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An overview of the Post-Mortem Interval and its Estimation from Dental Tissues

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ABSTRACT

The forensic odontology is one of the prominent and emerging branches in forensic sciences since decades. An important objective of post-mortem examination is to connect an accused to that moment of time to prove his guilt or innocence. The use of dental tissues and their role for the estimation of post-mortem interval (PMI) is evolving in the field of forensic medicine. The present review was focused on postmortem changes, the importance of PMI estimation and the various dental tissues used in PMI estimation. It was observed that the tissues like dental pulp, odontoblasts, enamel, dentin, periodontal ligament (PDL), and mitochondrial/nuclear DNA from coronal, radicular & cementum, and pulpal RNA, are an emerging aid in the estimation of PMI. These few studies have demonstrated the importance of dental tissues in the estimation of PMI with various techniques; most of them were related to dental pulpal tissue. Further, more studies are required to authenticate the role of other dental tissues to predict the PMI at an early stage with cost-effective and most convenient techniques.

Keywords:

Dental tissues, Forensic odontologist, Forensic medicine, Person identification, Post-Mortem Interval, Time Since Death.

Introduction

The forensic odontology is considered as one of the prominent and emerging branches in forensic sciences since decades. It was defined, as that branch of forensic medicine which in the interest of justice deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of the dental findings.(Keiser-Nielsen, 1980) This specialization plays an important role in person identification in mass disasters victims like terrorist attacks, earthquakes etc., and identification from decomposed, charred bodies like drowned and deep buried persons. Person identification by bite mark analysis, rugoscopy (palatal rugae study), cheiloscopy (lip prints study),odontometric measures(Sridevi et al., 2019) DNA and RNA analysis, etc(V et al., 2015) methods are used. Being an important member in the forensic investigative team, odontologist should have basic knowledge of the postmortem changes that takes place in the body tissues at macro- and micro-molecular level after death.

The post-mortem interval (PMI) *aka* time since death (TSD) is an important element of the forensic investigation and is a critical step in most homicide and un-witnessed death (including hospital deaths). It can be defined as the interval between death and time of examination of body or corpse discovery.(Abhishek Yadav et al., 2017)Despite the large number of researches have been undertaken since decade, it still remains as one of the most challenging variables to quantify and establish time since death. After death, many changes begin to take place in the human body due to physical, metabolic, autolysis, physicochemical and biochemical process.(Saukko & Knight, 2015) These changes progress in an orderly manner until the body disintegrates.The measurement of these changes along with the timeis used to estimate PMI. Forensic odontology being a one of important part of the forensic science, evidence from the dental tissues not only contribute for the person identification but also contribute for the accurate estimation of the time since death.

A plethora of literature have been published, stating the importance of dentists in ante-mortem and post-mortem person identification, sex-determination, disaster victim age- estimation etc., but there is no as such literature review with regards to dental tissues emergence in estimation of PMI till now.(Nuzzolese, 2018; Pradeep et al., 2009; Abhishek Yadav et al., 2017; Achla Yadav et al., 2012) Being a part of the forensic investigative team, knowledge about the post-mortem interval is essential. The present article describes an overview, sequence, and methods of estimation of PMI and its emergence from dental tissues through a review.

Why the PMI is important??

The estimation of PMI is an important objective of post-mortem examination which connects an accused to that moment of time to prove his guilt or innocence. It plays a vital role in investigation of medico-legal cases and helps the investigator to know about the knowledge of when an individual can help to determine the circumstances of their death which in turn can limit the number of potential suspects for the time of the crime. It

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also aids in the identification of unknown remains by restricting potential candidates to those known to have disappeared in the given time-frame.(Abhishek Yadav et al., 2017)

Gross body changes after death

Stages of death can be divided into somatic death and molecular death. Somatic death can be described as the permanent, irreversible death of an organism as a whole whereas molecular death is the death of all individual cells within the body. It usually occurs 2-3 hours after the somatic death. Somatic death is considered as ***legally a person is dead.***(Saukko & Knight, 2015)Different tissues die at different rates, the cerebral cortex being vulnerable to only a few minutes' anoxia, whereas connective tissues and even muscle survive for many hours, even days after the cessation of the circulation.Signs of death appear in the following order as immediate, early and late changes:(Aggrawal, n.d.; Swift, 2006)

Immediate changes Bichat proposed concept of tripod of life are based on the first three changes as (1). Insensibility and loss of voluntary powers, (2). Cessation of respiration, (3). Cessation of circulation.(Pinheiro, 2006; Swift, 2006)

Early changes include (a). changes in skin, (b). changes in the eye, (c). supra vital reaction of the skeletal muscle, (d). cooling of the body (algor mortis), (e). post-mortem lividity (livor mortis), (f). changes in the muscles (rigor mortis).

Late changes include (1). Decomposition (combination of autolysis and putrefaction) and (2). Modifications of putrefaction (adipocere, mummification).

The various gross changes in the body after death are used for giving opinion about PMI are:

- Pallor mortis takes place in 15 -20 minutes.
- Loss of corneal reflex and opacity in the cornea about 6 hours from death,fall of intraocular pressure (IOP) causes flaccid eyeball after a period of 8 hours. Absence of intraocular fluid suggests more than 4 days of since death.
- Cooling of the body-algor mortis occurs after death since all metabolism comes to a stop.
- Postmortem hypostasis- livor mortis also known as lucidity, staining or cogitation may take place between 8 to 10 hours of death.
- Rigor mortis- stiffness of muscles may takes place between 3 to 30 hours, and the following is a reasonable 'spot check' for use in average temperate conditions: in cases where changes in the body temperature felt as warm and flaccid, it is estimated as time of death is less than 3 hours. Similarly, if it is felt as warm and stiff, estimated as 3 to 8 hours since death and if is cold but stiff, then estimated to be from 8 to 36 hours, if along with cold, flaccidity is noted then time since death is estimated to be more than 36 hours. Many factors are tangled and are extremely critical to estimate the time of death depends on the rate of decomposition and the condition where corpse is found. Depending upon the climatic condition, there will be transformation in the body characteristics as either mummification or adipocere. Hot and dry climatic conditions causes body to dehydrate and skin to turn dark and leathery, as mummification changes. Adipocere changes can be when the body if found buried in cool and moist environment or in submerged water over a period of time where hard gray-white waxy substance is formed in the dependent parts of the body.

Various methods used to estimate PMI:

Various means of estimating PMIhave been developed and they can be broken down into three major categories(Bishop, n.d.)

i. last known activity of or contact with the decedent,
ii. those that rely upon the natural process of decay that begins after death, and
iii. those that depend upon knowledge of scavenging insects called as forensic entomology.

The first category is based on non-scientific observations such as last sighting, last phone call, opened/unopened mail, etc. No further discussion of this method will be presented in this article.

The second category is most accepted one, which includes the presence and/or absence of the *rigor mortis* and *algor mortis.* These two indicators can nearly accurately estimate PMI for a period of 2-48 hours after death.Additionally, visual identification of the stages of tissue decomposition due to autolysis and putrification, and biochemical changes within certain body fluids (for example changes in the chemistry of eye fluids) can be used to obtain an early PMI. Postmortem decomposition is affected by many environmental factors such as temperature, exposure to rainfall, composition of the surface on which the body is laid or buried, whether the corpse is clothed or wrapped in plastic, and these factors must be taken into consideration making the calculation of exact time of death difficult.The following methods have been applied to help determine the time since death for this category:(Li et al., 2016)

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- **Molecular biology methods** (degradation of DNA, RNA or proteins): RNA degradation was determined as *RNA integrity number (RIN) and followed by PCR* but used for short time frame PMI. Different tissue proteins showed different decay rates.
- **Spectroscopic technology** (Fourier transform infrared or Raman micro-spectroscopy); it presents several advantages and found a *significant linear correlation between the relative absorption intensity and PMI*. It is more convenient and easier to carry out than other methods, and just a few micrograms of sample are enough for detection. Various types of spectroscopic techniques have been applied, such as fluorescence, spectrophotometric analysis, laser-induced and UV-induced autofluorescence. However, the results are highly influenced by environment factors.
- **Estimation of energy changes in the body after death** (cooling or blood ATP levels); highly specific marker that can be useful for determining the PMI with varying causes of death in the range of 8–56 hr. Limitations are blood ATP levels are also affected by physical health, cause of death and environmental temperature.
- **Thanatochemistry methods** describes changes in the chemical composition (including *urea nitrogen, creatinine, uric acid, potassium, magnesium, sodium, chloride, calcium, hypoxanthine levels)* of various body fluids such as blood, serum, cerebrospinal fluid, vitreous humour and synovial fluid. The best fluids to study are the vitreous humour and the cerebrospinal fluid, to minimize the effects of the environment. However, these fluids are difficult to obtain. Quantitation of the increase of potassium ions within the inner eye fluid was first established in 1963 as a potential indicator of time since death up to 104 hours.(Saukko & Knight, 2015)
- **Other methods** such as stomach emptying, bone marrow cell change, imaging technology, electrophysiological methods, and enzyme activity.

The third category is entomological approach (either a carrion insect development or a succession model); mainly used for the estimation of longer PMI. Many insects have been identified as being useful for determining PMI. New techniques like optical coherence tomography, artificial neural networks and virtual forensic entomology have incorporated to explore the relationship between carrion insects and PMI. This type of study is known as ***forensic entomology.***Both Knight(Saukko & Knight, 2015)and Dimaio(Swift, 2006) have commented that all the methods used to determine PMI/TSD are unreliable and inaccurate, only the circumstantial evidences stand more accurate than the scientific methods.

Review of literature for use of dental tissues in PMI estimation

The determination of the PMI is one of the most challenging problems faced by forensic practitioners. A MEDLINE–PUBMED search engine wasused to review the literature using the keywords “teeth”, “dental tissues”, “post-mortem interval” and “forensic dentistry”.After the literature search, it has been observed that various dental tissues and techniques are used to determine PMI, which are subsequently discussed. The various dental tissues like dental pulp, odontoblasts, enamel, dentin, periodontal ligament (PDL) and mitochondrial/nuclear DNA from coronal, root and cementum and pulpal RNA have been used in estimation of PMI, of which, most of the articles were related to pulp tissues, followed by enamel, dentin,PDL, cementum and dental fillings. A study was conducted to explain the differences in morphological age-related changes in the teeth in terms of color, translucency, attrition, cemental apposition, secondary dentin, etc. by a computer-assisted image analysis. It was observed that these morphological changes were lower in freshly extracted teeth when compared with the teeth from human skeletal remains and they concluded that, the PMI affects the age-related morphological changes in the teeth.(Mandojana et al., 2001)These changes must be taken into consideration while estimating the age of the deceased.

Many factors influence the changes in the tooth after death. Enamel being the hardest tissue in the human body, morphological changes such as abrasions, cracking as shell appearance, erosions, mottling are seen which are caused by decomposers. Leaching of environmental factors such as soil, water into the dentinal tissues causes discoloration and crackling of the tissues as well.(Manoilescu et al., 2015) Soft tissue changes in labial mucosa, gingival tissues were also studied.(A. B. Yadav et al., 2015) Pink tooth phenomenon(Hartomo et al., 2019) is also noted because of the leaching of the pulpal hemorrhage into the dentin due to an increase in intracranial blood pressure and imbibition of hemoglobin and its degradation products into the dentinal tubules. Other morphometric variables like tooth length, tooth width, root length, and root area showed higher values in fresh extracted than skeletal remains teeth. They concluded that PMI affects age-related morphological changes, and therefore can be taken into account when the age of the corpse is diagnosed and is necessary when dental samples from human remains of unknown PMI are evaluated.(Solheim, 1993)

Another study was conducted to explain the microscopic changes of odontoblastic cells in PMI estimation.(Vavpotič et al., 2009)These are fixed post-mitotic cells and are the most characteristic cell of the dentin–pulp complex. The samples were obtained from 31 corpses with healthy oral status where the samples were divided into two groups at two different temperatures. Tooth was extracted during autopsy procedure every

day and stained with hematoxylin and eosin after decalcification. Densities of odontoblasts in the dental pulp were counted using light microscopy and logistic regression analysis was calculated for two different temperature samples. They have observed the reduced number of odontoblasts and their histological appearance may be one additional parameter in estimating the time since death in the early post-mortem period for up to 5 days. The limitation of the study was smaller sample size and further prospect with larger sample could provide us with more explanatory possibilities.

A study by Granrud et al. in 2012, conducted a preliminary study using exfoliation of incisor teeth as an estimator of the PMI.(Granrud & Dabbs, 2012)Accumulated degree days (ADD) were used to quantify the decomposition of the periodontal ligament, represented by post-mortem exfoliation of the incisors and data were correlated to provide an estimated length of thePMI.Daily average temperature was recorded on hourly basis using thermochrons for duration of 6 months. Six teeth were exfoliated during this period and average ADD was calculated. They also found no difference in ADD required for maxillary and mandibular teeth. Further research is necessary to solidify the data from the study by increasing sample size, increasing the observational time period and conducting human trials.(McKeown & Bennett, 1995)

In 2012, Raimann et al. conducted a study to recover DNA from the decomposed cadaver premolar and molar teeth with PMI of 2 months to 12 years using different protocol to extract DNA by PCR Amplification and noticed that, there is no significant difference in the amount of DNA obtained over a period of time duration.(Raimann et al., 2012) And also, concluded that, molar and premolar teeth stand as high potential teeth to obtain satisfactory amount of DNA for typing independent of decomposed corpse's time and laboratory procedures.

But later in 2013, Rubio et al. has concluded from his study that, quality and stability of the dental DNA decreases over a period of 1 month to 18 months duration when experimented at a room temperature which was determined using real time PCR. Also stated that, amount of the DNA obtained varies according to the gender and tooth position.(Rubio et al., 2013) They concluded as a potential effect of environmental conditions must be considered for dental DNA extraction for estimating PMI.

In 2013 Bytheway et al. estimation of the PMI was done using glycoproteinous adhesions deposits by Balanus improvises on human skeletal and dental remains.(Bytheway & Pustilnik, 2013) They concluded from their study that, typical feature of settlement indicates the remains were in fluvial environment for atleast 375-410 days and careful microscopic examination will be helpful for PMI. Further studies should be carried out on their predictability of life cycle and decay rate of adhesions to consider as a tool for PMI estimation.

Later in 2013, Young et al. has conducted a study to examine the time-dependent differences in RNA decay rates to extend the time frame over which early PMI estimates can be made from the tooth pulp of pigs.(Young et al., 2013) Comparison of the decay rates of large, labile and small stable segment of same RNA from the tooth pulp, the researchers were able to estimate PMI values of pigs buried within a shallow grave for up to 84 days. In addition to differences in RNA decay rates, morphological changes were observed in the pulp and quantitative measures was done using a simple colorimetric assay as its aged postmortem. They have concluded that combination of these two assays can create a more precise estimate of PMI.

Poor et al. in 2016 conducted a study to analyze the rate of RNA degradation in human dental pulp. Because of the confined nature of dental pulp tissue makes it an ideal candidate for PMI estimation, as the impact of environmental factors is reduced.(Poór et al., 2016) The RNA integrity analysis determined the time of post-mortem interval with high confidence level in the first 21 days. Apart from this, crude estimation of incubation time of teeth between 20 to 42 days was also performed with the help of PCR based technique. These results showed that this method might be a promising new tool for PMI estimation despite the limitations like ofinclusion of healthy teeth and did not assess the effect of environmental factors.

Higgins et al. in 2015 explained the advanced genetic analysis in combination with the histological examination of the DNA content and rate of DNA degradation from the human molar teeth for estimation of PMI.(Higgins et al., 2015) Out of 150 teeth samples DNA was extracted from coronal dentine, root dentine, cementum and pulp of 114 teeth, rest of them are examined histologically. They concluded as Real time quantification assays showed nuclear and mitochondrial DNA (mtDNA) degraded exponentially with different rates, depending on post-mortem interval and soil temperature. Also, they identified that, different tissues has differential survival rate of nuclear and mtDNA. Furthermore, dentine and pulp were destroyed in a relatively short period of time, but cementum showed little structural change upon histological examination. Finally, it was confirmed by Short Tandem Repeats (STR)-based genotyping method that, cementum from teeth buried upto 16 months can provide a reliable amount of source of nuclear DNA, without the need for specialised equipment or large-volume demineralisation steps.

Yadav et al. in 2015, conducted a study to evaluate the histologic post-mortem changes that take place in human gingival tissues and to correlate these changes with PMI estimation.(A. B. Yadav et al., 2015)Post-mortem gingival tissues were collected from 31 pools of decedents with different time interval 0-8 hours, 8-16 hours and 16 to 24 hours. Ante-mortem samples were collected from routine dental treatment patient's for comparison. Histological examination was done at epithelial, cytoplasm and nuclear levels, as the period of the post-mortem

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interval increased, loss of structural changes were noticed from epithelium to cytoplasmic to nuclear changes. With this they concluded that decomposition at cellular level started within 24 hours of death and other features will start decomposing subsequently. And it was stated that, gingival tissues can also be used in PMI estimation in the early post-mortem period. Limitation of other factors such as climatic and seasonal etc must be considered in future studies.

Mehendiratta et al. in 2015, made an attempt to observe various changes which occur during the process of putrefaction of the dental pulp in a coastal environment like Southern India.(Mehendiratta et al., 2015) Three different study setups at different times, at subsurface and surface soil level. This study explained the series of eventual changes at an interval every 24 hours in dental pulp in morphological and histological changes in size, color, consistency, and odor which can be interpreted up to 144 hours from burial, till the pulp ceases to exist. Various studieshave been conducted to determine the effect of environmental factors like average daily rainfall precipitation, temperature, soil humidity, soil temperature, and soil pH etc on the rate of putrefaction of dental pulp and hence, has to be considered during PMI.(Galloway et al., 1989)(Rodriguez & Bass, 1983)

Mahalakshmi et al. in 2016, conducted a study to estimate the postmortem interval from anti-mortem gingival tissue in histological changes over a time interval of 15, 30, 45 min, 1, 2, and 4 hours and to correlate these histological findings at an early stage of duration. There was a positive correlation (<1.0) between the time interval and the appearance of the histological changes.(Mahalakshmi et al., 2016) However, certain environmental factors and size of samples were not considered in this study which also plays a crucial role in the autolysis of the tissues.

Carrasco et al. in 2017, in this study explained the potential use of pulp post-mortem alteration as an indication for the PMI.(Carrasco et al., 2017) Histomorphological analysis was done over a period interval of 24 hours, 1 month, 3 months and 6 months. Microscopic analysis of the pulpal tissues showed progressive transformation in the cellular and fibrous component along PMI, however this study is limited to 6 days. Further research should focus on using a higher sample number and in different environment conditions to allow a wider application of the methodology and eventually to help narrowing the PMI time-frame estimations.

In 2019, Mansour et al. conducted a study to evaluate the effect of ante- and post-mortem factors on dental DNA-based identification as it has crucial effect in the elapsed PMI estimation.(Mansour et al., 2019) Total of 95 teeth from 39 corpses divided into 6 different post-mortem conditions, were used to evaluate amount of DNA concentration, which were measured using real time polymerase chain reaction (PCR). After the analysis of result, it was found that yield of DNA was best in first 10 days and later it was observed that decreased dramatically in the following time-period. Also, it was noted that, teeth extracted from burnt and fresh corpses yielded the highest amount of DNA, while skeletonized exhumed corpses resulted in the lowest DNA amount. Indeed, dry and indoor conditions demonstrated better results than those in water, outdoors, or buried in the ground. On the other hand, ante-mortem factors including sex, age, tooth type, and tooth root portions did not reveal significant effect on dental DNA yield. It was suggested that ante-mortem factors are considerably more subjected to individual variation while post-mortem factors including PMI, post-mortem conditions, and the relevant surrounding environments have considerable effect on the dental DNA amount yielded.

Till then many studies were conducted using dental pulp, but in 2019, Ishikawa N, et al. conducted a study using diatoms attached on the surface of dental enamel to determine PMI for underwater corpses by the Electron Probe X-ray Micro Analyzer.(Ishikawa et al., 2019) They concluded from the study as the immersion time increased, the quantity of sea water constituents like O, Si, Mg, K, Al, and S detected on the surface of dental enamel increased, while the quantity of the main dental components (Ca and P) that were detected gradually decreased. Based on their results, regression formula to estimate the immersion time was also established. This method was unique for estimating the postmortem interval as other conventional methods depending on the degree of decomposition of the corpse. Authors of this claimed that this method is a breakthrough technique for evaluating the time since death more objectively, compared to the conventional method of determination based on the degree of decomposition of the corpse, therefore concurrent use of this method may increase the accuracy of PMI.

Akbulut et al. 2020 conducted a study to determine PMI using micro-CT by evaluating the alternations in the hard tissues of tooth, mineral density of enamel and the surface abrasion of hard dental tissues in rats.(Akbulut et al., 2020) This study was conducted over a period of 12 week and at the end of 8^{th} week, it was observed that mineral density of the enamel tissues was decreased and so on significantly seen at 12th week. Similarly, surface abrasion on the hard tissues was decreased. They concluded that, evaluation of these parameter using micro-CT scan analysis can be considered as precise in PMI determination.

In 2020, Bhuyan et al. concluded in their study that, as pulpal tissue is enclosed and protected by the surrounding hard tissues, progressive degradation of the matrix and cellular constituents can be studied for a period of 24hours (h), 48 h, 72 h, 1 month, 3 months, 6 months, 1 year and 2 years by a cost effective method of morphologic and microscopic characteristics of the tooth pulp.(Bhuyan et al., 2020) Also, microbiological assessment was done up to 2 years by Gram staining for staphylococci and streptococci.

Salema et al. in 2020 conducted to study to evaluate the mechanical properties of the dental restorative materials after immersion in marine environment.(Salema et al., 2020) They concluded from their study that, significant changes in the increase in hardness and surface roughness and stated that, these findings could be helpful in the PMI estimation and further studies are required.

Turingan et al. in 2020, published an article about the Rapid DNA analysis for the identification of human remains.(Turingan et al., 2020) They concluded that, obtaining DNA from human remains using this technology can be as little as 2 hours. They used tooth sample of 12 month oldest and buccal tissue sample of 3 to 11 days old for PMI. Yet to be further studies with buried and decomposed bodies must be done using this technology.

Conclusion

The post-mortem interval plays a major role in forensic science for identification of a suspect. Currently, a few studies have been conducted to demonstrate the importance of dental tissues in estimation of PMI, in which most of the studies are related to dental pulpal tissue. Further, more studies are required to authenticate the role of other dental tissues to predict the PMI at an early stage with cost effective and most convenient technique. Forensic odontologists must also have a thorough knowledge, awareness regarding the importance of PMI from various dental tissues using different techniques. Inspite of the undisputed importance of PMI estimation, all the current applications suffer from a varying level of uncertainty. To improve accuracy and reliability, it is advised to perform several tests concurrently.

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