	
	14-20	21-30	31-40	41-50	51-60	Males	Females	Maxillary	Mandibular
Group I (n=26)	8	10	5	3	-	12	14	9	17
Group II (n=26)	11	6	3	4	2	11	15	11	15

Group I: Fresh teeth, Group II: Aged teeth (preserved for 6-12 months), n: number.

TABLE (2) Performance of the Dentin and the Pulp to Reference Blood Groups in the Study groups

	Total	Group I (n.=26)						Total	Group II (n.=26)					
		Pulp			Dentin				Pulp		Dentin			
		Positive %	Negative %	P-value	Positive %	Negative %	P-value		Positive %	Negative %	P-value	Positive %	Negative %	P-value
Reference ABO Blood group	26	24 (92.31%)	2 (7.69%)	0.94	22 (84.62%)	4 (15.38%)	0.84	26	21 (80.77%)	5 (19.23%)	0.51	19 (73.1%)	7 (26.92%)	0.08
A	11	10	1		9	2		10	7	3		8	2	
B	3	3	0		3	0		2	2	0		0	2	
AB	1	1	0		1	0		3	2	1		3	0	
O	11	10	1		9	2		11	10	1		8	3	

Group I: Fresh teeth, Group II: Aged teeth (preserved for 6-12 months), n: number, %; percent. P-value is considered significant at ≤ 0.05

TABLE (3) Performance of the Dentin and the Pulp to Reference Blood Groups in relation to Age

		Group I (n.=26)						Group II (n=26)						
		Pulp			Dentin			Pulp				Dentin		
Age (years)	N.	Positive %	Negative %	P-value	Positive %	Negative %	P-value	N.	Positive %	Negative %	P-value	Positive%	Negative %	P-value
14- ≤20	8	8 (100%)	0 (0.00%)	0.03	7 (87.5%)	1 (12.5%)	0.60	11	10 (90.9%)	1 (9.01%)	0.19	9 (81.82%)	2 (18.18%)	0.03
>20- ≤30	10	10 (100%)	0 (0.00%)		9 (90%)	1 (10.0%)		6	6 (100%)	0 (0.00%)		6 (100%)	0 (0.00%)	
>30- ≤40	5	4 (80.0%)	1 (20.0%)		4 (80%)	1 (20.0%)		3	2 (66.67%)	1 (33.33%)		1 (33.33%)	2 (66.67%)	
>40- ≤50	3	2 (66.67%)	1 (33.33%)		2 (66.67%)	1 (33.33%)		4	2 (50%)	2 (50.0%)		2 (50.0%)	2 (50.0%)	
>50- ≤60	-	-	-		-	-		2	1 (50%)	1(50.0%)		1 (50.0%)	1 (50.0%)	

Group I: Fresh teeth, Group II: Aged teeth (preserved for 6-12 months), N.: Number, %; Percent. P-value is considered significant at ≤ 0.05

TABLE (4) Performance of the Dentin and the Pulp to Reference Blood Groups in Males and Females

Sex	Total	Group I (n.=26)						Total	Group II (n.=26)					
		Pulp			Dentin				Pulp			Dentin		
		Positive %	Negative %	P-value	Positive %	Negative %	P-value		Positive %	Negative %	P-value	Positive %	Negative %	P-value
Male	12	11 (91.67%)	1 (8.33%)	0.90	10 (83.33%)	2 (16.67%)	0.21	11	9 (81.82%)	2 (18.18%)	0.91	8 (72.73%)	3 (27.27%)	0.97
Female	14	13 (92.86%)	1 (7.14%)		12 (85.71%)	2 (14.29%)		15	12 (80.0%)	3 (20.0%)		11 (73.33%)	4 (26.67%)	

Group I: Fresh teeth, Group II: Aged teeth (preserved for 6-12 months), n.: number, %; Percent. P-value is considered significant at ≤ 0.05

TABLE (5) Performance of the Dentin and the Pulp to Reference Blood Groups in relation to the Jaw Distribution of Teeth.

Jaw distribution	N.	Group I (n.=26)						N.	Group II (n.=26)					
		Pulp			Dentin				Pulp			Dentin		
		Positive %	Negative %	P-value	Positive %	Negative %	P-value		Positive %	Negative %	P-value	Positive %	Negative %	P-value
Maxillary	9	8 (88.89%)	1 (11.11%)	0.63	7 (77.78%)	2 (22.22%)	0.43	11	10 (90.91%)	1 (9.09%)	0.26	9 (81.82%)	2 (18.18%)	0.39
Mandibular	17	16 (94.12%)	1 (5.88%)		15 (88.24%)	2 (11.76%)		15	11 (73.33%)	4 (26.67%)		10 (66.67%)	5 (33.33%)	

Group I: Fresh teeth, Group II: Aged teeth (preserved for 6-12 months), N.: Number, %; Percent. P-value is considered significant at ≤ 0.05

TABLE (6) Correlation between the Pulp and the Dentin with Reference Blood Groups

Parameter	N. of positive results	N. of negative results	Coefficient correlation*
Group I (n=26)			
Pulp versus reference Blood Group	24	2	1.00
Dentin versus reference Blood Group	22	4	1.00
Pulp versus dentin	20	6	1.00
Whole teeth versus reference Blood Group	20	6	1.00
Group II (n=26)			
Pulp versus reference Blood Group	21	5	1.00
Dentin versus reference Blood Group	19	7	1.00
Pulp versus dentin	15	11	1.00
Whole teeth versus reference Blood Group	15	11	1.00

Group I: Fresh teeth, Group II: Aged teeth (preserved for 6-12 months), N.: Number. *Spearman correlation coefficient showing high positive correlation.

DISCUSSION

Human identification is a corner stone in forensic practice. Blood groups identification becomes difficult in putrefied bodies. Teeth provide a useful and an easily accessible tool in that respect [7].

In this study, we investigated the possibility of detecting the blood groups antigens in the hard dental material and the pulp obtained by splitting the teeth without heat using a new *Hand-Held Pulp Isolator* instrument and using a modified absorption elution technique. We studied our findings in relation to the age, sex, jaw distribution and time period since the teeth were extracted (Table 1).

The overall sensitivity of the pulp for ABO blood groups detection was 92.31% for fresh teeth and 80.77% for aged teeth, and that of the dentin was 84.62% in fresh teeth and 73.1% for aged teeth (Table 2).

Our results do not agree with **Ballal and David (2011)** [9] who investigated 30 teeth extracted within 6 months, for blood groups typing and stated that blood grouping from dentin was found to be not correlating with the reference blood groups, and that from the pulp was 90% correlating with the reference blood groups.

The investigators; **Ballal and David (2011)** [9], explained that the blood group substances may not be accessible in dentin material owing to a high degree of calcification. The same was believed by **Kramer (1957)** [10] and **Karzun (1978)** [11]**.**

This means that an advance has happened in methodologies and techniques that enabled using teeth as a tool for personal identification and that AE and modified AE methods have increased the sensitivity; allowing more of blood group antigens in teeth material to be detected. This was confirmed by many studies [1, 12, 13, 14, 15].

Introduction of more robust techniques has increased the accuracy of blood groups antigens detection from teeth materials. It was about a decade when absorption inhibition technique was replaced by absorption elution and then modifying the absorption elution technique to render it more accurate has increased its reproducibility in blood typing from teeth [8].

In our study, we split the tooth vertically with the *Hand-held pulp isolator* that had allowed extirpation of the pulp in one mass, which means maximum collection and minimal loss of the pulp material. Then, pulverization of the hard dentin material allows overcoming calcification that may prevent accessibility to any possible antigens and increases the surface area allowing giving more teeth material to be tested. Then we did the absorption, elution and agglutination steps. This increases the sensitivity of detection when compared to the ordinary AE and other older agglutination techniques [6].

The principle of AE test is the absorption of blood group specific agglutinin to the surface of a substance having blood group agglutinogens, then, elution of the absorbed antibody under a high temperature, and the agglutination of the blood cells against the corresponding antigens. Cold agglutination increases the agglutination intensity [7].

Also, use of BSA 10% (Bovine serum albumin) instead of physiological saline as a medium to carry out the elution is said to induce hemagglutination and to block the non-specific binding of antibodies two to eight times more than saline, due to the action of albumin. Centrifugation of the dentin powder after the elution of the antibodies and before the addition of the red cell suspension allows obtaining agglutinogens in a supernatant without the tooth powder that interferes with reading of agglutination results [16].

Moreover, aerobic gram negative bacteria in oral flora grow more in stored specimens or putrefying material and are believed to simulate blood groups activity causing false-positive reaction, or to cause loss of cells and then false-negative reaction [17].

Our results agree with the results of **Ramnarayan et al. (2013)** [16] who used AE technique and found 100% sensitivity of blood grouping from pulp at younger age groups and fresh teeth than the older age groups and stored teeth. They found the teeth pulp more sensitive than the dentin in blood groups testing and that males showed more correlated results to the reference blood groups than females. However, in our study, we did not find significant differences between males and females for both fresh and aged teeth groups. Also, the former investigators found no difference between maxillary and mandibular teeth except in aged teeth where maxillary teeth were more accurate in ABO antigens detection. In our study, the jaw distribution of teeth did not give any significant differences in accuracy of ABO antigens detection in both groups.

We found that most of teeth that showed negative results in pulp results were teeth in the age group of 40 years and above. The negative results of the pulp for blood grouping can be attributed to insufficient quantity of pulp, loss of the pulp tissue with increasing age, or due to increased calcification of the pulp canal [18].

In this study, the negative results of the pulp were 7.69% in fresh teeth and 19.23% in aged teeth. The negative results of the pulp for blood grouping can be attributed to insufficient quantity of the pulp, fibrosis and increased calcification of the pulp canal with increased age [18].

Ballal and David (2011) [9] tested ABO typing from fresh teeth using AE and found a sensitivity of 90% for the pulp. **Saxena et al. (2017)** [7] found 80% and **Ramnarayan et al. (2013)** [16] found 83.3% sensitivity of the pulp in both fresh and long-standing teeth.

The higher sensitivity found in this study may be attributed to a higher amount of the pulp material available for testing, which indicates that cutting the teeth by the new instrument '*Hand-held pulp isolator*' gives more amount of the pulp material available for testing, thus gives more accurate test results.

Sensitivity of the pulp for blood grouping was found to decrease with the increased age (P= 0.03) in the fresh teeth group. This agrees with **Ramnarayan et al. (2013)** [16], **Parekh et al. (1994)** [14] and **Lele (1970)** [19] and does not agree

with **Garg and Garg (1989)** [20] who found in his investigation of blood typing from teeth that age did not affect the pulp sensitivity.

There was found insignificant differences between males and females in pulp sensitivity for blood grouping (P=0.90).

This agrees with **Garg and Garg (1989)** [20], **Parekh et al. (1994)** [14] and **Saxena et al. (2017)** [7] and does not agree with **Ramnarayan et al. (2013)** [16] and **Lele (1970)** [19] who found males showing more positive results than females. They explained that by the larger size of teeth; hence more pulp material for testing, in males than in females.

Pulp sensitivity in fresh teeth (92.31%) was more than aged teeth (80.77%). This agrees with **Kumar et al. (2016)** [6] who stated that pulp sensitivity decreased in stored teeth for 12 months than fresh teeth. **Amit et al. (2017)** [21] agrees that teeth remain reliable for blood grouping even after 6 weeks and adverse environmental conditions (e.g. immersed teeth under water).

Regarding the dentin, it showed a sensitivity of 84.62% in fresh teeth and 73.1% in aged teeth. **Ballal and David (2011)** [9] did not find any positive ABO typing from the dentin and **Ramnarayan et al. (2013)** [16] found 80% and 76.7% in fresh and aged teeth, respectively.

Ballal and David (2011) [9] believed in **Kramer's (1957)** [10] opinion that the dental hard tissue cannot inform about blood grouping, because of inaccessibility of blood group substances in the dentin due to a high degree of calcification. They believed also in **Karzun's (1978)** [11] opinion who also believed that ABO blood grouping in hard dental tissue is unreliable. They said that the diffusion of ABH substances from the pulp cavity and from saliva to the dentin edge is insufficient to detect the ABH antigens in the dentin material. Those investigators used AE and mixed agglutination techniques in their studies.

Our investigation and many others succeeded to detect blood groups from dental hard tissue **(Ramnarayan et al., 2013** [16], **Kumar, 2016** [6], **Saxena et al., 2017**[7] **and Amit et al., 2017)** [21] this means that the advance in techniques and theories led to a subsequent advance in the forensic dental practice.

The negative results in dentin may be due to a failure of the technique, contamination of the sample, cell lysis, or lower amounts of blood groups antigens in the dental hard tissue [6]. This study is thus an addition to Forensic Odontology and how much more needs to be learned in this challenging branch of forensic sciences.

Teeth as a whole showed a sensitivity of 76.92% and 57.69% for blood group determination in Groups I and II, respectively. This is much higher when compared with the study by **Smeets et al. (1991)** [5]. This is probably due to the use of 10% BSA instead of saline as an elution and agglutination medium. This is also higher than the results obtained by **Saxena et al. (2017)** [7] and **Ramnarayan et al., (2013)** [16], who found accuracy of 73%, 66%, 73.3% and 66.6%, respectively.

CONCLUSION

Teeth revealed to be a helpful, inexpensive tool in blood groups identification, even after lapse of time. However, accuracy of blood groups typing decreases in aged teeth than in fresh teeth. The pulp showed higher accuracy than the dentin in this respect. The new *Hand-held pulp isolator* instrument showed a higher efficacy in isolation of the maximum amount of the pulp without generation of heat, hence more test accuracy. It is recommended to do the investigation on postmortem teeth samples as the contamination by plants and animals, and bacteria and fungi actions in putrefying materials, bring many blood group-like antigens that can lead to blood groups mistyping due to acquired antigen activity.

List of abbreviations

AE; Absorption elution, BSA; Bovine serum albumin, °C; degree centigrade, Inc.; incorporation, min.; minute, ml; milliliter, RBC; red blood cell, rpm; revolution per minute, %; per cent, ×10; times ten, ×40; times forty.

Conflicts of Interest

The authors of this manuscript declare that there are no conflicts of interests related to the concept of this work, its findings or any of its contents. There was no source of funding during preparation, analysis of data, editing or publishing of this work.

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