INTRODUCTION

Forensic odontology has established itself as an important and often indispensable science in medicolegal matters and identification of the dead.\(^1\) The forensic importance of dental tissue has been well recognized because tooth is the hardest of all human tissues. They are well preserved for a long period even after death,\(^2\) hence dental remains are the most stable biological evidence encountered in crime cases and yield useful information.\(^3\)

Abstract

**Objective:** The aim of the study was to determine blood groups and Rhesus factor from dentin and pulp using absorption-elution (AE) technique in different time periods at 0, 3, 6, 9 and 12 months, respectively.

**Materials and Methods:** A total of 150 cases, 30 patients each at 0, 3, 6, 9 and 12 months were included in the study. The samples consisted of males and females with age ranging 13–60 years. Patient’s blood group was checked and was considered as “control.” The dentin and pulp of extracted teeth were tested for the presence of ABO/Rh antigen, at respective time periods by AE technique.

**Statistical Analysis:** Data were analyzed in proportion. For comparison, Chi-square test or Fisher’s exact test was used for the small sample.

**Results:** Blood group antigens of ABO and Rh factor were detected in dentin and pulp up to 12 months. For both ABO and Rh factor, dentin and pulp showed 100% sensitivity for the samples tested at 0 month and showed a gradual decrease in the sensitivity as time period increased. The sensitivity of pulp was better than dentin for both the blood grouping systems and ABO blood group antigens were better detected than Rh antigens.

**Conclusion:** In dentin and pulp, the antigens of ABO and Rh factor were detected up to 12 months but showed a progressive decrease in the antigenicity as the time period increased. When compared the results obtained of dentin and pulp in ABO and Rh factor grouping showed similar results with no statistical significance. The sensitivity of ABO blood grouping was better than Rh factor blood grouping and showed a statistically significant result.

**Key Words:** Absorption elution technique, blood grouping, dentine, forensic odontology, pulp
Blood grouping has been one of the major factors for identification of biological materials in forensic investigations and is a widely used technique in forensic laboratories. The presence of ABO blood group and Rhesus factor is applied to inherited antigens detected on red cell surface by specific antibodies.\(^4\) Once the blood group and Rhesus factor are established, it remains unchanged throughout life.\(^5\)

Blood group substances are secreted in many body secretions including saliva. Kind in 1960 discovered the presence of ABO blood group in saliva by absorption-elution (AE) method.\(^6\) Blood grouping from dried stain by elution procedure was described more than 50 years ago but not employed widely in Forensic Serology, until 1960, when Kind refined this technique. AE technique/procedure originally devised by Siracusa\(^7\) is now employed in all forensic laboratories because it is proven to be most sensitive, reliable and reproducible.

Pulp tissue is one of the most protected tissues being surrounded from all sides by dental hard tissues. 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.\(^8\)

It is presumed that blood group substances in dentin are located in dentinal tubule.\(^8\) The distribution of ABO substances from the pulp cavity wall to the dentin edge and the enamel gradually reduces, because of fewer possibilities of diffusion of antigens from both blood and saliva.\(^7\) The existence of blood group antigens in tooth dentin and enamel and their nature has been substantiated by infusion — sedimentation phenomena combined with inherently present antigens. This theory describes the infusion of water-soluble antigens from saliva into the tooth tissue.\(^8\)

The presence of ABO blood group and Rhesus factor antigen in soft and hard dental tissues makes a possible contribution in human identification even in decomposed bodies. Mostly, teeth and bones are the only significant tissues remaining in mass disasters.

Therefore, blood group and Rhesus factor determination for biological evidence on tooth material is of great importance in Forensic Odontology. The aim of this study was to determine the ABO blood grouping and Rhesus factor from dentin and pulp of extracted teeth using "AE technique" at 0, 3, 6, 9 and 12 months after extraction.

MATERIALS AND METHODS

A brief case history with relevant medical history was recorded from patients selected for study and consent was taken. The blood groups were determined for all the study participants using capillary blood by slide agglutination method. The blood groups obtained were considered as control. Carious teeth and grossly decayed teeth were excluded; and teeth extracted for periodontal, and orthodontic purposes were included in the study. The extraction procedure was carried out under local anesthesia following the aseptic protocol in the Department of Oral and Maxillofacial Surgery. The extracted teeth were dried and stored in labeled bottles for a span of 3, 6, 9 and 12 months. The teeth were sectioned into two halves using a micromotor with a carborundum disc. The pulp was scooped with a spoon excavator and dentin was powdered using a straight fissure bur [Figure 1]. The blood grouping from teeth was performed by AE technique using powdered dentin and dental pulp. The pulverized dentin powder and pulp were divided into three equal parts and were taken into six sterile test tubes containing 2 ml of saline and labeled, respectively. To each of these test tubes, three drops of antiserum A, B, D was added respectively, and the test samples were sufficiently soaked with antiserum for 2½ h and left standing at room temperature. Then the excess antiserum was pipette out from the test tubes. Each sample was washed five times with cold saline solution by centrifuging it at 3000 rpm for 5 min and the supernatant was removed with pipette. Then two drops of fresh saline were added to the sample, and the test tubes were heated in a water bath at a temperature of 50–55°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 respective test tubes. The samples were incubated at 37°C for 30 min to enhance agglutination, and then, it was centrifuged at 1500–2000 rpm for 1 min. By gentle shaking of the test tube, the presence or absence of red cell agglutination was ascertained macroscopically and microscopically at a magnification of ×4 [Figures 2 and 3]. The results obtained were compared with control sample.

Statistical analysis

Data were analyzed in proportion. For comparison, Chi-square test or Fisher’s exact test was used for small sample. Two-tailed \(P < 0.05\) was considered as statistically significant, and \(0.01\) was considered as highly statistically significant. Data were analyzed using software SPSS version 16.0.

RESULTS

ABO blood grouping for dentin and pulp showed a gradual decrease in the sensitivity as the time period increased. In dentine, the sensitivity ranged from 100% to 73% and pulp sensitivity ranged from 100% to 80%. Overall pulp showed better sensitivity than dentin. There was no significant difference in the sensitivities of dentin and pulp [Table 1].
Rh factor grouping for dentin and pulp showed a gradual decrease in the sensitivity as the time period increased up to 9 months. There was a sudden decrease in the sensitivity at 12 months’ time period. In dentine, the sensitivity ranged from 100% to 40% and pulp sensitivity ranged from 100% to 23%. Overall pulp showed better sensitivity than dentin except at 12 months’ time period where dentin showed better sensitivity than pulp. Moreover, in comparison with the sensitivities of dentin and pulp, the P values obtained were not significant [Table 2].

When compared the sensitivity of ABO and Rh factor blood grouping of dentin and pulp in accordance to time period dentin and pulp showed almost similar results from 0 to 9 months. At 12 months both dentin and pulp showed vast difference in the sensitivities of ABO and Rh blood grouping with significant P value [Table 3].

**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 fingerprint 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 society. The use of blood group substances in medico-legal examination is grounded on the fact that once a blood group is established in an individual, it remains unchanged for a lifetime. 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

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**Table 1: ABO blood grouping for pulp and dentine**

| Period (number of samples) | Dentin sensitivity (%) | Pulp sensitivity (%) | P  |
|---------------------------|------------------------|----------------------|----|
| 0 month (30)              | 100                    | 100                  | 0.99 |
| 3 months (30)             | 93                     | 93                   | 0.99 |
| 6 months (30)             | 90                     | 90                   | 0.99 |
| 9 months (30)             | 83                     | 86                   | 0.71 |
| 12 months (30)            | 73                     | 80                   | 0.52 |
| Total (150)               | 88                     | 90                   |     |

**Table 2: Rh factor blood grouping for pulp and dentine**

| Period | Dentin sensitivity (%) | Pulp sensitivity (%) | P  |
|--------|------------------------|----------------------|----|
| 0 month | 100.00                | 100.00               | 0.99 |
| 3 months | 96.70                 | 100.00               | 0.31 |
| 6 months | 80.00                 | 93.33                | 0.13 |
| 9 months | 76.70                 | 93.33                | 0.07 |
| 12 months | 40.00                 | 23.30                | 0.16 |
| Total (150) | 78.7                  | 82                   |     |
employed. According to Kind, Nickolls and Periera and Outtridge, AE has proved to be markedly more sensitive than the absorption-inhibition test. AE has shown more success rate than mixed-agglutination for certain antigens.

Determination of Rh antigen was done in 1962 using AE technique from blood stains. We used the same techniques to determine Rh antigen from dentin and pulp. Rh blood group is considered to be the most complex genetically of all the blood type systems since it involves 45 different antigens on the surface of red cell.

Teeth are used for blood grouping and are considered as a hallmark for identification of biological materials in forensic investigations. Considering this fact in our present study, an attempt was made to detect ABO and Rh factor antigen from dentin and pulp over a time period of 0, 3, 6, 9 and 12 months. For ABO blood grouping, both dentin and pulp showed 100% sensitivity with the samples tested immediately after extraction and the sensitivity gradually reduced as time period prolonged. Pulp showed better sensitivity than dentin but the difference was statistically insignificant, suggesting both dentin and pulp have almost equal antigenic potential although pulp is better, the sensitivity weakened as the time period increased.

Till date, the various studies conducted by Smeets et al., Shetty and Premlata, Ballal and David and Ramnarayan et al. at different time period showed pulp to be better tool than dentin and there was a decrease in the sensitivity of the dentin 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 or time lapse for the procedure.

For Rh factor blood grouping, both dentin and pulp showed 100% sensitivity when the samples were tested immediately after extraction and the sensitivity gradually reduced as time period prolonged. Pulp showed better results than dentine, but the difference was statistically insignificant, suggesting both dentin and pulp have almost equal antigenic potential which weakened as the time period increased, similar to ABO antigens. This may be attributed to autolysis, dehydration and the loss of pulp antigens similar to ABO antigens. The study by Aswath et al. is the only study available in English language literature that was conducted for determination of Rh factor antigens along with ABO blood group antigens in freshly obtained pulp samples. In our study, both pulp and dentin samples were used and tested at 0 month to an extensive time period of 12 months.

ABO and Rh factor blood grouping for dentin and pulp showed a gradual decrease in the sensitivity as the time period increased. At 12 months, both dentin and pulp showed a drastic decrease in the sensitivity for Rh factor blood grouping than the ABO blood grouping with a statistically significant difference.

The sensitivity of pulp was better than dentin in both the blood grouping systems with an insignificant P value. ABO blood grouping was better than Rh factor grouping in both the teeth components and the P values obtained were significant. This indicates that the antigenicity of pulp is better than dentin in both ABO and Rh blood groups, and ABO antigens were better expressed than Rh factor blood group antigens. The outcome of our study showed that ABO and Rh blood group antigens could be detected up to 12 months, and there are no studies in the English literature to compare this study.

### Table 3: Comparison of ABO and Rh factor blood grouping for dentin and pulp

| Time period | Dentine | Pulp |
|-------------|---------|------|
| ABO blood group (%) | Rhesus factor blood group (%) | P | ABO blood group (%) | Rhesus factor blood group (%) | P |
| 0 month | 100 | 100 | 0.99 | 100 | 100 | 0.99 |
| 3 months | 93 | 97 | 0.99 | 93 | 100 | 0.99 |
| 6 months | 90 | 80 | 0.47 | 90 | 93 | 0.99 |
| 9 months | 83 | 77 | 0.99 | 87 | 93 | 0.67 |
| 12 months | 73 | 40 | 0.02 | 80 | 23 | 0.0001 |

### Table 4: Overall comparison of blood group systems and teeth components

| Blood group system | Teeth components | P |
|-------------------|-----------------|---|
| | Dentin (%) | Pulp (%) | |
| ABO blood group | 88 | 90 | 0.58 |
| Rhesus factor blood group | 79 | 82 | 0.46 |
| P | 0.03 | 0.04 |

Figure 4: Graph of comparisons of the overall sensitivity of ABO and Rh factor blood grouping of dentin and pulp
Pulp showed better agglutination than dentine for ABO and Rh factor blood grouping. The agglutinates for dentine and pulp weakened as the time period increased. This shows that pulp contains more amounts of antigen than the dentin and its antigenicity decreases with time. Blood grouping on teeth is not a straightforward technique; the concentrations of blood group antigens are low in the teeth when compared to other tissues and body fluids. In this study, dentin also showed the presence of blood group antigens almost as good as pulp. It is assumed that the origin of blood group antigen in dental hard tissue is based on the infusion sedimentation phenomenon combined with inherently present antigens.[7]

Considering all the factors that support the presence of blood group antigens in dentin and pulp and also the pitfalls of false positive result or mistyping of blood group, over a period in this study, we came to a close consequence that the results obtained with pulp were better than that of dentin. Another aspect to be highlighted here is ABO and Rh factor antigens were detected from both dentin and pulp even up to 12 months after extraction of the teeth. Blood grouping has been one of the bases for identification of biological materials in forensic investigations and ABO blood grouping is a widely used technique in forensic laboratories. Rh factor is the finer classification of ABO blood group system. Teeth can survive long after soft and skeletal tissues have been destroyed.[1] The presence of ABO blood group antigens along with Rh factor antigens in pulp and hard dental tissues makes it a potential substance in the identification of highly decomposed bodies or body part where teeth and bones are the only significant tissue remains.[6] Blood group substances in the hard dental tissues thus remain unaffected even in adverse environmental conditions.[13]

CONCLUSION

Teeth were used as a mode of identification of blood group in this study because teeth are one of the most indestructible parts of the body and exhibit the least turnover of natural structure. The presence of ABO blood group and Rh factor antigen in soft and hard tissue makes it possible for identification of the deceased. AE test to identify blood groups in teeth may be of immense value not only in the identification of accused but also in the investigation of mass disaster and fire victims.

On the basis of results obtained in our study, both dentin and pulp are reliable sources of blood group determination for up to 12 months for ABO and Rh factor blood grouping, especially where teeth ensues to be the only remnants existing for individual identity. Although expression of ABO blood groups and Rh factor was seen in both dentin and pulp, intensity of ABO blood groups and Rh factor was higher in pulp than dentin and ABO blood group antigens were better expressed than Rh factor antigens in both dentin and pulp.

Blood group determination from teeth warrants advance exploration as the establishment of identification of a person from the skeletal remains is of paramount importance to a Forensic Odontologist. Till date, in English language literature no studies have been conducted to detect ABO and Rh factor blood group antigens from tooth material for up to 12 months. This study detected blood groups antigens of ABO and Rh systems from tooth material over this extensive time period of 12 months. This study is thus a quantum of what has been known and learned and how much more needs to be understood in this challenging branch of Forensic Odontology.

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Conflicts of interest

There are no conflicts of interest.

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