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Forensic identification of abo blood grouping from teeth under different conditions using absorption elution method

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ABSTRACT

Human identification is one of the biggest challenges faced, but with the advances in science, various techniques such as blood grouping have been used to identify different individuals. Forensic odontology analysis of dental evidence found that teeth are among tissues that can resist adverse environmental conditions, hence ensuring their features remain stable for a long period of time including blood group antigens which are known to be unique and unchangeable throughout an individual's lifetime. This study explores the use of techniques like the absorption elution method in ABO blood grouping analysis to determine the reliability of teeth found in different conditions such as in normal environmental conditions, drowning cases, and burial cases for ABO blood grouping and explores the impact of long-term storage of teeth under the conditions on ABO blood group antigens, their preservation as well as advances in blood group determination to provide reliable information for forensic purposes.

Keywords: *Forensic science, Blood group determination, Absorption elution technique, Teeth, preservation of blood groups, Advances in blood grouping.*

INTRODUCTION

The area of dentistry known as forensic odontology is concerned with the proper handling, examination, and presentation of dental

evidence and discoveries in the interest of justice.(Shamim, 2012) Teeth identification has been used since early time in the identification of dead bodies in AD 49, Julia Agrippina, the wife of a Roman emperor Claudius was able to identify Lollia by her canines, and in 1453, the identification of John Talbot, the England's first earl of Shrewsbury by his missing left molar and others. (Balachander et al., 2015)

Forensic odontology plays a major role in the identification of crime victims and deceased people of either mass disaster or others through the evaluation of anatomical components, dental appliances, and dental restorations. Through the years, there has been an evolution in identifying deceased subjects not only from skeletal remains but with blood grouping techniques, The ABO blood group system was first described by Karl Landsteiner in 1900 and remains the cornerstone of forensic blood group investigation.(Vala et al., 2017)

The term ABO blood grouping includes red blood cell (RBC) antigens whose specificity is determined by several genes that can be allelic or extremely closely spaced on the same chromosome, and once a blood group is established, it remains unchanged for a lifetime. (Mitra et al., 2014) Teeth and Bones are the major tissues that can survive for a long time even after mass calamities like fire or bomb blasts and others. Since tooth pulp contains a lot of blood vessels, blood group antigens are nearly clearly bound to be present in tooth pulp. Dentin is more durable than bone and makes up most of the teeth, it has a higher cell-to-matrix ratio than bone making it easier to get blood components from it.

Blood group compounds in dentin are thought to be found in dentinal tubules. The presence of blood group antigens in dentin and enamel has been demonstrated by infusion and sedimentation phenomena in combination with naturally occurring antigens, which is described as the infusion of water-soluble antigens from saliva into tooth tissue. (P. Kumar et al., 2016)

The principle of the Absorption elution method lies in the absorption of blood group-specific agglutinin on the surface of a substance having blood group agglutinogens. This technique was devised by Siracusa in 1923 and refined by Kind to use in teeth blood typing in forensic science. (Vala et al., 2017)

Various strategies and techniques were employed to determine the ABO blood group from teeth (pulp, enamel, and dentin) as well as the reliability of the absorption elution method. Methods

employed were the absorption elution method, slide agglutination method, absorption inhibition method, and different extraction methods. The samples analyzed were both extracted freshly, and others stored in different mediums or conditions for corresponding durations.

The aim of this study is to outline the use of the absorption elution technique for ABO blood grouping from teeth under different conditions for forensic purpose, highlighting its significance and methodology.

ABO blood grouping in forensic science

The blood groups are known to reside on the surface of red blood cells also called erythrocytes, having a concave disk shape with the role to distribute oxygen to tissue cells with the help of haemoglobin as structure referred to as blood group antigens. ABO blood grouping was first discovered by an Austrian scientist Karl Landsteiner in 1900, where he described three blood groups A, B and C which was later changed to blood group O. Later in 1901, Alfred von Decastello and Andriano Sturli discovered the AB blood group. Blood group antigens consist of proteins, peptides and polysaccharides which make up their complex structure. These blood groups are made up of end terminal sugars such as N-acetyl-D-galactosamine as a dominant sugar for A antigen (group A) and D-galactose for B antigen (group B), for group AB both sugars are attached on the red cells as for group O no sugar is attached to the red blood cells. ABO blood groups have naturally occurred antibodies anti-A and anti-B which attach themselves to the red cell surfaces of different red cells leading to clumping known as agglutination and all the blood groups have ABO genes located on chromosome 9.(H. Graham, 2023)

ABO blood group antigens are found at birth and stays even after death for a long period of time in body tissues even in decomposed bodies with an estimated post-mortem interval less than 85 hours. This is helpful in forensic science, mostly in human identification and case solving as it can give information about the suspect, victim or missing person based on the presence or absence of specific blood groups. It was observed that in cases where DNA analysis is not possible, ABO blood grouping remains reliable for the identification even in various environmental conditions.(K. Kumar et al., 2021)

In cases of rape where there is lack of nuclear material for DNA analysis due to the washed off blood stains or absence of semen, ABO blood grouping can be effective as the antigens remain

stable even in adverse conditions proving the reliability of ABO blood grouping even in this advanced era of DNA profiling.(Palaskar SG, 2015)

ABO blood grouping was used since early 1900s by forensic medicine to differentiate human and non-human blood for legal purpose, it was also employed in anthropology for insights into gene flow and racial origins, in paleo-serology to compare modern populations with their ancestors and other fields to improve the value of blood evidence in forensic investigations.(Smith Madeleine, 1960)

The rise of criminal cases as well as paternity disputes in forensic investigations enhanced the use of ABO blood grouping to establish genetic connections between individuals involved in legal disputes. Blood group antigens were used as genetic markers to determine parentage and biological relationship between individuals based on the inheritance pattern.(Bangham, 2014)

Absorption elution method for ABO blood grouping

The absorption elution method is a serological assay for blood group identification where antibodies are attached to antigen receptors on dry blood stains, which are then eluted for absorption to indicator cells to provide agglutinations observed under the microscope. This technique uses ABO blood groups, MNSs and Rhesus systems for the blood grouping in forensic analysis. It is used for typing blood stains, salivary stains, nails, seminal stains, hair, and other tissues. The principle of the absorption elution method involves the elution of antibodies which have been previously attached to antigen receptors to be absorbed onto the appropriate indicator cells. In various countries such as Japan it is performed before DNA profiling.(Sen et al., 2015)(Kobayashi et al., 1999)

The absorption elution method starts with ammonia extraction which was first discovered by Kind and Cleverly in 1969 and later modified by Chisum in 1971. The sample is placed in a test tube containing 5% ammonia solution until a dark brown color is seen indicating the extraction of blood components. The fluid is then used for the absorption elution method which involves the absorption of antibodies to the sample at low temperature and their elution at a higher temperature to detect blood grouping by observing agglutinations under the microscope.(Sen et al., 2015)(Kobayashi et al., 1999)(Wyatt & Parker, 1972)

The purpose of using this technique is to provide definite evidence for identification individuals involved in criminal activities by adding to the individuality of bloodstains, making them more specific in identity for forensic investigations. The modified absorption elution method was introduced by reducing the elution temperature leading to more definite agglutination patterns improving the blood group determination accuracy, this method permits the rapid grouping of blood stains making it more effective for forensic science applications.(Sen et al., 2015)

Studies have shown that Monoclonal antibodies can be used as substitute of polyclonal antibodies due to their sensitivity to erythrocytic antigens to determine the ABO status in various forensic samples. These Monoclonal antibodies are produced by a single clone of cells specific to certain antigens such as erythrocytic antigens specifically, blood group antigens. The solvent displacement is said to enhance the specificity and sensitivity of monoclonal reagent for certain samples which improves the accuracy of forensic blood grouping analysis. The absorption elution method takes place into two phases: The absorption phase and the elution phase. The monoclonal antibodies are added to the sample such as blood stain, salivary stain, semen stain or tissues where they bind to the ABO antigens present on the erythrocytes in the sample in the absorption phase, the sample is washed and heated to elute the antibodies which are then mixed with red cell suspensions to observe agglutination. However, the use of monoclonal antibodies can be affected by the solvent used, as displacing the solvents containing bovine serum albumin (BSA) or human AB serum can enhance the hemagglutination reaction which affects the specificity and sensitivity of monoclonal antibodies hence improving the accuracy of ABO blood grouping analysis in forensic science.(Kobayashi et al., 1999)

The absorption elution method was also found reliable for Rh (Rhesus) antigens identification from dried blood stains for solving criminal cases and paternity disputes in forensic investigations. This method was also used to test for specific reactions like anti-D, anti-C, anti-Cw and anti-E, however, it was found that the specificity of anti-c and anti-e antigens was challenging but showed potential for Rh antigen.(Bargagna & Pereira, 1967)

MATERIAL AND METHODS

A thorough search of literature was conducted using databases such as PUBMED, SCOPUS, ScienceDirect, ResearchGate and Google Scholar. Keywords used for the search were Blood

grouping determination in teeth, ABO blood grouping in teeth using the absorption elution method, Advances in blood grouping and others. Both review articles and original articles were considered as well as books related to the topic of interest. The exclusion included studies not related to the topic as well as those about blood grouping but not in teeth. Finally, all studies were reviewed to select the most relevant ones based on the topic. The study was then divided into the blood grouping analysis from teeth, preservation of blood group substances in teeth under various conditions and methodological advances in forensic abo blood group determination from teeth.

BLOOD GROUPING ANALYSIS FROM TEETH

Human teeth were used for generations in individual identification using bitemarks and the teeth arrangement for determining the suspects involvement. Until now research is still going on to prove the uniqueness of each person's dentition and its impressions.(D. Sweet & I. A. Pretty, 2001)

Human teeth carry blood group antigens in blood vessels especially present in the pulp where they are more concentrated. These antigens can also be found in other secretions as well as in dentin and enamel, as they diffuse from the pulp cavity wall to the enamel. However, this diffusion leads to a decrease in concentration in enamel. The ABO blood grouping from teeth made it easy for individual identification especially in mass disasters or adverse environmental conditions due to their unique resistance and morphological characteristics.

The determination of ABO blood grouping of an individual is done using the absorption elution method and the absorption inhibition method. This is important in forensic applications as ABO blood grouping identification from teeth remains a reliable source when other tissues are unavailable especially in cases of decomposition. Various studies have highlighted the reliability of teeth in ABO blood grouping identification as well as its significance in forensic science.(Shah et al., 2019)

Absorption elution method in teeth

The absorption elution method in teeth starts with the extraction of pulp and dentine. The word extirpation means to root out, to eradicate or to wholly get rid of or expel something. So, pulp extirpation is the removal of pulp from the tooth.(Ingersoll, 1880) From various studies, a modeling wax block was used for embedding and the sectioning of extracted teeth was done using micromotor and carborundum disc longitudinally as shown in Fig. 1(Das et al., 2023) and a hand-

held Pulp Isolator device designed to split the pulp without a heat generator.(Motawei et al., 2018) A drop of normal saline is then placed on the extirpated pulp to wet it. The pulp was removed using the excavator spoon or tweezers, then kept in a test tube where it was later used for the blood grouping identification(Das et al., 2023). The remaining tooth was pulverized using mortar and pestle to get a fine powder which was stored dry in sterile test tubes.

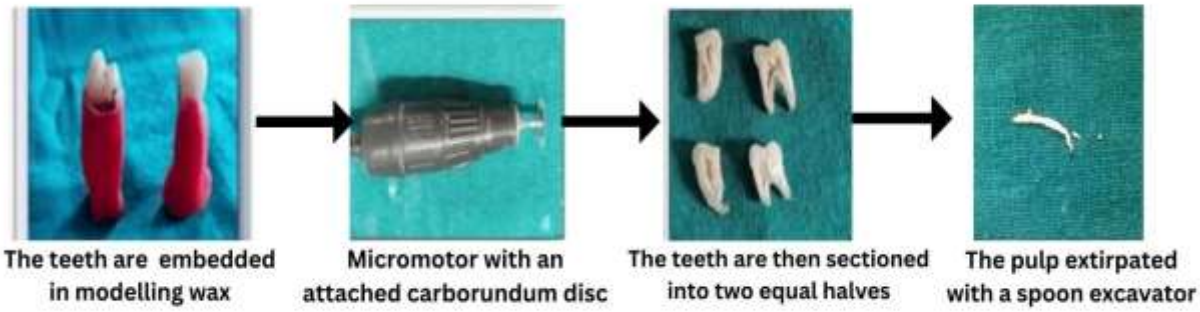

Figure 1: Illustrates the mounting and sectioning of teeth.(Das et al., 2023)

The process of the absorption elution method on teeth.

Antiserum A, B, and D with 0.5% red cell suspension of A, B, and O known blood group are used in the absorption elution method for blood group identification. They were mixed and kept at 4 degrees Celsius overnight, then later washed 3 to 6 times to remove the antisera. Then observed for agglutination in all test tubes, the whole process is shown in Fig. 2.

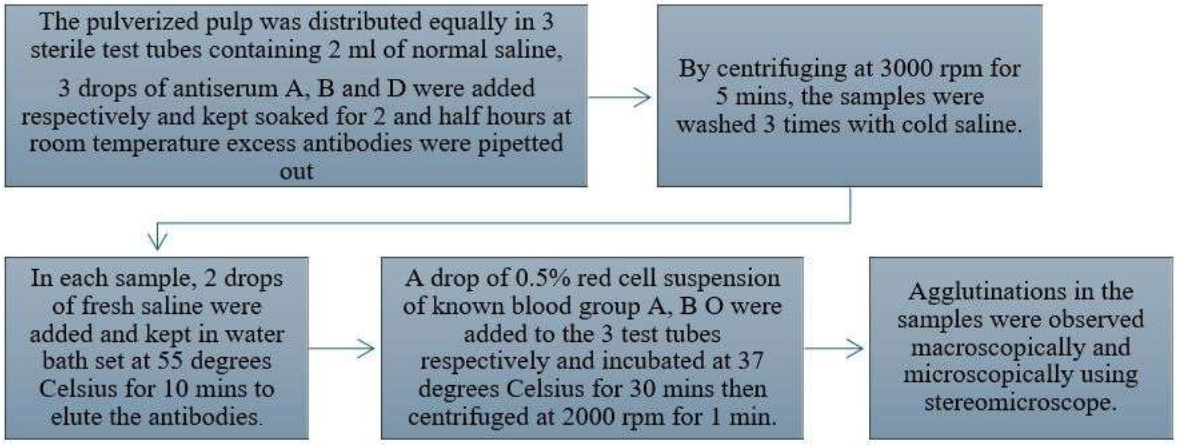

Figure 2: a flowchart describing the process of ABO blood grouping from dental pulp using absorption elution technique.(Das et al., 2023)

PRESERVATION OF BLOOD GROUP SUBSTANCES IN TEETH UNDER VARIOUS CONDITIONS

Blood group substances are known to be found in the dental pulp protected within the enamel and dentin as well as in the dentinal tubules filled with dentinal fluid for preservation. Teeth are known as indestructible and capable of surviving for a thousand years making it a stable source for blood grouping under different environmental conditions.(Mishra et al., 2021)(Prabhawati Inamdar P. I. et al., 2011)

Various studies have found that blood antigens are not affected by environmental conditions and remain stable over a period. Blood group antigens in teeth are mostly preserved by the structure of the tooth as it can withstand even the highest and lowest temperature without losing its microstructure. Blood group antigens as well as DNA can be extracted from teeth after exposure to high temperature but beyond 200^{o}C it was found that only teeth protected by soft tissues like dental pulp can still maintain the ABO antigen activity. This shows that blood group antigens can remain stable and resist incidents such as fire accidents or explosions. In cold environments such as below -20oC, blood group antigens and DNA are found to be preserved in the dental pulp leading to a significant amount of amplifiable DNA as well as blood group antigens valuable in the identification of decomposed bodies found in avalanches or other cold weather disasters. At room temperature, dental pulp was found to be effective for blood grouping even after six months.

Apart from the resistance and stability of teeth in different temperatures, environmental factors such as soil humidity and PH in soil burial cases were found to affect the decomposition rate but the teeth remained reliable for DNA and blood grouping extraction even in teeth buried for up to 80 years.(Singh et al., 2019)

Studies have found that the highest percentage of positive results for blood grouping was given by permanent teeth compared to deciduous teeth either on the day of extraction or after 14 days, the effects of autolysis and dehydration on pulpal antigens was found to affect the rate of positive results of blood grouping as the time passed.(Prabhawati Inamdar P. I. et al., 2011)

Blood group antigens preserved in blood vessels within the dental pulp at a pH of 4 and pH of 7, were found to show 100% preservation for blood grouping while those at a pH of 10, dropped to 10% preservation rate.(Shah et al., 2019)

Teeth remains sensitive for the determination of blood grouping even after harsh conditions making it a valuable source in forensic investigation even when other sources may not be available such as DNA profiling.(Prabhawati Inamdar P. I. et al., 2011), (Singh et al., 2019)

BLOOD GROUPING FROM TEETH UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

As teeth are known to be one of the hardest structures which can resist various adverse environmental conditions (Prasath & Athanari, 2021), different studies were conducted to identify the reliability of ABO blood grouping using the absorption elution method in teeth retrieved from different environmental conditions such as normal conditions (dry), drowning cases (water) and burial cases (soil).

Normal conditions (dry)

Various studies were conducted over the years illustrating the determination of ABO blood grouping from teeth under normal condition or stored in dry condition and to assess their preservation and reliability as well as the impact of these conditions on the absorption elution method. Researchers have analysed blood grouping from teeth, especially from pulp. The dentin and enamel were found to contain organic components where the enamel contains 1 to 3% of the components and the dentin has 28%, these are known to affect the presence of blood group antigens while the pulp with its protected nature was found to contain antigens detectable even after a long time such as 6 weeks, and 6 to 12 months, making the pulp more reliable for blood grouping than the dentin and enamel. The dentin was also found reliable for blood grouping due to the presence of blood group antigens present in the dentin tubuli, but the reliability of the enamel was found to be low due to the limited possibility for diffusion of antigens from saliva and blood into enamel reducing the presence of blood group antigens.(Smeets et al., 1991)

ABO blood grouping was also determined from the dentin and pulp of deceased individuals extracted and stored at room temperature for 6 months using the absorption elution method. The pulp showed a high sensitivity of 96.7% for ABO blood grouping even after 180 days post-mortem with no significant difference between the blood group from teeth and that from reference samples while the dentin the dentin may not be reliable after a long time.(Shetty, 2010)

Another study confirmed the identification of ABO blood grouping and rhesus factor from the deceased teeth using 60 samples where 57 of them showed positive results with a high correlation in comparison with the blood grouping of capillary blood using slide agglutination.(Aswath et al., 2012)

The use of the modified absorption elution method was employed to identify blood grouping from hard and soft tissues of teeth in a study comprising of 60 samples stored for 6 weeks, 6 months and 12 months in dry conditions for the comparison purpose, it was found that after 6 weeks, the pulp exhibited a sensitivity of 93.3% and 86% for the hard tissue which later decreased after 6 to 12 months to 83.3% for soft tissues and 76.6% for hard tissues which proved the reliability of teeth for blood grouping even after extended storage periods. The overall teeth (the combination of pulp and dentin) showed 66.6% after 6 to 12 months storage. It was also found that the sensitivity decreased as the age increased, to mean older teeth showed less pulp sensitivity and the maxillary teeth were found to give better results than mandibular teeth after 6 to 12 months storage in male samples.(Ramnarayan et al., 2013)

Later, a study showed that dental pulp was 100% accurate for ABO blood grouping determination after 2 days of extraction in normal environmental conditions, however after 6 weeks the accuracy decreased, still with a high positive correlation with the reference blood samples, revealing its significant help in forensic investigations.(Nayar et al., 2017)

Similar findings were found in a study of Farhan, where the blood group from dental pulp was found to have a high correlation with venous blood as control samples till 6 months storage in dry conditions. The rhesus factor from teeth also showed a high correlation which decreased with time based on the storage duration showing the effects of the storage condition on ABO blood grouping and rhesus determination from teeth as well as the accuracy of the absorption elution method.(Farhan dil & Asfandyar ahmed, 2018)

The ABO blood group determination using the absorption elution method was found to provide good internal consistency compared to the conventional blood typing. With the teeth stored dry for 2 months providing a sensitivity of 80% shows that the storage does not affect the blood group identification as well as limit the accuracy of the absorption elution method proving that dental pulp can be used for personal identification even when found in mass disasters.(Vrinda Saxena et al., 2017)

The pulp from aged teeth stored for 6 to 12 months was found to be less accurate compared to that from freshly extracted teeth but still reliable for ABO blood grouping determination while for the dentin which also showed a high correlation with the reference blood groups, its accuracy was found to decrease not only with the storage duration but also with the increase in age of the individual. However, it was found that the age, sex, and jaw distribution of the individual does not significantly affect the accuracy of both pulp and dentin making the reliable for ABO blood grouping even after a long-time storage.(Motawei et al., 2018)

In a study conducted by Das M et al, freshly extracted teeth stored at room temperature for different durations (immediately, after 3 months and after 6 months) were analysed, it was found that the correlation with the reference blood groups decreased over time as those analysed immediately exhibited 86.67%, after 3 months it was 76.67% while after 6 months only 33.33% was found. It was revealed that the decrease in sensitivity after 6 months was due to the decrease in pulp antigenicity showing the importance of storage conditions over the sensitivity of the absorption elution method.(Das et al., 2023)

The reliability of dental pulp in ABO blood grouping for human identification has been thoroughly investigated throughout several decades of research. The usefulness of blood grouping on tooth pulp for identification after a relatively long storage period and from deceased person highlighted a high correlation between the dental blood group mainly the pulp and reference blood groups, with a high sensitivity of the absorption elution method which was revealed to decrease over time in some studies. These investigations illustrate the consistent efficacy of teeth in ABO blood grouping for forensic identification, providing useful insights for a variety of identifying situations.

Drowning cases (water)

In cases related to drowning (water), several studies were conducted to illustrate the reliability of teeth for ABO blood grouping obtained from those cases using the absorption elution method. In a study conducted by S. Ragul Prasath and Dr. Abirami Athanari, the reliability of teeth for ABO blood grouping stored under water simulating drowning cases was evaluated. It was found that water does not significantly affect the determination of ABO blood grouping using the modified absorption elution method as the pulp showed a positive result with 100% sensitivity in blood

group estimation immediately and after one week, this proved that the blood group antigens in teeth can remain stable for at least up to 6 months when in water.(Prasath & Athanari, 2021)

Later, the effectiveness of the absorption elution method and the absorption inhibition method was assessed for the determination of ABO blood grouping from teeth specifically dentin and pulp stored in seawater. The absorption elution method was found to be more reliable compared to the absorption inhibition method with a correlation with the control sample of 80% in pulp and 47% in dentin while the absorption inhibition method gave 21.7% in pulp and 5% in dentin. As for the reliability of teeth in determining ABO blood grouping, the pulp was found to be more reliable compared to the dentin as in comparison with the reference sample, the pulp exhibited 90% positive result without any storage medium to mean when dry, and 75% after 1 month same as after 2 months in seawater, while the dentin positive rate decreased to 55% without storage, 45% after 1 month in seawater and 20% after 2 months in seawater.(Dineja Raman et al., 2023)

Recently another study was conducted to determine the ABO blood grouping from teeth using the absorption elution method and to determine whether the identification could vary based on age and sex. The pulp and powdered dentin were used to test for the presence of rhesus and ABO blood group antigens where both exhibited accurate results even when stored underwater. At room temperature, the pulp showed 97.78% accuracy with 82.22% for dentin, while under water, the pulp exhibited 93.3% accuracy with the dentin showing 77.78% accuracy. The identification of blood grouping based on age and gender revealed no significant difference.(Rao et al., 2024)

The decrease in the sensitivity of the absorption elution method while determining the ABO blood grouping from teeth was found to be due to cell lysis or degradation and associated with the increase in age in most studies but still it was found that despite the effects of the environmental conditions on the tooth, teeth stay as a reliable source for ABO blood group determination in forensic investigation for individual identification mainly the pulp due to its protected nature to resist harsh conditions.(Dineja Raman et al., 2023)(Rao et al., 2024)

Burial cases (soil)

Burial cases or teeth samples found in the soil are among the most adverse conditions for ABO blood grouping analysis. Different studies were conducted to determine the reliability of ABO blood grouping from teeth found in burial cases.

Soil simulating burial cases was found to have similar impact on teeth as water (Drowning cases) while determining the ABO blood grouping, 100% positive result was found in blood group estimation from teeth stored in mud in the study of S. Ragul Prasath and Dr. Abirami Athanari when stored up to 6 months, this showed that the blood group antigens in teeth remain stable even after a long duration in mud which is helpful in individual identification.(Prasath & Athanari, 2021)

The comparison between the absorption elution method and the absorption inhibition method showed that the absorption elution method is more reliable when determining the ABO blood grouping from teeth stored in soil than the absorption inhibition method. ABO blood group determination from pulp and dentin after 4-6 months of storage in soil showed 78% sensitivity in pulp and 4% in dentin in correlation with reference blood groups proving the pulp to be more accurate compared to the dentin. When comparing methods, a sensitivity of 95.83% was found using the absorption elution method and 93.75% when using the absorption elution method. This proved that the soil does not significantly affect the reliability of the techniques when determining the ABO blood grouping from teeth.(A.NILOPHAR, 2016)

In another study, when determining the ABO blood grouping and rhesus from teeth stored in soil using the absorption elution method, the pulp showed 93.3% accuracy with 77.78% accuracy for dentin, revealing that soil exposure does not significantly affect the accuracy of teeth in ABO blood grouping. This identification was similar either based on age or sex which showed that the age and sex of the individual did not significantly affect the ABO blood grouping determination from teeth. both the pulp and dentin were found effective in the determination of ABO blood grouping despite the storage condition and duration with the pulp providing a slightly higher accuracy compared to the dentin.(Rao et al., 2024)

Research investigated the reliability of teeth in ABO blood grouping under soil conditions (burial cases). Using the absorption elution method, pulp showed a high sensitivity compared to the dentin

sensitivity. Age-dependent differences were seen, where both pulp and dentin gave mainly negative results with the older the age.(A.NILOPHAR, 2016)

METHODOLOGICAL ADVANCES IN FORENSIC ABO BLOOD GROUP DETERMINATION FROM TEETH

Despite the advances in human identification using DNA typing, ABO blood grouping still stands as one of the helpful tests in forensic investigation especially in cases when DNA is not likely available, blood group antigens can resist even the most adverse conditions. Despite the easy application and cost effectiveness of the traditional method of ABO blood group identification involving serological methods, they were found to have some limitations leading to inconclusive results in some cases. Factors like dehydration autolysis, contamination, and loss of antigens in some cases were found to affect the accuracy of the serological method in ABO blood group identification. Indirect typing methods involving *histochemical techniques* have been employed for ABO blood group determination in teeth. However, these techniques were found to be time consuming and required high performance skills to provide better results affecting their use in forensic analysis. The advance in DNA technology has evolved the ABO typing methods for forensic analysis provide more accurate results in comparison to the traditional serological methods. *DNA analysis* for ABO blood group determination from teeth offers more reliable results due to its high sensitivity and specificity.(Ghosh Sandip, 2022)

The development of different *Genotyping methods* for ABO blood group determination has contributed to the forensic investigations in individual identification. The ABO gene is localized on chromosome 9 with major alleles such as A, B and O having various single nucleotide polymorphism (SNP) sites. It consists of 7 exons encoding for the glycosyltransferase that helps in ABO blood group determination. The ABO genotypes are identified with the use of single nucleotide polymorphism (SNP) profiles made from the patterns of nucleotides at each SNP locus. The detection of the specific nucleotides at each SNP locus is required to determine the pattern of the single nucleotide polymorphisms (SNPs).(Taira et al., 2018)

The polymerase chain reaction (PCR) based genotyping is the most used for the determination of ABO blood group from teeth as it allows the amplification of the specific DNA regions particular to the ABO blood group alleles for blood grouping. This method is highly sensitive and specific to

the blood groups enabling the overcoming of the limitations of the traditional methods of blood grouping. It includes techniques such as polymerase chain reaction- single specific primer (PCR-SSP), droplet allele specific polymerase chain reaction (Droplet-AS-PCR), polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) and polymerase chain reaction sequencing based typing (PCR-SBT).(Ghosh Sandip, 2022) (Hui Li Qing Chen et al., 2022)

Another method for genotyping ABO blood groups from teeth is *short tandem repeat (STR) analysis* which involves the analysis of variations in specific short tandem repeat loci associated with the ABO blood group gene located on chromosome 9. Other methods of genotyping include SNaPshot, Matrix assisted laser desorption/ ionization time of flight mass spectrometry (MALDI-TOF MS) and the Next generation sequencing (NGS) which involves the whole genome, exome, and the targeted sequencing to enhance the accuracy of ABO blood grouping providing reliable results and characteristics of ABO blood group antigens for the forensic investigations.(Ghosh Sandip, 2022) (Hui Li Qing Chen et al., 2022)

The introduction of *microfluidic paper-based analytical devices* in 2007 provided a robust outcome in the determination of ABO blood grouping due to their simplicity, rapidity, portability, and economical functionality for blood grouping it is the mostly use due to its ability to passively transport fluids by capillary action and eliminating the need power or external pumping. The two main mechanisms of the microfluidic paper based analytical devices blood typing includes the lateral chromatographic flow method and the vertical flow through method to detect blood group antigens visually. The ABO blood group determination using paper- based analytical devices (PADs) in teeth has contributed to a significant level in forensic investigation mainly for the on-site analysis of blood group antigens. These devices are portable and biocompatible making them suitable for point of care testing which includes teeth, especially where the traditional laboratories may not be available. The advantages of PADs can also be applied in blood group identification from teeth samples enhancing quick and reliable results as well as the efficiency of investigations.(Ebrahimi Fana et al., 2021)

CONCLUSION

A high sensitivity of ABO blood grouping from teeth under different adverse environmental conditions using the absorption elution method was observed. The pulp of the tooth was found to

be more sensitive than the dentin and the comparison between the absorption elution method and the absorption inhibition method showed that the former is the most appropriate method to use in blood grouping in most of the cases. It was also shown that an individual's age might influence sensitivity, with younger people exhibiting greater beneficial outcomes. Blood group antigens were found to be preserved mostly in the pulp even after long storage time. Through an analysis of the data obtained from the three different environmental conditions—soil, water, and dry it was found that blood grouping on tooth pulp may be useful for forensic investigations and the identification of missing persons, particularly in times of disaster. With the development, Advances in blood grouping were established to provide quick and accurate results compared to the traditional method of blood grouping.

FUTURE PERSPECTIVES

Advanced studies are required to validate and expand upon these findings, including exploring the reliability of tooth pulp blood grouping in different populations as well as other cases such as arson cases or burn cases and investigating or researching on other dental components for blood grouping analysis.

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