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Survival of Dental Pulp Tissue under Different Climatic Conditions: A Review

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Abstract:*The established importance of Forensic Dentistry for human identification, mainly when there is little remaining material to perform such identification (e.g., in fires, explosions, decomposing bodies or skeletonized bodies), has led dentists working with forensic investigation to identify the deceased individual and the culprit in medico-legal cases. The blood group once it is formed in an individual can't be changed in their life time and dental pulp is protected by the surrounding calcified structures namely enamel and dentin, so blood group and dental pulp use has its own significance in identity. This review discusses about the survival of dental pulp under different climatic conditions.*

Keywords:*Forensic odontology, Human identification, Pulpal blood group*

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I. Introduction

Forensic identification has been defined as the multidisciplinary team effort which relies on positive identification methodology and also on presumptive or exclusionary methodologies. This is a branch, which deals with identification and has many maxims, the best known of which, is that every contact leaves its trace. Typically, this effect involves the cooperation and co-ordination of law enforcement officials, forensic pathologists, forensic odontologists, serologists and criminologists.[1]

Keiser-Neilson defined forensic dentistry as "that branch of forensic dentistry that in the interest of justice deals with the proper handling and examination of dental evidence and the proper evaluation and presentation of dental findings.[1]This branch of dentistry aims at investigating physical, chemical, psychological as well as biological phenomena which not only covers human beings (alive, dead or body fragments) but also the comprehending aspects of human identification; criminal, civil, labor and administrative forensic investigation; forensic tanatology; legal documents; forensic traumatology; image (x-ray, tomography) examinations; saliva analysis; and other aspects involving a multidisciplinary team.[2]Forensic Odontology, as a science, did not appear before 1897 when in a doctoral thesis entitled "L' Art DentaireenMedecineLegale" written by Dr. Oscar Amoedowhich enlightened about the utility of dentistry in forensic medicine with particular emphasis on identification.[3]A remarkable work has been done by Forensic Dentistry in the process of human identification.

Dental professionals can carry out investigations involving biological materials with the aim of establishing human identity derived from the human body in various conditions (quartered, dilacerated, carbonized, macerated, putrefied, in skeletonization and skeletonized).[2]
According to American board of forensic odontology dental identification can be divided into four types:[4]

1. Positive identification: The ante-mortem and postmortem data match to establish that it is from same individual;
2. Possible identification: The ante-mortem and postmortem data have few consistent features, but because of quality of the records it is difficult to establish the identity;
3. Insufficient evidence: The data is not enough to from the conclusion;
4. Exclusion: The ante-mortem and postmortem data clearly inconsistent.

Antemortem dental record is a data that contains information of treatment undergone and dental status of a person during his/her lifetime. Postmortem dental record is a data that contains information that is obtained from autopsy report, photograph, radiograph, finger print, lip print, finger nail scrapping and bite mark.[5] The ante-mortem records are compared with the dental status of the cadaver giving strong evidence of the identity of the cadaver. A thorough dental profile is taken in case where there is no dental anamnesis. This process helps in sorting the already existing ante-mortem material and results in selecting the information that most fits to the profile of the cadaver.The importance of identification of human remains with methods of high accuracy is better understood in cases where the identification of the cadavers is impossible due to the deformities caused by a disease that ailed the person and finally leaded to his/her death or by a natural or an aviation disaster.[5]

The most indestructible component of the human body is the Teeth may remain more or less intact for many years beyond death. They are biologically stable and contain information about the physiological and pathological events in the life of an individual which remain as markers within the hard tissues of the teeth. During a disaster even after the other tissues are destroyed, teeth have been found to survive. That's why teeth are the most durable organs in the body which can withstand the temperature of 1600°C without the loss of its microstructure.[6]For the human identification, Fingerprints have been from a long time. But in situations like fire and skeletonization, fingerprints are very easily wrecked. In addition, prior to the death of the victim, experts need to deploy comparative elements of the victim which includes the dental records so as to carry out the identification process. However, this documentation may be unavailable or incomplete. In the present scenario, with the use of bio-molecular resources for human identification, it has become possible to identify a person with the use of small amounts of deteriorated biological material, conditions that are relatively frequent in forensic analysis.[2]A practical explanation of this fact could be demonstrated after the South Asian tsunami disaster on December 26th in the year 2004, where the most diverse techniques were used for identification of thousands of victims, such as forensic pathology, forensic dentistry, DNA profiling and fingerprinting. In this calamity, about 99% of the cadavers were identified using dental records or fingerprints and from DNA profiling, only 1% of forensic identification was made.

The prime exogenous factors associated with fire like flames, heat and explosions may curb the recuperation of information from body remnants and thus restrict the procedure of human identification.[7]On this matter, the teeth, due to their high uniqueness of dental characteristics in addition to the relatively high degree of physical and chemical resistance of the dental structure, play an important role in identification and criminology.[2]Because of their ability of ensuring environmental changes, teeth also represent an excellent source of DNA because of the reason that this biological material may provide the necessary relation for identification of an individual in cases where there have been failure of conventional methods for dental identification.[7]Though advances have been made in terms of Deoxyribonucleic acid (DNA) analysis, rugoscopy, cheiloscopy (lip prints), fingerprinting, etc., blood grouping still has a major role in forensic science in the field of person identification, paternity dispute, and other scenarios. This is attributed to the fact that genetic and antigenic constituents of an individual are not affected by environmental conditions.[8]

Lattes has aptly said "The fact that belonging to a definite blood group is a fixed character of every human being and can be altered neither by the lapse of time nor by intercurrent disease".[8] Blood group like fingerprint is an unalterable primary character. The need for personal identification arises in natural mass disasters like earth quakes, tsunamis, landslides, floods etc., and in man-made disasters such as terrorist attacks, bomb blasts, mass murders, and in cases when the body is highly decomposed or dismembered to deliberately conceal the identity of the individual.[9] Because of the resistant nature of dental tissues to environmental assaults, such as incineration, mutilation dental tissues offer an excellent source in forensic analysis.

The presence of blood group substances and other genetic markers such as enzymes in soft and hard dental tissues makes it possible to assist in the identification of highly decomposed bodies.[9] Pulpal tissue being contained within dental hard tissues; the post-mortem changes are seen very late. The pulp which is enclosed by these hard tissues is well protected which carries the ABO blood group antigens in a comparatively larger proportion than dentin and enamel. In dentin, it is presumed that these substances are located in the dentinal tubules.[8]

The possible distribution of ABO substances from the pulpal 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. It undergoes degeneration, necrosis and putrefaction inside an exfoliated tooth which takes a period of weeks to months.[8]To check the usefulness of the pulpal remains during and after a period of time lapse at various environmental conditions are very necessary. So far not much is known about employing these time-related

changes of the dental pulp, hence this review is an attempt to determine time-related changes in dental pulp samples from teeth under different climatic conditions.

a) Thermal Changes

Teeth resist long exposure to soil or water and also relatively high temperatures. It has been shown that even when practically all other parts of the body have been changed to such a degree that they are no longer recognizable, the teeth may be present without any noticeable changes.

(i) Extreme heat

Korszun A.K et al in 1978[10] studied the thermo-stability of ABO blood group antigens in dental pulp using differential scanning calorimeter. 30 non carious teeth were taken from donors of ABO blood group. The heat diffusion study shows that dentin and enamel were poor insulators and give inadequate thermal protection to the pulp when the external temperature risesto 200°C or more. Therefore in 200°C only those teeth protected by the tongue and cheeks or by bone would be expected to exhibit ABO antigen activity. Out of three antigens tested, B antigen showed greatest resistance to heat.[10]

Schwartz TR et al in 1991[11] determined the effects of varying temperature changes on the deoxyribonucleic acid (DNA) obtained from dental pulp. They isolated high molecular weight (HMW) DNA from teeth at 4°C upto6 weeks. At 25°C, HMW DNA was isolated after 19 years and at 37°C, teeth can yield HMW DNA following storage for 6 months.[11]Another study was by BregtSmeets et al in 1991, where they found tooth pulp with lot of blood vessels and showed blood group antigens are bound to be present in the pulp.[12]Among the several cases described in the literature with DNA isolation from teeth, a very important report was published by Sweet and Sweet in 1995.[13] This paper presented a case of human remains identification, by a preserved un-erupted third molar which enabled 1.35 µg DNA extraction from the dental pulp of the victim body burnt at approximately1093°C for30-40 minutes.[13]In a study done by Shetty V et al, DNA quantification was done on 128 teeth samples using nano-drop spectrophotometer after heat treatment under different ranges of temperatures. It showed amplifiable DNA present at the temperature range of 100°C, 200°C, 500°C, 600°C, 700°C and 800°C, with variations after 500°C. Thus, it is possible to depend on the results of ABO blood grouping after fire accidents to certain levels of temperatures.[14](Table 1)

Temperature	Histopathology of pulp
100°C	Pulp intact, displays fibroblasts, blood vessels and collagen fibers, tissue destruction seen to some extent
200°C	Pulp in multiple bits, most of the cells undergo degeneration
500°C	Carbonated tissue remains are seen

Table1. Histopathology of Pulp

(ii) Extreme cold

Violence and crime in human lives from bomb explosions, wars, plane crash and natural disaster make identification of victims difficult.It's also important to identify the Climbers, backcountry skiers and the army officials who die under the snow when the Avalanche hits. The oral health care providers have played a vital role in the recovery of human remains, when the identification is not established by conventional means. During the 9/11 disaster strike, an entire dental team which was assisted by the DMORT, which is part of the National Disaster Medical Service (NDMS), played a relatively significant role in disaster response.[15]

Various studies have been planned to evaluate the changes in teeth after exposure to a decreased temperature simulating real life disasters for forensic identification. A study was designed by Burger et al[16] in 1999 to study on the effect of environmental factors on the preservation of DNA in teeth and concluded that teeth which are preserved at -20°C yield enormous amount of amplifiable DNA.A study was done in 2016[17] to evaluate to quantity of DNA obtained from pulpwhere the teeth were stored at-80°C in a deep freezer (REMI). It was found that 3.01 µg/ml of DNA was recovered after storing for five days. This indicated that preserving the human tooth at a decreased temperature does not alter the quantity of DNA present. Hence storing teeth at lesser temperatures canyield a significant quantity of DNA.

b) Under soil

Shortly after the death of an individual, decomposition of tissue starts leading to a series of changes which can be observed morphologically and histologically. These changes occurring in a specific sequence can also be observed in dental pulp tissue retrieved from the jaws of buried dead bodies.TC Boles in 1995[18] could successfully extract DNA from teeth that had been buried up to 80 years. It is possible to discriminate one

individual from all others with a high level of confidence by starting with only 1 ng or less of target DNA whereas, the amount of DNA that can be recovered from molar teeth with pulp volume of 0.023-0.031cc is nearly 15-20 mg.[18]

Also, a study done by Shetty and Premalatha, [19] three different study setups at different times, followed one by other were created. In each setup, 10 specimens of porcine jaws with teeth were buried in surface soil and 10 specimens in subsurface soil. Dental pulp was retrieved at an interval of every 24 h to see for the various changes. All the environmental parameters including average daily rainfall precipitation, temperature, soil humidity, soil temperature, and soil pH were recorded.Dental pulp buried in a coastal environment goes through a specific series of morphological and histological changes which can be interpreted up to 144 h from burial, after which pulp ceases to exist.[19]

c) At room temperature

A study was done in 1991 to determine ABO blood grouping on the pulp on 35 teeth using absorption elution technique. Twenty teeth were examined within six weeks of extraction and 15 teeth after 6-10 months. It was found that blood grouping on pulp gives fairly good results. It was concluded that blood grouping on tooth pulp is of great help in identification even after six months of death.[20]This was in accordance with the study conducted by Shetty and Premalatha in which ABO blood grouping was conducted on tooth material of 60 extracted teeth, which were collected from dead bodies brought to the Mortuary for medico legal autopsies. The extracted teeth were stored for a span of 6 months at the room temperature without any preservative and they suggested that sensitivity of pulp in determining blood grouping is significantly high (96.7%) even after storing dry for 6 months.[19]

Ballal and David[21] in their study to determine ABO blood grouping from dentine and pulp showed a statistically significant positive results (90%) in determining ABO blood groups from dental pulp which is in unison with the other studies.[8,22,23]In a recent study done by Saxena V in 2017,sensitivity of pulp was found to be 80% in determining the blood groups. Thus, the author came to conclusion that the dental pulp can be used to establish identity, where teeth happen to be the only remnants available for personal identification.[24]

II. Discussion

Identification of ABO blood grouping and Rh factor in 1940 by Dr. Karl Landsteiner 1900 opened a new vista in studies on blood grouping.[25]Since a long time, in the medico legal examinations, information from studies on blood grouping has been applied.

The basis of the use of blood group substances in medico legal examinations is done on the fact that once a group is established in an individual it remains unchanged throughout his life.[26]For several decades forensic scientists have been searching for a reliable method for blood typing of teeth and it was possible to distinguish blood groups not only from fresh teeth but also from old teeth that has been left standing in room temperature, embedded in sand, or left standing in running water for as long as 2yrs to simulate postmortem deterioration. Teeth being made up of hardest tissue in the body, retain their characteristics even in the most adverse environmental condition thus, have been identified as the most stable biological material. On the other hand, additional means of identification like facial and dermatoglyphic characteristics, tattoos, marks etc. fail owing to mutilation, decomposition and charring.[27]The most protected tissue of the oral tissues is the Pulpal tissue present inside the root canal that carries blood vessels and nervesas it is surrounded on all sides by other dental hard tissues.

It was suggested in a review done by Karthika and Elumalalai on the use of dental pulp in identification of blood group of deceased humans that pulpal tissue from teeth can be used for blood grouping and has been attributed for identification of biological material in forensic investigation.[26]In 2012, an evaluation was done by Berketaetal regarding forensic odontology involvement in DVI and was of the opinion that forensic odontology is one of the three primary identifiers designated by Interpol to identify victims of mass casualty events.[28]

As dental structures are the most durable of human tissue, the utilization of odontology continues to provide evidence of identification of victims subjected to the extremes of heat, trauma or decomposition. Even when victims are not severely compromised, forensic odontology proves to be rapid and cost effective relative to DNA analysis.Hinchliffe in his paper [29] emphasized on the need of a structured and sensitive response which would help in the identification of victims of mass fatality incidents. He also talked about the need of trained team responseswhich requires the efficient use of dental tissue in victim identification providing evidence. Hence, the dental team has a major role in disaster incidents when there are accurate and available ante-mortem dentalrecords.

III. Conclusion

Blood group determination is an important step in personal identification in forensic sciences. Surrounding environment has a definite role in preservation of pulp tissue. The blood group antigen from pulp remains stable for long because of the reason Teeth being the hardest and the most stable biological material which can withstand adverse environmental conditions and the pulpal tissue inside the teeth remains well protected. The pulp tissue from these teeth can provide DNA for blood group determination and other DNA analysis procedures. Using a large sample sizeand various other environmental conditions, this technique can be systematized in determining the blood group from pulp tissue, thus allowing benefits to the society when need arises.

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