Original Article

Determination of ABO Blood Grouping from Dentine and Pulp by Absorption-elution Technique

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Abstract

Introduction: Blood grouping has been one of the cornerstones of identification of biological material. Mostly, teeth and bones are the only significant tissues remaining in mass disasters such as aircraft crash or, bomb blasts and hence, used in human identification. It has, also, been suggested that blood group antigens in the pulp and dentine are preserved even up to 2 years after the death of an individual. **Aim:** In the present study, an attempt was made to determine the ABO blood grouping from the dentine and pulp by absorption-elution (AE) technique. **Materials and Methods:** The study group included sixty patients requiring extraction due to periodontal or, orthodontic purposes. The extraction procedure was carried out under local anesthesia following an aseptic protocol. After extraction, the socket blood was collected for blood group determination which served as control for the study. The blood grouping was performed by AE technique using powdered dentine and dental pulp. **Statistical Analysis:** The statistical analysis was carried out using Statistical Package for Social Sciences version 19. The statistical analysis for comparison of teeth component with ABO blood groups with the age period and gender differentiation was done using Chi-square test. $P < 0.05$ was considered as statistically significant. **Results:** Out of sixty samples tested for ABO blood grouping, dentine and pulp showed no significant difference with age and gender; results were more positive in the age group in which individuals were <20 years of age with the sensitivity decreasing with increasing age of the individuals, while pulp was better than dentine in expressing ABO antigens. **Conclusion:** On the basis of the results obtained from the present study, it could be concluded that both dentine and pulp are reliable sources of blood group determination for ABO blood grouping where teeth happen to be the only remnants available for personal identification.

Keywords: *ABO blood grouping, absorption-elution technique, dentine and pulp*

Received: April, 2017.
Accepted: May, 2017.

Introduction

Forensic odontology is an investigative aspect of dentistry that analyses dental evidence for human identification. Identifiable information from oral structures is more than any other part of the body.[1] Blood grouping has been one of the cornerstones of identification of biological material. The term blood group is applied to inherited antigens detected on the red blood cell surface by specific antibodies.[2] The ABO blood group system, first described by Karl Landsteiner in the year 1900, remains the bulwark of forensic blood group investigation. The reasons for this are manifold. It is the primary, most common, conspicuous and easily detectable system.[2] Teeth can survive for a long time even after soft and skeletal tissues have been destroyed. Blood grouping from teeth could be a source of personal identification.[3] The use of blood group substance in the medicolegal examination is based on the fact that once a group is established in an individual, it remains

Access this article online

Quick Response Code:

Website: www.ijofb.org

DOI: 10.4103/ijofb.ijofb_4_17

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How to cite this article: Vala D, Nayyar AS, Pooja VK, Kartheeki B, Patel N, Vala D, *et al.* Determination of ABO blood grouping from dentine and pulp by absorption-elution technique. Int J Orofac Biol 2017;1:70-80.

unchanged throughout life.[3] Absorption-elution (AE) technique was devised by Siracusa in the year 1923 and has been refined by Kind who employed it almost exclusively for blood typing of teeth in forensic science laboratory.[3] It was thought to be of interest to apply the method to calcified tissue such as dentine. Dentine was chosen as it has a higher ratio of cell substance to matrix than bone and is easier to obtain blood.[4] The presence of ABO blood group from soft and hard dental tissues makes it possible to assist in human identification even in decomposed bodies. Mostly, teeth and bones are the only significant tissues remaining in mass disasters such as aircraft crash or, bomb blasts and hence, used in human identification.[4] Pulp tissue is one of the most protected tissues being surrounded from all sides by dental hard tissues. Postmortem changes in pulp are seen very late and also, pulp remains one of the most protected tissues and therefore, could be readily available for examination. Pulp contains numerous blood vessels and blood group antigens are certainly present in tooth pulp. Blood group substances are presumed to be present in dentinal tubules also.[3] In the dental pulp, vascular endothelium and red blood cells are regarded as a source of ABH antigenicity.[3] It has been suggested that blood group antigens in the pulp and dentine are preserved even up to 2 years after the death of an individual.[5] It is presumed that blood group substances in the dentine are located in the dentinal tubules, however, it is still a subject of controversyand furthermore, complete removal of the pulp components from the dentine sample was not confirmed.[6] The odontoblasts, which form a single layer lining the periphery of the pulp and have their processes extending into the dentinal tubules, would, also, have a cell component with the epitopes of the ABH blood groups although antigenic activities on the cells have not been demonstrated histologically.[7] The distribution of ABO blood group antigens from the pulp cavity wall to the dentine edge and to the enamel gradually decreases because of fewer possibilities of diffusion of the antigens from both the blood and saliva.[6] The existence of blood group antigens in tooth dentine and enamel and their nature has been substantiated by infusion-sedimentation phenomena combined with inherently present antigens. The infusion-sedimentation theory describes the infusion of water-soluble antigens from saliva into the tooth tissue.[8] Therefore, blood group determination for biological evidence on tooth material is of great importance in forensic odontology. Teeth are resistant to environmental assaults such as incineration, immersion, trauma, mutilation, and decomposition, therefore, teeth represent an excellent source in the identification of an individual. Blood grouping from teeth could be a significant source of identification.[4] However, there is a possibility of loss of the pulpal antigens due to autolysis and dehydration in long-standing tooth remains. Therefore, it was justifiable to study the blood group antigens of the dental pulp.[7] In the present study, an attempt was made to determine the ABO blood grouping from the dentine and pulp by AE technique in freshly cut tooth. The objectives of the study were to determine ABO blood group from the dental pulp and dentine in permanent teeth, to correlate the blood group with the blood collected from the respective extraction sockets and to compare and evaluate the sensitivity of dentine and pulp in determining blood group in an individual.

Materials and Methods

Source of data

Patients coming to the outpatient department of the institution for extraction of teeth due to periodontal or, orthodontic purposes were selected for the study.

Sample size

Sixty patients were selected randomly.

- Inclusion criteria
  1. Both male and female patients in the age group of 13–70 years
  2. Teeth which were extracted for periodontal or, orthodontic purposes; and
  3. Only permanent teeth
- Exclusion criteria
  1. Age groups below 13 years and above 70 years were excluded
  2. Carious teeth, root canal-treated teeth; grossly destructed teeth with exposed pulp cavity were excluded; and
  3. Deciduous teeth were excluded.

Materials

a. Gloves, face masks, surgical spirit, glass slides, gauze pieces, blood lancets, Anti-coagulant solution-EDTA and Anti-sera A and B; [Figure 1]
b. Lathe, carborundum disc, spoon excavator and straight fissure bur; [Figure 2]
c. Centrifuge; [Figure 3]
d. Hot water bath; [Figure 4]
e. Incubator; [Figure 5]
f. Glass slides, gauze pieces, blood lancets, micro-pipette, saline, EDTA and Anti-sera A and B added blood samples in test tubes, A and B Red Blood cell suspension; [Figure 6]

Study design

The study group included sixty patients requiring extraction due to periodontal or, orthodontic purposes. A brief case history with relevant medical history was recorded, and a detailed clinical examination under artificial illumination was done.

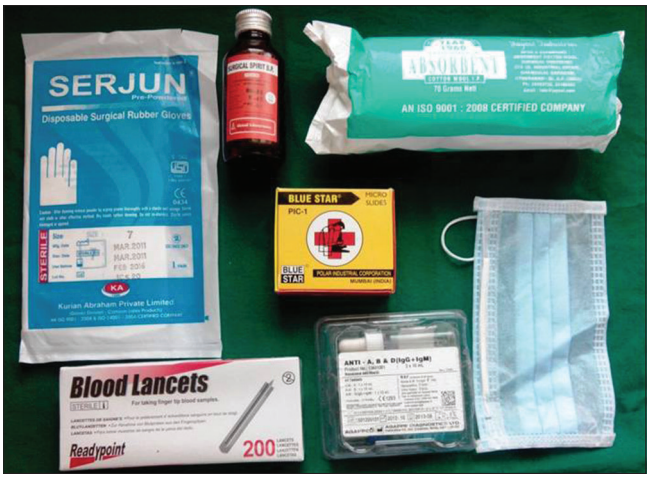


Figure 1: Materials used for blood grouping

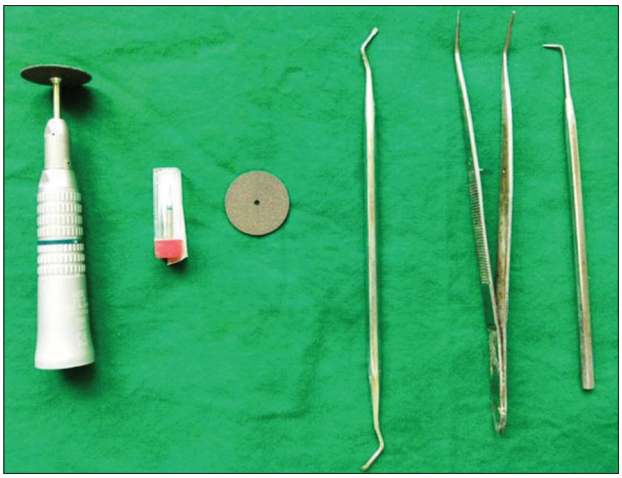


Figure 2: Equipment used to dissect and pulverize the tooth sample

Figure 3: Centrifuge machine

Figure 4: Samples in water bath for elution

Figure 5: Samples in incubator

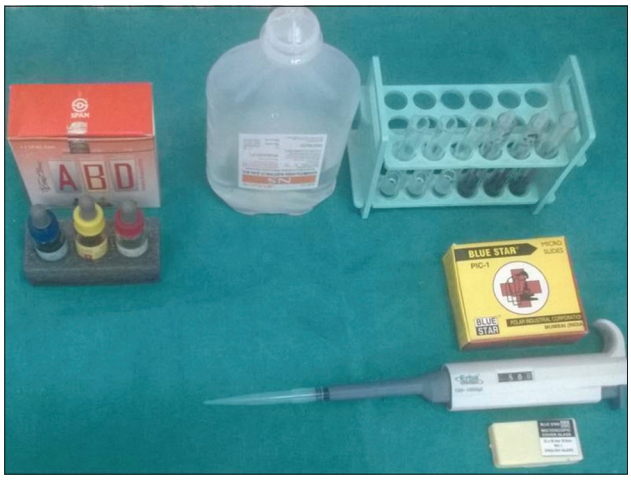


Figure 6: Materials used to detect blood groups

Extraction of teeth

The extraction procedure was carried out under local anesthesia following an aseptic protocol. After extraction, the socket was compressed with a sterile gauze piece, and socket blood was collected for blood group determination which served as control for the study.

Detection of blood group from the patient

Anti-coagulant (EDTA) was added to the socket blood, and one drop of this blood was placed on the slide, and a drop of anti-serum-A was added. If agglutination occurred on the addition of

anti-serum-A, blood group was recorded as an A blood group and vice-versa.

Storage of the teeth

The extracted teeth were washed under running water, and the debris was removed with the probe and wiped with gauze and kept in bottles which were numbered for identification.

Sectioning of the teeth: The teeth were sectioned into two halves using a micromotor with a carborundum disc [Figure 7]. The pulp was scooped out with a spoon excavator [Figure 8] and dentine was powdered using a straight fissure bur [Figure 9].

Red blood cell suspension

Blood samples belonging to A, B, and O were collected from three different patients, respectively. The samples were centrifuged and a suspension was obtained. To this suspension, 5 ml of saline was added.

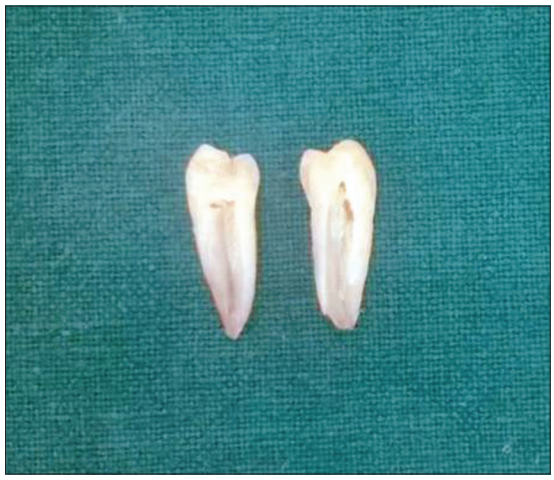


Figure 7: Dissected tooth samples

Figure 9: Pulverized dentine

Detection of blood group from the teeth

The blood grouping was performed by AE technique using powdered dentine and dental pulp. The tooth was completely trimmed to remove the enamel and cementum with lathe and further split vertically with carborundum disc, and the dental pulp scooped out with spoon excavator [Figures 7 and 8]. The remaining tooth consisting of dentine was pulverized with straight fissure bur [Figure 9]. The pulverized tooth powder and the pulp were kept in four test tubes with the label pulp with antisera A and pulp with antisera B and dentine with antisera A and dentine with antisera B [Figure 10]. To each of these test tubes, three drops of antisera A and B were added, and it was ensured that the test samples were being sufficiently soaked with the antisera for 2½ h and left standing at room temperature. After removing antisera, each sample was washed three times with cold saline solution and centrifuged at 3000 rpm for 5 min, and the supernatant was sucked with pipette. Then, two drops

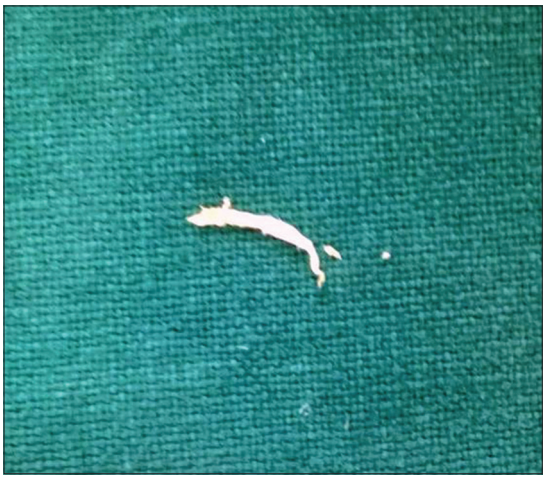


Figure 8: Excavated pulp tissue

Figure 10: Labeled test tubes and test tube rack

of the fresh saline were added to the sample, and the test tube was heated in a water bath at 50°C–55°C for 10 min to elute the antibodies. A drop of 0.5% red cell suspension of known blood group A, B, and O was freshly prepared and immediately put into the respective test tubes. The samples were incubated at 37°C for 30 min to enhance agglutination and then, were centrifuged at 1500–2000 rpm for 1 min. By gentle shaking of the test tubes, the presence or absence and grading of red cell agglutination was ascertained macroscopically [Figures 11 and 12] and then, at a magnification of ×400 microscopically for the pulp [Figure 13a and b] and dentine [Figure 14a and b] samples, respectively. The results obtained were compared with the control samples.

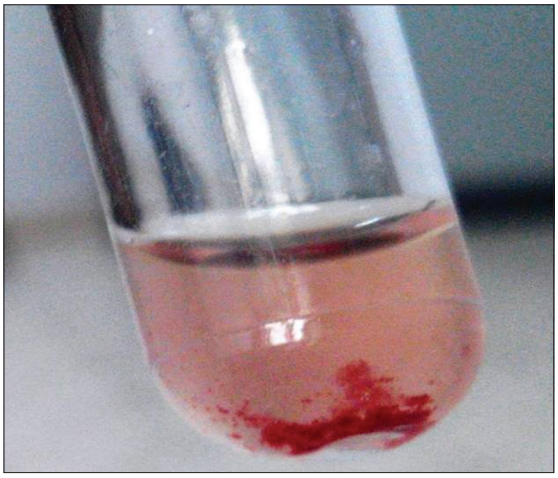


Figure 11: Macroscopic observation of agglutination in pulp samples

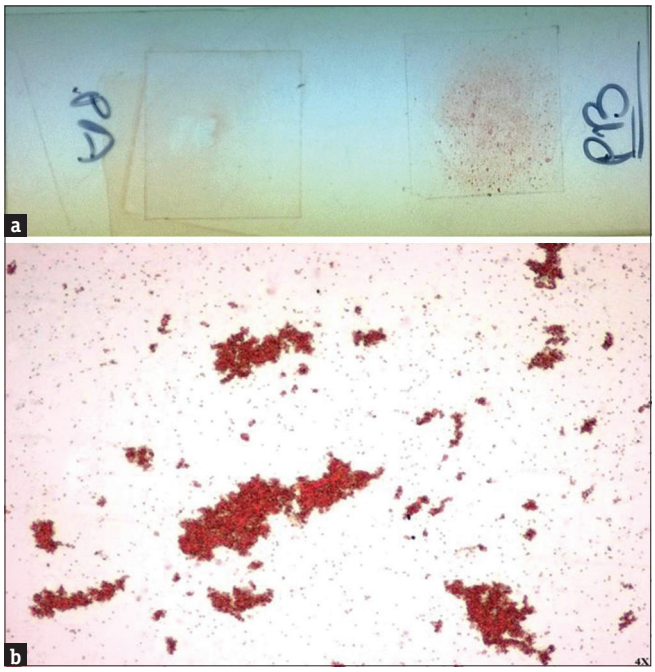


Figure 13: (a) Microscopic observation of agglutination in pulp samples, ×400. (b) Microscopic observation of agglutination in pulp samples, ×400

Statistical analysis

The statistical analysis was carried out using Statistical Package for Social Sciences version 19 (SPSS Inc., Chicago, USA). Descriptive statistics such as mean, standard deviation, and proportion were used. The statistical analysis for comparison of teeth component with ABO blood groups with the age period and gender differentiation was done using Chi-square test. $P < 0.05$ was considered as statistically significant and 0.0001 was considered highly significant.

Results

The study was conducted to identify blood groups from dentine and pulp. The study group included sixty patients, among whom 16 patients were <20 years of age, 11 were in between 21 and 40 years and 33 belonged to the age group of 41–60 years while 24 (40%) were females and 36 (60%) were males, requiring extraction due to periodontal or, orthodontic purposes [Graph 1].

Figure 12: Macroscopic observation of agglutination in dentine samples

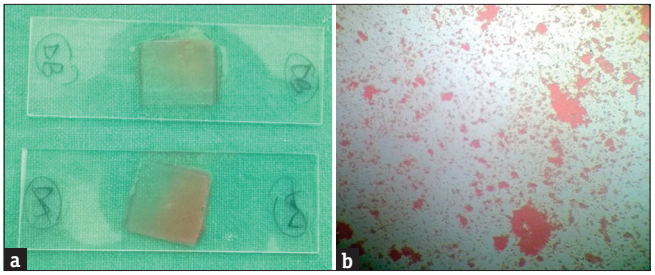


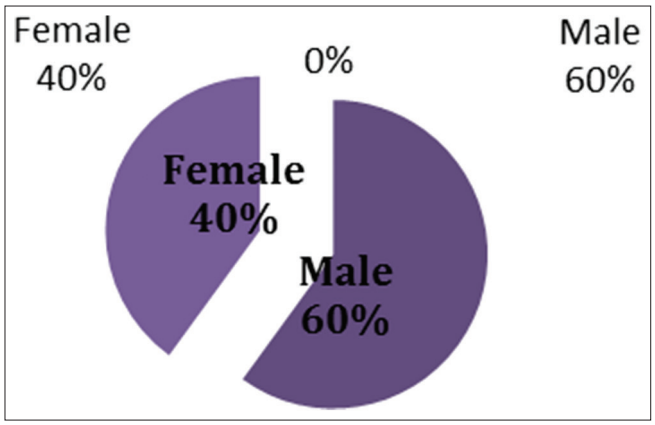
Figure 14: (a) Microscopic observation of agglutination in dentine samples, ×400. (b) Microscopic observation of agglutination in dentine samples, ×400

ABO blood grouping based on pulp

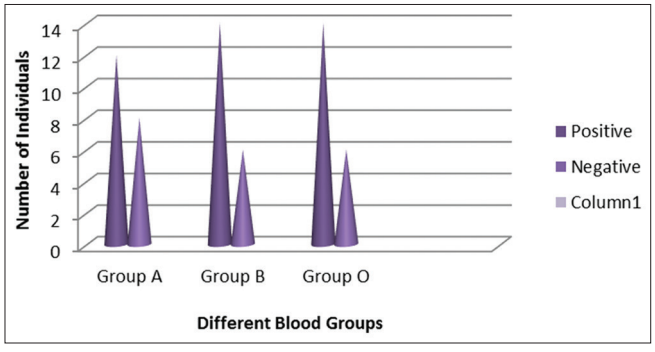
When ABO Blood Grouping was done based on pulp, 16 (80%) out of twenty samples were found to be positive for blood group A while 4 (20%) were found negative; 18 (90%) out of 20 samples were found to be positive for blood group B while 2 (10%) were found negative; 16 (80%) out of twenty samples were found to be positive for blood group O while 4 (20%) samples were found negative. Overall, 50 (83.3%) samples tested positive while 10 (16.6%) samples tested negative and the *P* value obtained was found to be statistically insignificant ($P = 0.619$) [Graph 2].

ABO blood grouping based on dentine

In the same way, when ABO blood grouping was done based on dentine, 12 (60%) out of twenty samples were found to be positive for blood group A while 8 (40%) were found negative; 14 (70%) out of twenty samples were found to be positive for blood group B while 6 (30%) were found negative; similarly, 14 (70%) out of twenty samples were found to be positive for blood group O while 6 (30%) samples were found negative. Overall, 40 (66.6%) samples tested positive while 20 (33.3%) samples tested negative and the *P* value obtained was found to be statistically insignificant ($P = 0.714$) [Graph 3].



Graph 1: Distribution of male and female patients



Graph 3: Determination of ABO blood group from the dentine

Age-wise distribution of blood group A from the pulp

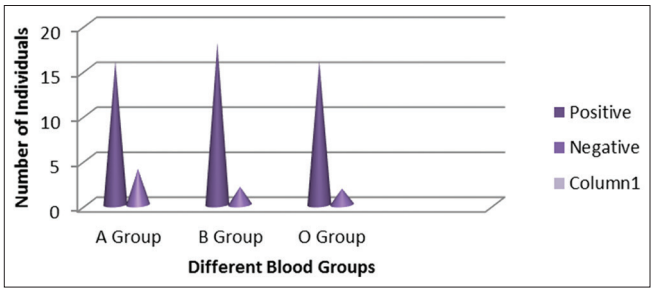
The pulp samples tested in the age group <20 years showed 4 (80%) positive results out of five samples while 1 (20%) sample tested negative. In 21–40 years of age group, 1 (100%) sample showed positive result out of one sample while in 41–70 years of age group, 11 (78.5%) samples showed positive results out of the 14 samples while 3 (21.4%) samples tested negative. *P* value obtained was 0.87 which was found to be statistically insignificant [Graph 4].

Age-wise distribution of blood group A from the dentine

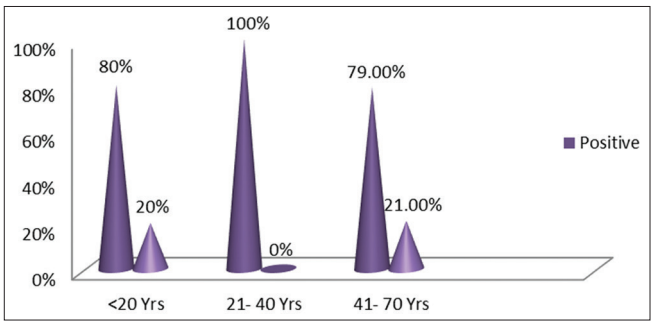
The dentine samples tested in the age group <20 years showed 4 (80%) positive results out of the five samples while 1 (20%) sample tested negative. In 21–40 years of age group, 1 (100%) sample showed positive result out of one sample while in 41–70 years of age group, 7 (50%) samples showed positive results out of 14 samples while 7 (50%) samples tested negative. *P* value obtained was 0.35 which was statistically insignificant [Graph 5].

Age-wise distribution of blood group B from the pulp

The pulp samples tested in the age group <20 years showed 5 (100%) positive results out of five samples. In 21–40 years of age group, 7 (87.5%) samples showed positive result out of the 8 samples while 1 (12.5%) sample tested negative while in 41–70 years of age group, 6 (85.7%) samples showed positive results out of the seven samples while 1 (14.2%) sample tested



Graph 2: Determination of ABO blood group from the pulp



Graph 4: Age-wise distribution of blood group A from the pulp

negative. *P* value obtained was 0.68 which was, again, statistically insignificant [Graph 6].

Age-wise distribution of blood group B from the dentine

The dentine samples tested in the age group <20 years showed 5 (100%) positive results out of five samples. In 21–40 years of age group, 4 (50%) samples showed positive result out of the 8 samples while 4 (50%) samples tested negative. In 41–70 years of age group, 5 (71.4%) samples showed positive results out of the seven samples while 2 (28.5%) samples were found to be negative. *P* value obtained was 0.15 which was found to be statistically insignificant [Graph 7].

Age-wise distribution of blood group O from the pulp

The pulp samples tested in the age group <20 years showed 6 (100%) samples to react positive out of the six samples. In 21–40 years of age group, 5 (100%) samples showed positive result out of the five samples while in 41–70 years of age group, 5 (55.5%) samples showed positive results out of the nine samples while 4 (44.4%) samples were found to be negative. *P* value obtained was 0.047 which was found to be statistically insignificant [Graph 8].

Age-wise distribution of blood group O from the dentine

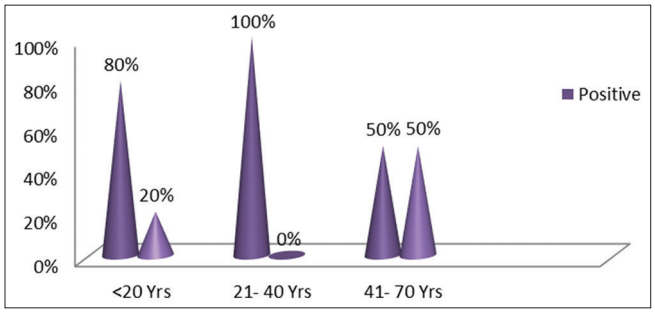
The dentine samples tested in the age group <20 years showed 6 (100%) samples showing positive results out of the six samples. In 21–40 years of age group, 4 (80%) samples showed positive result out of the five samples tested while 1 (20%) tested negative. In 41–70 years of age group, 4 (44.4%) samples showed positive results out of the nine samples while 5 (55.5%) samples were found to be negative. *P* value obtained was 0.06 which was close to being statistically significant [Graph 9].

Gender-wise distribution of blood group A from the pulp

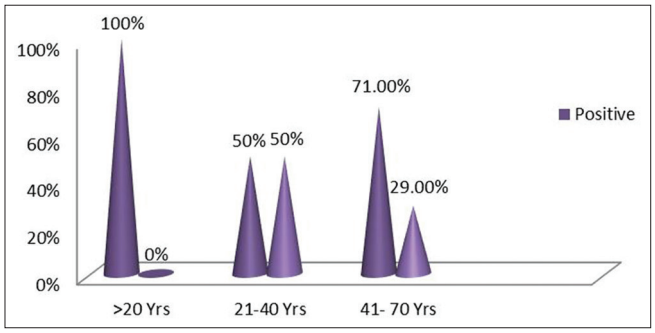
Out of the twenty samples, 11 (55%) of the samples were of males while 9 (45%) samples were of females. In males, 9 (81.8%) samples out of 11 tested positive while 2 (18.8%) tested negative and in females, 7 (77.7%) samples out of 9 tested positive while 2 (22.2%) tested negative. *P* value obtained was 0.62 which was found to be statistically insignificant [Graph 10].

Gender-wise distribution of blood group A from the dentine

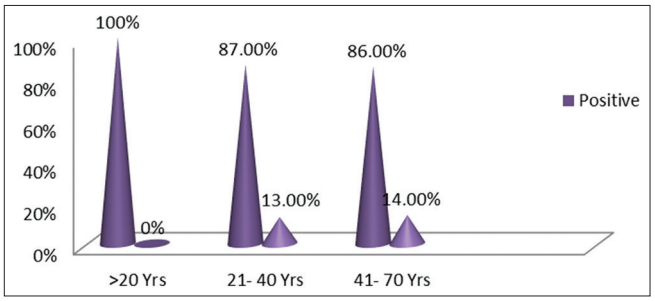
Out of the twenty samples, 11 (55%) of the samples were of males while 9 (45%) samples were of females. In males, 6 (54.5%) samples out of 11 tested positive while 5 (45.4%) tested negative and in females, 6 (66.6%) samples out of 9 tested positive while 3 (33.3%) tested negative. *P* value obtained was 0.00 which was found to be statistically significant [Graph 11].



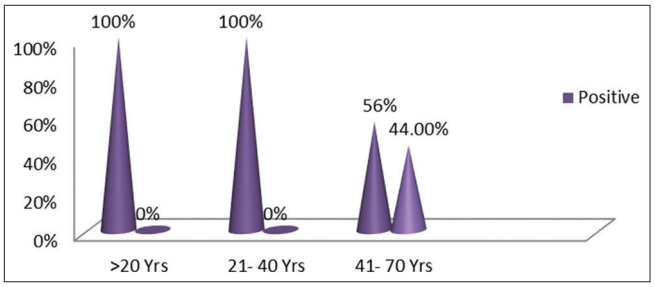
Graph 5: Age-wise distribution of blood group A from the dentine



Graph 7: Age-wise distribution of blood group B from the dentine



Graph 6: Age-wise distribution of blood group B from the pulp



Graph 8: Age-wise distribution of blood group O from the pulp

Gender-wise distribution of blood group B from the pulp

Gender-wise distribution of blood group B from the dentine

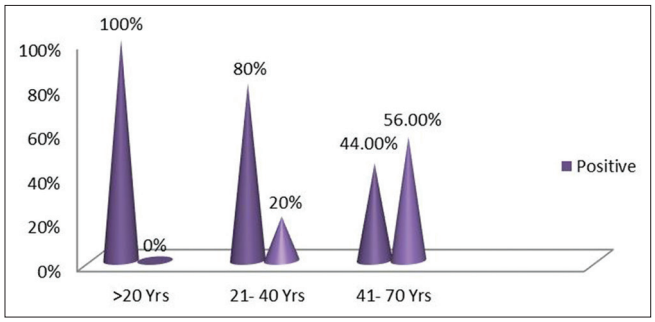
Out of the twenty samples, 13 (65%) of the samples were of males while 7 (35%) samples were of females. In males, 10 (76.9%) samples out of 13 tested positive while 3 (23%) tested negative and in females, 4 (57.1%) samples out of 7 tested positive while 3 (42.8%) tested negative. *P* value obtained was 0.33 which was found to be statistically insignificant [Graph 13].

Gender-wise distribution of blood group O from the pulp

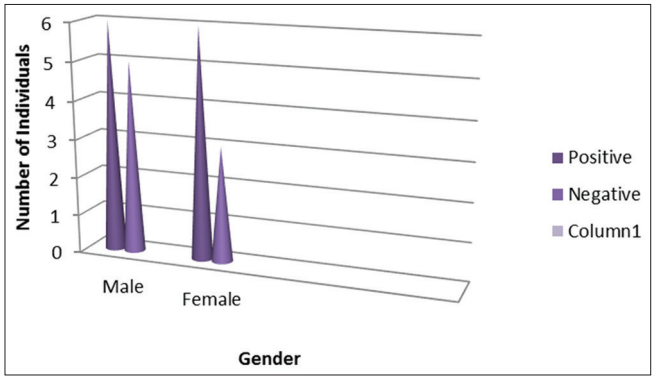
Out of the twenty samples, 11 (55%) of the samples were of males while 9 (45%) samples were of females. In males, 10 (90.9%) samples out of 11 tested positive while 1 (9%) tested negative and in females, 6 (66.6%) samples out of 9 tested positive while 3 (33.3%) tested negative. *P* value obtained was 0.21 which was found to be statistically insignificant [Graph 14].

Gender-wise distribution of blood group O from the dentine

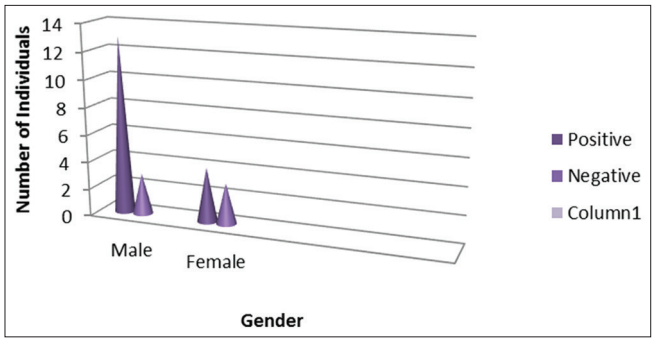
Out of the twenty samples, 11 (55%) of the samples were of males while 9 (45%) samples were of females. In males, 8 (72.7%) samples out of 11 tested positive while 3 (27.2%) tested negative and in females, 6 (66.6%) samples out of 9 tested positive while 3 (33.3%) tested negative. *P* value obtained was 0.57 which was found to be statistically insignificant [Graph 15].



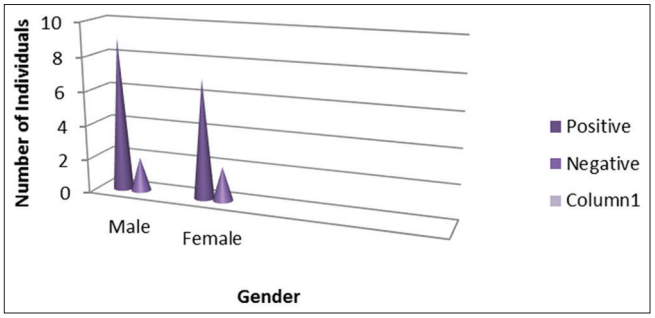
Graph 9: Age-wise distribution of blood group O from the dentine



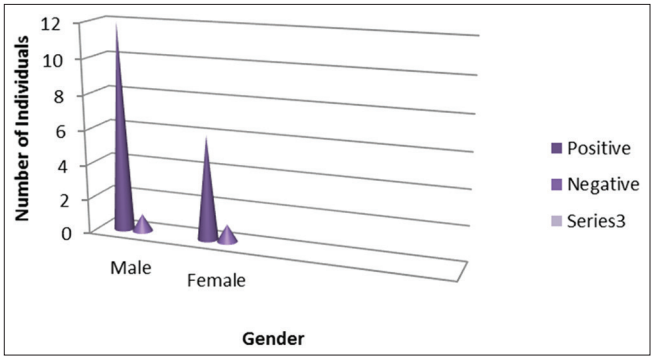
Graph 11: Gender-wise distribution of blood group A from the dentine



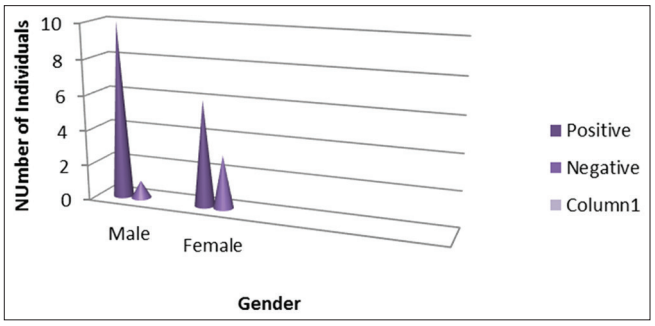
Graph 13: Gender-wise distribution of blood group B from the dentine



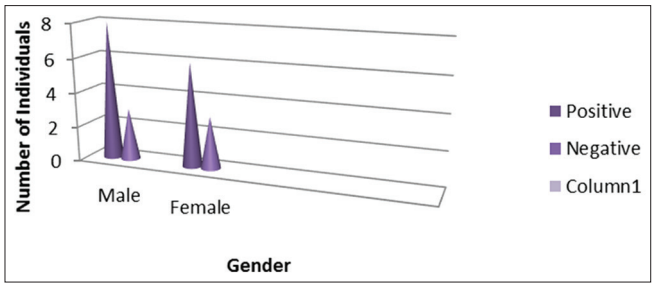
Graph 10: Gender-wise distribution of blood group A from the pulp



Graph 12: Gender-wise distribution of blood group B from the pulp



Graph 14: Gender-wise distribution of blood group O from the pulp



Graph 15: Gender-wise distribution of blood group O from the dentine

Discussion

Lattes have rightly said the fact that belonging to a definite blood group is a fixed character of every human being and can be altered neither by lapse of time nor by intercurrent disease. Blood group like fingerprints is an unalterable primary character. Human identification is the mainstay of civilization and the identification of unknown individuals has always been of paramount importance to the society. The use of blood group substances in medicolegal examination is based on the fact that once a blood group is established in an individual, it remains unchanged throughout life.[3] The term blood group is applied to inherited antigens detected on the red cell surfaces by specific antibodies.[2] The ABO blood group system, first described by Karl Landsteiner in 1900, remains the bulwark of forensic blood group investigations. The reasons for this are manifold. It is the primary, most common, conspicuous, and easily detectable groups.[2] Teeth can survive for long time even after soft and skeletal tissues have been destroyed. Blood grouping from teeth could be a source of personal identification.[3] Even in case of mass disaster such as fires and plane crash, the teeth remain unaffected. Teeth are the most durable organs in the body and can be heated up to 16,000°C temperatures without appreciable loss of the microstructure.[5] Pulp tissue is one of the most protected tissues being surrounded from all sides by the dental hard tissues. Postmortem changes in pulp are seen very late and also, pulp remains one of the most protected tissues and therefore, could be readily available for examination. Pulp contains numerous blood vessels and blood group antigens are certainly present in tooth pulp. Blood group substances are presumed to be present in the dentinal tubules, too.[3] It has been suggested that blood group antigens in the pulp and dentine are preserved even up to 2 years after the death of an individual.[5] The distribution of ABO blood group antigens gradually decreases from the pulp cavity wall to the dentine edge and from there to the enamel because of the fewer possibilities of the diffusion of these antigens from both blood and saliva.[7] For several decades, forensic scientists have been searching a reliable method for blood typing of teeth. The technique AE is the most sensitive and the most widely employed. According to Kind, SS[9,10] and Outtridge RA[11], Absorption-Elution AE technique has proved to be markedly more sensitive than the absorption-inhibition one. AE has shown more success rate than mixed-agglutination for certain antigens.[12] The determination of Rh antigen was done in 1962 using AE technique from blood stains. Rh blood group is considered to be the most complex, genetically, of all the blood group systems since it involves 45 different antigens on the surface of red cells.[13] Teeth are used for blood grouping and are considered as a hallmark for the identification of biological materials in forensic investigations. Considering this fact, in the present study, an attempt was made to detect ABO blood group antigens from dentine and pulp in freshly extracted teeth. The blood group determination was done and compared with their control blood group which was obtained from analyzing the blood obtained from the tooth sockets by slide agglutination method. The samples taken for the study ranged from 14 years to 70 years of age. Among them, the maximum numbers of samples were in the age group of 41–60 years. A total of 16 samples belonged to the age group of <20 years of age. 11 samples belonged to the age group of 21–40 years and 33 samples belonged to the age group of 41–70 years of age. Out of sixty samples, 36 samples were from males while 24 samples were of females. Pulp showed maximum positive results (83.3%) while dentine showed 66.6% positive results. In pulp, blood group A showed 80% positive results while blood group B showed maximum positive results (90%) and blood group O showed 80% positive results. In dentine, blood group A showed 60% positive results while blood group B and O showed 70% positive results. On comparison of pulp results among all the age groups, there were minimal variations in the < 20 years of age group and 21–40 years of age group while sensitivity decreased in 41–60 years of age group. The differences, though, were found to be statistically insignificant. On comparison of overall positive results of dentine and pulp according to age, the positive results of pulp decreased in 41-60 years age group while pulp showed better results than dentine in all the age groups. The findings suggest that the pulp shows decrease in antigenicity after 40 years of age. It has been observed from histological studies that there is an apparent decrease in the number of blood vessels and nerves that supplied the pulps of older teeth along with a progressive deposition of calcified bodies and fibrosis.7 There is no study conducted as yet showing more positive results in dentine than pulp.

ABO blood grouping of dentine and pulp according to gender and age

Out of 60 samples, 36 samples belonged to males while 24 samples belonged to females. The 36 samples of dentine which belonged to males, also, showed a gradual decrease in the positive results with the increase in age. The 36 samples of pulp which belonged to males showed a variable decrease in the positive results along with the age. The 24 samples of dentine and pulp which belonged to females tested showed a gradual decrease in the positive results with the increase in age. On comparing the results of dentine and pulp with gender, the results obtained from males were found to be better than the females for both dentine and pulp. Pulp was better than the dentine in both the males and females although the results were not found to be statistically significant. There were no statistically significant age and gender differences in the results obtained by dentine and pulp for ABO blood grouping. The results of the present study were found to be in agreement with the observations made by Ramnarayan B *et al*[2], Lele MV *et al*[14], Garg RK and Garg S[15] and Aswath N *et al*[16]. Furthermore, various studies conducted by Ramnarayan B *et al*[2], Shetty M and Premalatha K[3], Ballal S and David MP[4] and Smeets B *et al*[6], at different time periods, showed pulp to be better than dentine and there was a decrease in the sensitivity of the dentine and pulp as the time periods increased. The overall decrease in the sensitivity could be due to dehydration, the loss of pulp antigens, insufficient quantity of pulp, calcification of the canals, cell lysis, contamination of the tooth, and time lapse for the procedure.

Distribution of individual blood groups of dentine and pulp for ABO blood grouping

Out of the twenty samples for the blood grouping, blood group A in the pulp showed maximum positive results in the 21–40 years of age group and followed by <20 years age group and 41–70 years. At the same, dentine showed maximum positive results in the 21–40 years age group followed by <20 years of age group and 41–70 years age group whereas out of the twenty samples for the blood grouping, blood group B in the pulp showed maximum positive results in <20 years of age group while minimum positivity was seen in the 41–70 years of age group. In dentine, blood group B showed maximum positive results in <20 years of age group and minimum positivity in the 21–40 years of age group. Out of twenty samples for the blood grouping, blood group O showed maximum positive results in <20 years and 21–40 years of age and least positivity in 41–70 years of age. It is suggested that sensitivity decreased with increasing age. A recent study conducted by Ramnarayan *et al*[2] analyzed the individual blood groups in pulp and hard tissues and showed blood group O with maximum positivity followed by A, B, and AB blood groups. Aswath *et al*[16]. analyzed the individual blood groups in pulp and showed blood groups O, B, and A blood groups with maximum positivity followed by the AB blood group. These variations in the results obtained might be due to a smaller sample size and the time period variations considered in the studies. Blood grouping on teeth is not a straight forward technique; the concentrations of blood group antigens are low in the teeth when compared to other tissues and body fluids. In the present study, dentine showed presence of blood group antigens almost as good as pulp. It is assumed that the origin of blood groups antigen in dental hard tissues is based on the infusion sedimentation phenomenon combined with inherently present antigens. Considering all the factors that support the presence of blood group antigens in dentine and pulp and also, the pitfalls of false positive results or, mistyping of blood groups, it can be concluded that the results obtained with pulp were better than that of dentine.

Conclusion

All humans have an identity in life; certain humanities require identity even after death in some circumstances. One of the ways of identification is an individual's blood group. Teeth were used as a mode of identification of blood group in the present study because teeth are one of the most indestructible parts of the human body and exhibit the least turnover of natural structure. The presence of ABO blood group in soft and hard tissues makes it possible for the identification of the deceased. AE technique to identify blood groups in teeth may be of immense value not only in identification of the accused but also in investigation in mass disasters and fire victims. There was no significant age and gender difference in the results obtained in blood grouping from dentine and pulp, however, pulp showed better positive results than dentine, the difference, however, was not found to be statistically significant suggesting both dentine and pulp having almost an equal antigenic potential which weakens as the time period increases. Individual blood group analysis of ABO blood groups for both dentine and pulp showed maximum positivity for B blood group followed by A and O blood groups. Positivity of pulp was better than dentine in both the blood groups although with insignificant *P* values indicating that the antigenicity of pulp is better than the dentine. Positivity of both dentine and pulp decreased with increasing age suggestive of decreasing potential of antigens in both dentine and pulp with increase in age. On the basis of the results obtained from the present

study, it could be concluded that both dentine and pulp are reliable sources of blood group determination for ABO blood grouping where teeth happen to be the only remnants available for personal identification. Although expression of ABO blood groups was seen in both dentine and pulp, ABO blood group antigens were found to be better expressed in the pulp. Blood group determination from teeth warrants advance exploration as establishment of identity of a person from the skeletal remains is of paramount importance to a forensic odontologist. The present study detected blood groups antigens of ABO blood group system from the tooth tissues. The present study is, thus, a quantum of what has been learned and how much more is needed to be learned in this challenging branch of forensic odontology.

Acknowledgment

To all the patients who contributed in the study without whom this study would not have been feasible.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Inamdar PI, Inamdar P, Rai K, Mirajkar AM, Venkatesh SP. Teeth-hidden treasure of blood group. Indian Journal of Forensic Medicine and Pathology 2011;4:113-8.
2. Ramnarayan B, Manjunath M, Joshi AA. ABO blood grouping from hard and soft tissues of teeth by modified Absorption-Elution (AE) technique. J Forensic Dent Sci 2013;5:28-34.
3. Shetty M, Premalatha K. Original research paper ABO Blood Grouping from Tooth Material. J Indian Acad Forensic Med 2010;32:336-8.
4. Ballal S, David MP. Determination of ABO blood grouping from Dentin and pulp. Pak Oral Dent J 2011;31:3-6.
5. Stimson PG, Mertz CA. Forensic Odontology. Forensic Dentistry. CRC Press Book. South Australia. 1997; p. 23-4.
6. Smeets B, van de Voorde H, Hooft P. ABO blood grouping on tooth material. Forensic Sci Int 1991;50:277-84.
7. Nakayama Y, Aoki Y. Distribution of ABH Blood group Epitopes on Inner surface of Dental Hard Tissue: Serological, Immuno-histo-chemical and Ultra-structural study on Odontoblast. Tohoku Journal of Expert Medicine 1998;184:267-76.
8. Xingzhi X, Ji L, Hao F, Ming L. ABO Blood grouping on dental tissue. Journal of Forensic Science 1993;38:956-60.
9. Kind SS. Absorption-Elution (AE) grouping of dried blood smears. Nature 1960;185:397-8.
10. Kind SS. The ABO blood grouping of blood stains. Journal of Criminal Law and Criminology and Police 1962;53:367.
11. Outtridge RA. Bloodstain grouping-Elution V. Inhibition. Journal of the Forensic Science Society 1963;4:87-90.
12. Karthika B, Elumalai M. Identity of blood group from dental pulp of deceased human. International Journal of Pharma and Bio Sciences 2013;4:1000-4.
13. Aye UT. ABO Grouping of blood stains on sweaty garments by absorption elution method. Forensic Sciences 1977;16:8-12.
14. Lele MV, Malvekar AG, Dang AH, Madiwale MS. Detection of ABH blood group substances in human dental pulp. J Indian Acad Forensic Sci 1977;16:2-3.
15. Garg RK, Garg S. The detection of ABO (H) blood groups specific substances from human dental hard tissue using low ionic strength solution. J Forensic Med 1989;5:3-6.
16. Aswath N, Selvamuthukumar SC, Karthika B. Role of dental pulp in identification of the deceased individual by establishing ABO blood grouping and Rhesus factor. Indian J Dent Res 2012;23:811-3.