

Determination of age, sex, and blood group from a single tooth

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Introduction

Human identification is one of the most challenging subjects that human has been confronted with.^[1]

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Abstract

Background: Human identification is one of the most challenging subjects that human has been confronted with. Through the ages, odontological examinations have been a critical determinant in the search of human identity. Data in the form of age, gender, and blood group might provide vital clues in such investigations. In the recent times, it has been often desirable to preserve tissues for further investigations following the unfolding of certain events or discovery of new data. Hence, it is important to gather as much data as possible using less tissue. **Aims:** The purpose of this study was to determine age, sex, and ABO blood group of individual from a single tooth, to determine the effect of different environmental conditions, and to extract maximum information also at the same time preserving some tissue for the further investigation whenever needed. **Materials and Methods:** The study sample consisted of sixty teeth divided into four groups under different environmental conditions and time. The teeth were sectioned longitudinally in the buccolingual plane along the midline. Longitudinal ground sections of each tooth were prepared for age determination from cemental lines. Pulp removed was divided into two halves thereafter sex and blood group was determined. **Statistical Analysis:** For correlation of age between estimated age and actual age, using cemental lines Pearson's correlation coefficient was applied. Further for determination of both sex and blood group between groups, Chi-square test was applied. **Results:** A strong positive correlation was found between the estimated age and actual age of the study groups. Moreover, there was no significant difference between the actual and determined sex and blood group of the study groups. **Conclusion:** Although age, sex, and blood group are more reliably determined in freshly extracted teeth, these variables may be of significant help in identification even after a period of 6 weeks postextraction.

Key words: Absorption-elution, age determination, amelogenin, blood group determination, sex determination, total cementum annulation

Recognition of the importance of human teeth in personal identification has been recognized from time immemorial as they remain well preserved in a reasonably variable

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environment and even long after death.^[2] Data in the form of age, gender, and blood group might provide vital clues in identification.

Dental age estimation makes use of morphological, radiographic, histological, and biochemical methods to examine age-dependent changes in teeth.^[2] It is been hypothesized that the incremental lines in the tooth cementum can be used as a marker more reliable than any other morphological or histological traits in the human skeleton. Acellular cementum bands viewed in transmitted polarized light are characterized by alternating parallel opaque and translucent (wider) bands.^[3]

Gender identification has evolved over time, till the use of advanced techniques like polymerase chain reaction (PCR).^[4] The amelogenin gene is a discrete and major constituent, unique to the developing enamel and contains high concentrations of proline, glutamine, leucine, and histidine. The gene structure of this protein has been demonstrated, and it is confirmed that there are two amelogenin genes, one on the X chromosome and the other on the Y-chromosome in humans. The amelogenin gene is a single copy gene, homologs of which are located on Xp22.1–Xp22.3 and Yp 11.^[5] The difference in the pattern of these two genes is sufficient enough to be used as a sensitive sex determinant. In our study, we have used AMEL gene marker for gender identification.

In addition to the age and sex, the identification of blood group can increase the possibility of a positive match manifolds. Blood grouping from absorption-elution procedure devised by Vittorio Siracusa in 1923 is now employed in all forensic laboratories because it is proven to be most sensitive, reliable, and reproducible in identifying blood group of deceased individuals.^[6,7] The distribution of ABO substances from the pulp cavity wall to the dentin edge and to the enamel gradually decreases because of fewer possibilities of diffusion of antigens from both blood and saliva. The infusion sedimentation theory describes the infusion of water-soluble antigens from saliva into the tooth tissue.^[8]

Hence, in this study, using three parameters, under water, in normal environment for a time period between 2 days and 6 weeks after the tooth extraction, we made an attempt to extract maximum information from the teeth as this might help in forensics when there is a lack of available data for the identification of an individual.

Aim and objectives

The aim and objective of the present study were to determine age, sex, and ABO blood group of individual from a single tooth and to determine the effect of different environmental conditions on the same. The objective of this study was to extract maximum information from a single

tooth and at the same time also preserving some tissue for the further investigation whenever needed.

Materials and Methods

In this double-blinded study, a total of sixty extracted teeth were collected as a sample. The age of individuals (when the tooth was extracted) ranged from 18 to 72 years. Teeth were extracted either with periodontal disease or indicated by orthodontic or prosthetic reasons. The date of birth, date of extraction, gender, tooth identification, and the reason for extraction were recorded for each tooth, and each tooth was assigned a distinct serial number. The teeth were subjected to study under the distinct serial numbers with the information on age, gender, and blood group kept secret. The results were later correlated with the initially captured data. Ethical clearance from the Institutional Ethical Committee was obtained. Blood group identification was done during the routine hematology prior to extraction.

The extracted teeth were washed under running water for 5 min to remove blood, saliva, and debris attached to it and divided into four groups [Table 1]. Thereafter, the teeth were sectioned longitudinally in the buccolingual plane along the midline. A groove was made by a double-sided diamond disc mounted on a straight micromotor handpiece under continuous saline irrigation and then split using a hammer and chisel. The pulp was removed using a sterile spoon excavator for each sample to avoid contamination. The removed pulp was placed on a sterile glass slab and cut into half. Half of the pulp was kept in a sterile test tube containing normal saline for the blood group determination by absorption-elution method.^[9] Other half of the pulp was stored in T.E. buffer and subjected to gender determination using amelogenin protein as a marker in PCR technique. PCR primers were used to amplify 112 and 106 bp product of Y and X band, respectively.^[10]

Forward primer

5'-CCC TGG GCT CTG TAA AGA ATA GTG-3'.

Reverse primer

5'-ATC AGA GCT TAA ACT GGG AAG CTG-3'.

For determination of age using cementum incremental lines, one-half of the sectioned teeth was ground using Arkansas's stone to prepare longitudinal ground sections, which were examined under light microscope. Ground section of the teeth was prepared based on the method described by Stott

Table 1: Subgroups of the sample collected

Group	Enviornmental conditions	Number of days/weeks
I	Normal environmental condition	2 days
II	Normal environmental condition	6 weeks
III	Under saline water	6 weeks
IV	Under saline water	2 days

et al.^[11] The resulting slides were mounted and photographed under an optical microscope, and the resulting digital images were enhanced using Image J software, version 1.43s, NIH, USA aiming to enhance the cementum lines present in the image (by contrast enhancement), without alteration.^[12] The counting of cementum lines was made according to Kagerer and Grupe,^[13] where an observer had his scores confirmed by two other independent members (control observers). Each observer performed count in the region with the best visualization of the cementum lines.

By adding the average age of eruption in years (for each tooth presented) in the counted number of incremental lines, the chronological age of the individual was obtained.

$E = n + t$: Where, estimated age (E) = number of incremental lines (n) + eruption age of the tooth (t). This was compared with the prerecorded data.^[14]

The obtained results were statistically analyzed. The P values were obtained using Chi-square test. To check the correlation between known and estimated ages from cemental lines, Pearson's correlation coefficient was used. Further, the one-way statistical test method was also applied to check the mean age error between the known and estimated ages across all the groups and space saver reliability test was used to check the reliability of the test.

Results

In our study, to check the correlation between known and estimated ages within the four groups by counting the cemental incremental lines [Figure 1], the Pearson's product moment correlation coefficient (r) showed a high degree of correlation between the known and estimated ages among all the four groups [Table 2 and Graph 1]. The results of one-way statistical test method showed that the mean age error varied between - 0.282 in Group I to a maximum of 1.762 in Group II. The average mean age error among all the subgroups as calculated was 0.776. The F analysis of

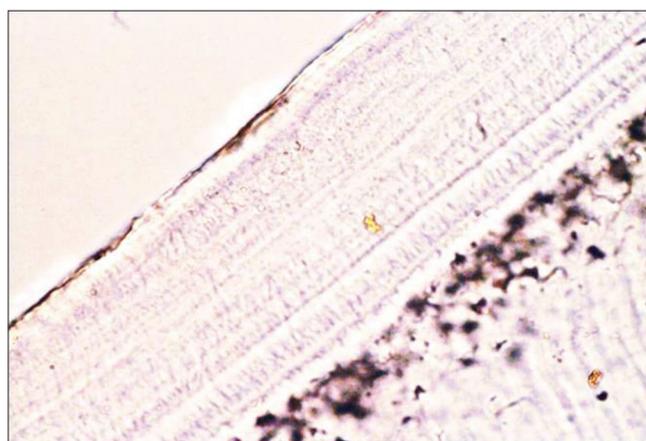


Figure 1: Pictomicrograph showing cemental incremental lines at x40

variance test was applied for comparing the calculated mean age errors for statistical significance. The F value calculated for this study was 2. The P value calculated was 0.057 (which is >0.05) thus proving that the mean age error difference between and within the subgroups is insignificant.

To check the reliability for the test method between the actual and determined age, the Space saver reliability test was used (the valid results of 55 were taken for the study and 5 invalid results were excluded for the same).

Sex determination was done with PCR method [Figure 2] which showed 100% accuracy in Group I, 86% accuracy in Group II, 73% in Group III, and 86% in Group IV [Graph 1]. On applying the Chi-square test (3.75), the P value between the groups is 0.2957 which is more than 0.05 thus indicating that there is no significant difference between the groups.

The determination of blood group [Figure 3] was done with 100% accuracy in Group I and Group II, while Group III shows an accuracy of 66% and Group IV shows an accuracy of 73% [Graph 1]. Statistically upon applying the Chi-square test (10.2), a significant difference between all the with P value (0.0126) which is <0.05 , while there is no significant difference among the other groups whose P value >0.05

Discussion

The teeth have for long been known to be resistant to environmental changes and have thus been used reliably for identification in disaster situations. In an attempt to increase reliability, this study was conducted to attempt determining three variants (age, sex, and blood group) from a single tooth.

Table 2: Pearson's product moment correlation coefficient between the known and estimated ages among all the four groups

Group	Pearson's moment correlation coefficient value
I	0.990
II	0.986
III	0.992
IV	0.994

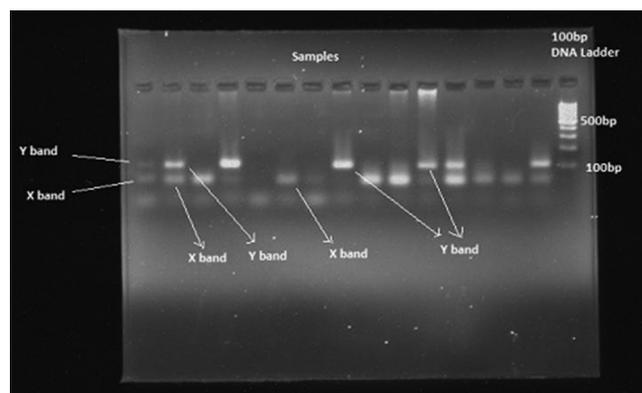


Figure 2: Electrophoresis in gel of amelogenin gene amplified

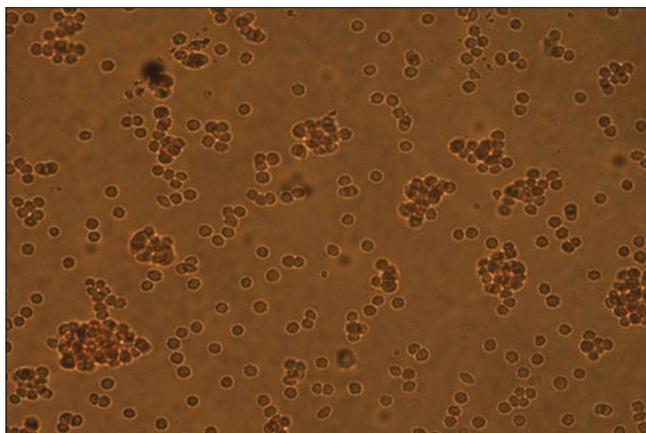
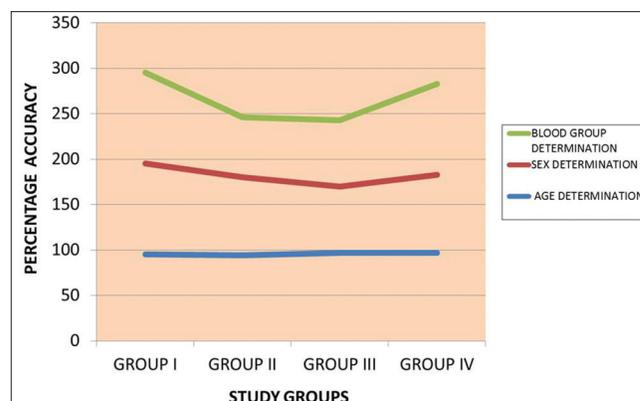


Figure 3: Pictomicrograph showing agglutination at x40

In the present study, acellular cementum in the longitudinal sections was used to calculate incremental lines as it is clearer because of the absence of cementocytes. In our study, we were able to determine the age with an age accuracy of 95% in Group I, 94% in Group II, and 97% in Group III and Group IV. Our results showed a high degree of correlation between the known and estimated ages among all the four groups by applying the Pearson's product moment correlation coefficient signifying the accuracy of the procedure. These results are in accordance with the studies conducted by Aggarwal *et al.*,^[15] Avadhani *et al.*,^[16] and Dias *et al.*^[14] The age estimation from teeth subjected to adverse environmental conditions or those studied after a reasonable time period were comparable to those from those in standard conditions. This indicated that the procedure may be reliably used in a variety of disaster situations. This finding is in accordance with Grosskopf (1989) who showed that this method is applicable to historical skeletons and cremations.

Further, we found that the interobserver reliability among the observer was statistically high which proved that the use of a photomicrograph lends a reasonable degree of objectivity to the calculation of cemental annulations. However, as noted in previous studies, the reliability of age estimation decreased with the increasing age particularly above the 55 years of the age. This may be attributed due to the prevalence of periodontal disease in the elder age group as in accordance with the studies done by Dias *et al.*^[14] Moreover, our findings showed that the teeth submerged under water also gave reliable age estimation.

Gender determination from the pulp tissue, our second parameter in the present study, showed high specificity using PCR technique in all the samples, and there is no significant effect of time or environmental conditions (water) on the sex determination from the pulp. The identification of the pattern of the AMEL gene on the X- and Y-chromosomes has been proved to be a sensitive test for gender determination. Our study shows a high specificity in all the samples for



Graph 1: Comparison of accuracy of results of all the three parameters among the predetermined four groups

gender identification using this system. Moreover, there was no significant change in specificity of the test even after submerging the teeth to environmental conditions for 6 weeks. This study highlights the use of pulp as a source of DNA for gender determination, as well as the sensitivity of the PCR technique. Our results were in accordance to the study done by Urbani *et al.*,^[17] Ghraeab,^[18] and Sivagami *et al.*^[19] The results showed that dental pulp serves as an excellent source of DNA, also yields a good quality of DNA which can be used for PCR-based amplifications. Any tooth regardless of the sex or age can be used and DNA extraction could be performed. We also collude with the opinion of Leo D and other coworkers who have established that pulp is a rich source of genomic DNA. In our study, the results show that the PCR-based method is a sensitive technique for sex determination, which can be done with a complete specificity even in adverse environmental condition or after prolong period of time. Our results are consistent with other authors who have utilized pulp as a source of DNA.

Although the identification of gender with reliability is sufficient in many cases, it is often desired to add more variables to narrow down the identification process.

Our third parameter, i.e., blood group determination showed 100% accuracy of blood group determination from pulp after 2 days of extraction in normal environmental condition and under the saline water in group I and group IV. These findings are in accordance to the study done by Smeet *et al.*,^[20] Xingzhi *et al.*,^[6] Shetty and Premalatha,^[9] and Inamdar *et al.*,^[21] who also showed highly accurate results in blood determination of individuals from the pulp of the extracted teeth. However, the reliability of the test decreased 6 weeks after extraction in both environmental conditions, which is 66% in Group II and 73% in Group III. These findings are similar to those observed by Inamdar *et al.*^[21] and in contrary with Sharma and Chattopadhyay,^[22] Haertig *et al.*^[23] stated that the overall decrease in reliability in the success rate may be due to the cell lysis, contamination of the tooth, or time lapse for the procedure. In our opinion,

the cell lysis or degradation of cells may be a reason for failure rather than contamination as we have taken adequate measures to avoid contamination and have got 100% results in the group examined 2 days after extraction.

Conclusion

Our study concludes that even though age, sex, and blood group are more reliably determined in freshly extracted teeth, these variables may be of significant help in identification even after a period of 6 weeks postextraction. As per the review of scientific literature, this is the first kind of study in which three parameters: age, sex, and blood group have been determined from a single tooth. Hence, further studies are needed with a larger sample size, under different environmental conditions to prove the accuracy of the present study. We hope that our attempt in extracting maximum information from a single tooth will be of benefit to society in disaster situations.

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Conflicts of interest

There are no conflicts of interest.

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