Determination of ABO Blood Grouping from Dentine and Pulp

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ABSTRACT

This study was carried out to determine the blood group from dentine and pulp and to correlate the same with blood collected from respective extraction socket.

30 teeth were extracted and stored dry for 6 months. An attempt to establish the blood group from dentin and pulp was made by Absorption–Elution method.

The pulp showed positive results for 27 out of 30 teeth (sensitivity 90%). Two teeth showed negative results, wherein one tooth showed mistyping of blood grouping. Blood grouping from dentin was not correlating with control group.

In view of the particularly significant positive results of ABO blood groups obtained from dental pulp in this study, it can be concluded that dental pulp can be used to establish identity, where teeth happen to be the only remnants available for personal identification. Dentine may not be reliable source for blood group determination and this warrants further research.

Key words: ABO blood group, dental pulp, dentin, Absorption–elution method

INTRODUCTION

Teeth are an invaluable source of personal identification in the field of forensic medicine as they are resistant to environmental assaults. Blood grouping from them could be a source of personal identification. The term blood group is applied to inherited antigens detected on red cell surface by specific antibodies. The use of blood group substances in medico-legal examination is based on the fact that once a group is established in an individual it remains unchanged throughout his life. It was thought to be of interest to apply the method to a calcified tissue such as dentine. Dentin was chosen as it has higher ratio of cell substances to matrix than bone and is easier to obtain blood. Blood group substances have been detected from the pulp. Post mortem changes in pulp are seen very late and also pulp remains one of the most protected tissue and therefore could be readily available for examination. Absorption Elusion procedure for blood group determination was originally devised by Siracus is now employed in all forensic laboratories because it is proven to be most sensitive, reliable and reproducible. Blood group substances are secreted in many body secretions, such as saliva. Kind in 1960 discovered the presence of ABO blood group in saliva by absorption elution method.

In this study an attempt was made to determine ABO blood group from the dental pulp and dentin by absorption–elution method. Since tooth pulp contains lot of blood vessels, blood group antigens are most certainly bound to be preset in tooth pulp. It is presumed that blood group substances in dentine were located in dentin tubuli. The existence of blood group antigens in tooth dentin and enamel, and their nature has been substantiated by infusion – sedimentation phenomena. The infusion sedimentation theory describes the infusion of water soluble antigens from saliva into the tooth tissue. Teeth are resistant to environmental assaults, such as incineration, immersion, trauma, mutilation and decomposition, therefore teeth represent an excellent source for identification. Blood grouping from them could be a source of identification.

METHODOLOGY

30 teeth which were extracted from patients in the age range of 13–60 years due to poor periodontal status or for the purpose of orthodontic treatment were collected. Exclusion criteria: Carious teeth were excluded because of the possibility of showing false positive results.

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A brief case history with relevant medical history was recorded from patients selected for the study. Subjects were examined under artificial illumination. Extraction procedure was carried out under local anesthesia following the aseptic protocol. After the extraction the socket was compressed with sterile gauze piece and blood collected from this was considered as control group in this study. The normal saline and anticoagulant was added and diluted. One drop of diluted blood was placed on the slide and a drop of Antiserum-A was added. Ifagglutination occurred when Antiserum-A was added the blood group belonged to group A and vice versa. By extending this logic, grouping according to ABO system were recorded.

The extracted teeth were washed under running water and debris were removed with the probe, and wiped with gauze and kept in numbered bottles. The teeth were dried and stored for a span of 6 months (Fig 1).

The blood grouping was performed by absorption elution test using dentin and dental pulp, which was later, compared with control sample in blind study. The tooth was completely trimmed to remove the enamel and cementum with lathe. The tooth was further split vertically with carborundum disc and the dental pulp was scooped out with spoon excavator, thus the remaining tooth consisted of dentin, which was pulverized, with straight fissure bur (Figs 2 and 3). The pulverized tooth powder was put in two test tubes, to each of this test tubes 3 drops of antiserum A, B was added and confirming the test samples being sufficiently soaked with antiserum for two and half hours and left standing at room temperature (Figs 4 and 5). After removing antiserum, each sample was washed three times with cold saline solution (it was centrifuged and the supernatant was sucked with pipette). Then two drops fresh saline was added to the sample and the test tube were heated in a water bath (50-55°C) for 10 minutes to elute the antibodies (Fig 6). A drop of 0.5% A or B group red cell suspension was immediately put into each respective test tube of known blood and the samples were again put in humified recipient and were incubated at 37°C for 30min to enhance agglutination and after this procedure it was centrifuged at 1,500-2000rpm for 1 min. By gentle shaking of the test tube the presence or absence of red cell agglutination was ascertained with microscope at magnification of 100X. The p values were obtained by javascript pre-post prepared proportion test.

RESULTS

The clinical study consisting of 30 teeth was undertaken to evaluate the sensitivity of dentin and pulp in determining the blood group. Thirty teeth were examined for blood grouping maximally 6 months after extraction. Blood grouping from dentin was not correlating with the control group, but sensitivity of pulp in

<table>
<thead>
<tr>
<th>Blood group</th>
<th>Control (n=30)</th>
<th>Pulp (n=30)</th>
<th>P value</th>
<th>Goodman-Kruskal Gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11 (36.7%)</td>
<td>10 (33.3%)</td>
<td>P&gt;0.05</td>
<td>0.82</td>
</tr>
<tr>
<td>AB</td>
<td>1 (3.3%)</td>
<td>1 (3.3%)</td>
<td>P&gt;0.05</td>
<td>1.0</td>
</tr>
<tr>
<td>B</td>
<td>18 (60.0%)</td>
<td>17 (56.7%)</td>
<td>P&gt;0.05</td>
<td>0.89</td>
</tr>
<tr>
<td>Overall</td>
<td>30 (100.0%)</td>
<td>28 (93.3%)</td>
<td>P&gt;0.05</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Inference Sensitivity of Pulp in relation to Control is 90% (Pick-up rate of Pulp) and overall correlation 0.87 (very large correlation)
Determination of ABO Blood Grouping from Dentine & Pulp

Fig 1: Preserved tooth with label

Fig 2: Sectioned tooth

Fig 3: Dentine powder

Fig 4: Dentin and Pulp in antisera A and antisera B

Fig 5: Pulp in antisera A and antisera B

Fig 6: 0.2% red cell suspension
relation to control was 90% and overall correlation was 0.87% (very large correlations). Two teeth showed negative results, wherein one tooth showed mistyping of blood grouping.

**DISCUSSION**

The discovery of ABO blood grouping by Land Steiner in 1900 opened a new complex field of study with many practical applications. Over the past three-quarter of century, information from studies on blood grouping has been applied in medico-legal examinations. The use of blood group substances in medico-legal examinations is based on the fact that once a group is established in an individual it remains unchanged throughout his life.1

Teeth have been identified as the most stable biological material. Being made up of hardest tissue in the body, they retain their characteristics even in the most adverse environmental condition, whereas other means of identification like facial and dermatoglyphic characteristics tattoos marks etc fail owing to mutilation, decomposition and charring.6 Hence teeth are used for blood grouping and are considered as a hallmark for identification of biological material in forensic investigation. Initially conventional Absorption inhibition was employed for hard dental tissue. However, the method used was complicated. Pretreatment decalcification and the results were less than ideal. About one decade later the absorption elution technique was substituted for Absorption inhibition technique and is most exclusively used for this purpose in forensic laboratories.3

In this study 27 teeth out of 30 showed positive results in pulp. This showed 90% sensitivity. This finding is consistent with the studies done by Xingzhu Xu et al3 and Smeets.5 The negative results in two samples and mistyping in one sample could be attributed to insufficient quantity of pulp, reduction in fibrosed tissue in the pulp with increasing age and also increased calcification of the canal.7 In living people, a large number of aerobic gram-negative bacteria are present in saliva or on dental tissues. It should be kept in mind, that together with yeasts, the aerobic gram-negative bacteria tend to grow explosively in contaminated stored specimens or putrefying material and they tend to over grow other species. Particularly in those situations, the massive growth of such bacteria with blood group simulating activity may become a more important source of blood typing error than would be expected at first sight from their presence relative to other bacteria in the natural oral flora of the living. It is therefore, possible that the occasional mistyping of blood groups from oral material (e.g. teeth and alveolar bone) may be caused by the aerobic gram-negative oral flora, especially in heavily contaminated or putrefying material.8

In the present study blood grouping from dentin was not found in any of the samples. This is consistent with the study done by Kramer in which it was thought that negative finding might be a result of inaccessibility of blood group substances in the dentine because of high degree of calcification. Karzun9 also accepted the fact that detection of ABO blood grouping activity in hard dental tissue is unreliable. The distribution of ABH substances from the pulp cavity wall to the dentine edge and to the enamel decreases gradually because of fewer possibilities for diffusion of antigen from both blood and saliva.

Pulpal tissue is one of the most protected tissues of the oral tissues as it is surrounded from all sides by dental hard tissues.9 From positive results of ABO blood groups obtained from dental pulp in this study, it can be concluded that pulps possess a high potential value for application in forensic odontology. This study is thus a quantum of what has been learned and how much more needs to be learned in this challenging branch of forensic odontology.

**REFERENCES**