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REVIEW

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Advances in histological profiling of hard tissue injuries for forensic identification: A narrative review

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ABSTRACT

Background: Injuries to hard tissues, including teeth and maxillofacial bones, are essential for forensic identification, trauma assessment, and determination of the postmortem interval. Histological analysis of dental and skeletal tissues yields significant insights owing to their resilience and resistance to postmortem deterioration.

Objective: The objective of this narrative review is to critically synthesize existing data on histological, radiological, and morphological changes in hard tissue injuries, with a particular emphasis on dental trauma and its forensic implications.

Methods: A thorough examination of the literature pertaining to forensic odontology, anthropology, and pathology was conducted, concentrating on maxillofacial trauma patterns, thermal modifications of teeth, and histomorphological irregularities in dental pulp pertinent to trauma timing and postmortem interval assessment.

Results: The studies demonstrate that hard tissues exhibit unique injury patterns that aid in distinguishing between antemortem, perimortem, and postmortem trauma. Histological changes in tooth pulp, marked by progressive vacuolization and cellular degeneration, show potential as reliable indicators for determining postmortem interval. Thermal exposure induces noticeable macroscopic, radiographic, and microscopic changes in dental tissues that can aid in forensic identification after fire-related events.

Conclusion: This review highlights the forensic importance of histological examination of hard tissue injuries and demonstrates the necessity of interdisciplinary collaboration among forensic odontologists, anthropologists, and pathologists. While current data supports the forensic utility of these procedures, further standardized and quantitative research is required to validate their regular application.

KEY WORDS

Dental trauma; Hard tissue injuries; Forensic odontology; Histological analysis; Forensic identification

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Introduction

Forensic odontology is a branch of dentistry that utilizes the oral and para-oral characteristics of human remains, including teeth, mandibles, and maxillae, to aid in criminal investigations and identification. This is particularly useful in cases involving catastrophes and accidents where the human remains are decomposed, burned, or reduced to skeletons [1]. Forensic odontology, a combination of dental and legal investigations, is an essential discipline in the identification of individuals, particularly when conventional procedures prove inadequate [2]. According to the guidelines set forth by the International Criminal Police Organization, comparative dental examination is a crucial method for human identification (INTERPOL) [3]. Skeletal trauma refers to any injury that occurs in living organisms and affects 'bones', 'teeth', or other 'hard tissues'. An analysis of skeletal injuries is a crucial component in the investigation of human remains, as it aids in both the identification of the individual and the determination of the cause of death. When doing a study on trauma, it is more reliable to examine rigid tissues such as 'bone', 'teeth', and 'cartilage' rather than flexible tissues. The reason for this is that soft tissues are prone to significant alterations following an injury, while hard tissues retain a lasting record of the traumatic incident [4] .

Historically, the job of forensic anthropologists was restricted to the reconstruction of biological profiles based on the examination of dry bones. In the past, providing information about the reason for death or the circumstances surrounding it was deemed to be outside of their area of competence. This viewpoint has shifted significantly since the late 1970s as a direct result of the study and casework cooperation that has taken place between forensic pathologists and anthropologists. The interpretation of traumatic events may be improved significantly using trauma analysis, which involves the study of injuries sustained during an autopsy and after any soft tissue has been removed from the body. Analysis of trauma necessitates careful observation and thorough documentation, but its interpretation must consistently remain unbiased and based on dependable scientific methodologies and criteria [5]. Nevertheless, the assessment of bone injuries should consistently rely on the specific circumstances of the case.

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Interpreting bone fractures outside of their original context is unhelpful and may result in an inaccurate diagnosis [6]. Forensic anthropologists have a crucial role in understanding trauma within the area of forensic anthropology. Their function is critical for comprehending the traumatic event and its relationship to the cause of death. An examination of the skeletal remains of the deceased person may offer the coroner or medical examiner with information that is very helpful in determining the method in which the person died as well as the cause of death. Forensic anthropologists are receiving more requests to assess bone trauma due to the valuable information it can provide for forensic investigations [7]. This narrative review seeks to clarify the histological and morphological features of hard tissue injuries pertinent to forensic identification and trauma assessment.

Literature Review

Consequently, there is an increasing number of scientific studies focused on trauma analysis. Although the topic is important, there is a lack of bibliographic references on maxillofacial and dental trauma in the field of forensic odontology. These references often only included in publications that deal with forensic anthropology. There is not a lot of information available in the scientific literature on trauma analysis from a forensic odontology point of view. In the context of forensic science, the purpose of this work is to conduct a review of the skeletal trauma that may occur in the maxillofacial region using the concepts of forensic anthropology and odontology. Trauma to the maxillofacial region may impact the 'cranial vault', the 'upper face' third area comprising the 'orbits', the 'mid-facial' third structures including the 'nasal bones', 'zygomatic', 'maxilla', and 'mandible', and the 'lower facial' third structures including the 'maxilla' and 'mandible'. In addition, damage to the teeth and the structures connected with them is often present when trauma is done onto the skull. Fractures occurring in the 'cranial vault', 'midfacial structures', 'mandible', and 'teeth' exhibit distinct fracture characteristics due to the variations in structure and morphology of these maxillofacial components.

Cranial vault fractures: The cranial vault, also known as the neurocranium, is a rounded structure that is made up of three layers: the outside, also known as the ectocranial layer; the inner, also known as the endocranial layer; and the diploe, which is a spongy layer that is placed in between the inner and outer cortical layers [8].

Mandibular fractures: The various types of mandibular fractures include symphyseal, para symphyseal, body, angle, ramus, condyle, and coronoid process fractures [Figure 1]. The most common fractures in the mandible are condyle fractures, which make up 30% of all mandibular fractures, followed by 'body' and 'angle fractures', accounting for 25% of 'mandibular fractures', and 'symphyseal and parasymphyseal fractures', accounting for 15% of mandibular fractures. 'Ramus fractures', comprising 3% of mandibular fractures, and 'coronoid process fractures', comprising 2% of mandibular fractures, are less common [8]. Depressed fractures, linear fractures, and penetrating wounds can all occur because of trauma to the cranial vault. When the skull is impacted, it applies compressive loads on the outer layer of the cranial vault and tensile forces on the inner layer. The inner layer of bone is typically the primary site of fracture because bone undergoes tensile failure prior to compressive failure [3], [8].

Figure 1. Mandibular fractures classification according to their location: (a) Symphseal, (b) Parasymphyseal, (c) body, (d) Angle, (e) Condyle, (f) Coronoid process, (g) Coronoid process.

This means that the exterior and inner layers of bone must always be looked at when analysing cranial vault fractures. Direct contact across the malar eminence often causes zygomatic fractures. Displacement type and fracture pattern are two of the main criteria used to categorise these breaks. Although zygomatic fractures may happen by itself, they often occur in tandem with other facial bones being broken as well [9], [10]. Unilateral fractures of the zygoma or zygomatic orbital complex are more common than bilateral fractures of the skull base [9]. Orbital fractures can cause injury to the 'rim', 'floor', 'medial', or lateral walls of the 'orbit', and can occur either with or separately from the 'zygomatic complex' [10]. Rim fractures that occur in isolation are often caused by a blow to the upper or upper lateral edges [11]. The 'orbital floor' and 'medial orbital' wall are easily broken, and it's true whether other nearby bones (zygoma, maxilla, nose, and ethmoid) are also broken. Zygoma fractures often occur with lateral wall fractures. Orbital roof fractures are equivalent to skull base fractures. These fractures almost seldom happen by themselves [12].

Different types of orbital fractures exist depending on what parts of the body are broken. Nasal fractures are the most common kind of face fracture, and they often include several broken bones [10], [13]. The structures involved, the degree of comminution, and the impact orientation (lateral vs. frontal) may all be utilised to categorise a nasal fracture [10]. Fractures may be induced by low-energy collisions when caused by lateral forces but need higher-energy impacts when caused by frontal forces [13]. 'Le Fort' classification is the standard for

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categorising maxillary fractures. 'Le Fort I' fractures separate the 'upper palate' from the remainder of the maxilla and continue between the 'maxilla' and the 'nasal' opening. Typically, strikes to the alveolar process or infranasal region cause these fractures [14]. 'Le Fort II' fractures traverse the 'maxilla', the orbit's inferior border, and the upper nasal bones. These breaks often occur from a direct hit to the middle of the bone. Le Fort III fractures cross the bridge of the nose and the upper orbits. These breaks happen when the upper midface takes a direct hit from above [15] [Figure 2]. Le Fort fractures that occur in isolation are uncommon in clinical practise. Le Fort II fractures are often seen on the unaffected side of the skull, whereas Le Fort III fractures are located on the more severely afflicted side [16].

Figure 2. Le Fort classification of maxillofacial fracture: (a) Le Fort I, (b) Le Fort II, (c) Le Fort III.

In addition, there are three distinct types of palatal fractures. Fractures in the sagittal plane, which occur at or around the midline; Coronal transverse and oblique fractures of the palate; Comminution of palate fractures is another possibility. It seeks to enhance the comprehension of dental trauma patterns, advance knowledge in the domain of forensic odontology, and enhance the capacity to identify and distinguish individuals in legal and forensic contexts. To perform histological exams for the purpose of analysing various types of oral trauma and investigating the forensic implications of such injuries.

Antemortem trauma

Trauma that happens before death is called antemortem trauma, and it triggers a cascade of highly controlled inflammatory, reparative, and morphological responses [17]. Blood artery disruption inside the injured region is a direct result of trauma and subsequent bone fractures, and the healing process after trauma is long and intricate. Many variables, including the patient's age, overall health, the site and degree of the injury, and whether surgical intervention was necessary, might determine how long it takes for bones to recover. Closed and open surgical devices and methods are available for the reduction and stabilisation of maxillofacial fractures, respectively [18]. During closed therapy, the fracture is reduced with blind manipulation of the bones, whereas during open treatment, the fracture is exposed, reduced anatomically, and internally mended using surgically inserted implants. Tooth avulsion, tooth luxation, and tooth intrusion are all forms of dental trauma that may lead to the need for reimplantation, splinting, occlusal correction, and even root canal therapy. When dealing with tooth fractures limited to the enamel, it is common practise to round off any jagged edges and release any occlusal pressure. Dentoalveolar maxillary and mandibular fractures may be stabilised temporarily with the use of a tie wire to avoid uncomfortable mobility. Intermaxillary fixation and maxillomandibular fixation are fewer common treatments that include securing the upper and lower jaws together with 'arch bars', 'hooks or eyelets', 'circum dental wires [19].

Rigid or semi rigid fixation is the gold standard for treating craniofacial and mandibular fractures. There is no motion across the fracture site when it is rigidly fixed, and the fracture is adequately stabilised for healing when it is semi-rigidly fixed, but there is some "micromovement" across the fracture site. Replacement options for teeth lost due to trauma include Osseo integrated dental implants, detachable dental prostheses, and fixed dental prostheses [20]. Forensic odontologists must be able to compare 'postmortem' results with 'antemortem' data and have expertise with craniofacial and mandibular fracture repair techniques and dental appliances. Evidence of maxillofacial and/or dental care may be inferred from the discovery of dental implants, fixation devices, or treated dental injuries in skeletal remains. 'Bony callus' or any surgical method or device used to minimise the fracture should allow 'forensic odontologists' and 'anthropologists' to establish 'antemortem craniofacial trauma'. Bone damage that occurred before to death might be difficult to recognise in the early phases of healing but can be easily classified as antemortem after a callus has formed or a fracture fixation device has been used. Because teeth do not rebuild after a fracture, various criteria must be utilised to diagnose 'antemortem' trauma in 'dental' structures compared to 'bone'. Antemortem dental trauma can be inferred from dental fractures exhibiting worn edges and dental therapy including the surgical insertion of devices such as 'plaques' and 'screws' [21].

Periomortem trauma

The term "perimortem trauma" relates to an injury that occurs at the time of death or during the immediate pre-death period. The onset of trauma caused by skeletal parts is less precise compared to the timing of damage based on soft tissues [22]. This is because bones and teeth have specific qualities. Because of this, forensic anthropologists and pathologists have quite different perspectives on how to use the terms perimortem and postmortem respectively. The process of death, which might begin with an accident and/or sickness and end with the loss of somatic existence, is what forensic pathologists mean when they refer to the perimortal period. Nevertheless, forensic anthropologists analyse the reaction of bone tissue, whether it is fresh or dried, to a mechanical force coming from the outside. Fractures that occur during the perimortem period will exhibit the new bone reaction, but there will be no evidence of healing [23]. When writing a report on trauma analysis, it is important to consider the use of specialized language specific to a certain area. This is especially relevant in situations where both a

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forensic pathologist and an anthropologist may be required to provide testimony regarding the same piece of evidence [2,3].

When assessing the bone, the criteria for categorizing trauma as postmortem are based on the lack of evidence of bone healing or infection, the presence of recent bone characteristics, and a fracture pattern that aligns with a final event. For the trauma to be classified as postmortem, all these criteria must be present. Teeth fractures with sharp edges suggest trauma that occurred around the time of death. However, the characteristics of oral trauma at the time of death are sometimes unclear and should be evaluated along with injuries to the skull and other parts of the body. Depending on the force that was responsible for the damage, perimortem trauma in the craniofacial region might display a variety of distinct features. Therefore, diverse characteristics are shown in the maxillofacial structures by high-velocity projectiles, sharp forces, blunt forces, and thermal stress respectively. In the next sections of this article, the features of the many trauma mechanisms that might affect the maxillofacial and dental structures will be explored.

In the event of a catastrophic fire, the answers to the following questions are of the utmost importance: (1) What started the fire, and (2) Who was subjected to combustion. Heat has the capacity to damage or modify the distinctive features of a corpse, particularly its soft tissues. As a result, investigating the cause of death and determining the identity of the deceased becomes more challenging. Teeth, being the most durable formation in the human body, exhibit exceptional resistance to decay and can withstand prolonged periods of burial. Teeth can endure temperatures as high as 1100 degrees Celsius. The dental hard tissue analysis can help solve specific puzzles for these reasons [24]. To accurately understand the effects of intense heat on both hard and soft tissues, it is crucial to possess knowledge of the specific changes that occur, both in terms of physical characteristics and histological properties. [25]. Studies by Nisha et al stated that fire intelligence serves as the multidisciplinary foundation for reconnaissance, encompassing the determination of the origin, cause, and identification of fire victims. Fire is a potent agent of destruction that can cause considerable harm. The destruction of soft tissue in fire disasters significantly complicates victim identification. Teeth exhibit hardness and resilience, enabling them to endure such conditions. The analysis of morphological, stereomicroscopic, histological, and gravimetric findings yields valuable insights from dental evidence in forensic investigations. Thirty-six mandibular premolar teeth, extracted for therapeutic purposes, were subjected to high-temperature gradients. Analyses including macroscopic, stereomicroscopic, histological, and dry weight assessments were conducted at each temperature gradient. The color of the teeth transitioned from yellowish orange to metallic black bronze and finally to chalky white. Stereomicroscopy revealed intact teeth at 100°C, the emergence of micro-cracks at 500°C, and complete crown fracture at 900°C. Decalcified sections demonstrated dilatation of the dentinal tubular pattern at 300°C. Dentinal tubules exhibited the formation of vapor bubbles at 400°C, leading to a loss of their characteristic architecture. Alterations in the scalloping nature of the dentino-enamel junction, coalescing radicular dentinal tubules, and the sand cracking appearance of teeth were observed at temperatures of 100°C, 300°C, and 900°C, respectively. Higher temperatures resulted in notable reductions in the weight of the tooth samples. The morphological, histological, and gravimetric alterations in a tooth resulting from fire exposure may provide insights into the temperature, duration of exposure, and the fire's origin [26]. The preservation of fragile incinerated teeth is essential in fire investigations related to the temperature exposure and the identification of victims. Preservation is essential for conducting both macroscopic and microscopic ultra-structural examinations, which yield important insights into the structural changes that dental tissues experience when subjected to varying temperature ranges.

The objective is to examine the macroscopic alterations and the microscopic ultra-structural modifications of dental hard tissue in both permanent and deciduous dentition utilizing a stereomicroscope and scanning electron microscope (SEM). The research involved 40 healthy, freshly extracted teeth, comprising 20 permanent and 20 deciduous specimens. These teeth were exposed to specific temperatures of 200 °C, 400 °C, 600 °C, and 800 °C for a duration of fifteen minutes in a muffle furnace. Teeth were examined using a stereomicroscope, followed by processing for scanning electron microscopy (SEM) at a magnification of 1000×. The parameters for macroscopic observation include color, translucency, and surface texture of enamel and cementum. The parameters utilized in the microscopic observation of enamel include pit and fissure morphology, prism pattern, crack and fracture lines, microporosity, debris, and erosion. In contrast, the parameters for cementum encompass crack presence, fissure morphology, collagen bundle arrangement, pattern, and debris. Observations of dentition, both macroscopic and microscopic, were quantified at various specific temperatures using percentage calculations. The differences in macroscopic and microscopic changes between permanent and deciduous teeth were analyzed using the chi-square test. No significant correlation was observed in the macroscopic and microscopic changes between permanent and deciduous teeth. Observations of dentition at specific temperatures, both macroscopic and microscopic, indicated a significant reduction in the presence of each selected parameter in enamel and cementum. The study demonstrated notable macroscopic morphological changes and consistent microscopic ultra-structural pattern alterations that were clearly observable at designated temperatures. Scanning electron microscopy (SEM) offers significant potential for improving victim identification and advancing forensic odontology through the examination of burnt dental remains [27].

Gross changes observed

The crown's colour and smoothness were unaffected by temperatures of 200 degrees Celsius. When examined closely, the root's surface revealed a few uneven fissures. The cementoenamel junction (CEJ) gaps, the crown becomes brownish black, and the root turns black at 400 degrees Celsius. At such temperature, the teeth become more fragile. There were a lot of weird fissures on the surface. Surface irregularity and peeling of hard tissue occurred at 600 degrees Celsius. The enamel had flaked off the dentin. Discoloration ranging from black to grey was seen at the crown and the root. At 800 degrees Fahrenheit, the tooth looked completely charred. The colour of the root and crown changed to white. It was discovered that the exposed dentin is blue at the pulpal area and fades to white at the tooth's edges. The crown disintegrated at 1000 degrees Fahrenheit. Blue dentin faded to grey, with white highlights at the edges. The molars had centrally originated, radiating, vertical fracture lines. The root continued to be a chalky white and had developed several irregular fissures [28].

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Radiographic changes observed

Radiographic analysis showed no changes in tooth structure after exposure to 200 degrees Celsius. The dentin enamel junction (DEJ) and cementoenamel junction (CEJ) showed gapping when subjected to temperatures of 400 degrees Celsius. At 600°C, the DEJ and CEJ completely dissociated from the surrounding hard tissues. Fracture lines were observed in both the crown and root at a temperature of 800°C. Additionally, there was tissue separation at the DEJ and CEJ. The crown underwent total liquefaction at a temperature of 1000 degrees Fahrenheit. The stem was entirely cut [29].

Histological changes

At temperatures of '400 °C' or higher, it was discovered that teeth of all ages totally disintegrated. This prevented the teeth from being processed and sectioned. Only teeth heated to '200'°C yielded usable stained pieces. H&E-stained tissue slices revealed two patterns upon closer inspection: a vapour bubble pattern and a 'wicker basket pattern'. There was no variation in radiographic, morphologic, or histologic tooth appearance between teeth of different ages. A set of teeth subjected to direct heat showed increased crown cracking at '400°C', and full 'enamel' destruction at '600°C'. Teeth that were directly exposed to heat exhibited higher structural damage on x-rays. Histologically, there was no discernible variation [30].

Histo-morphological and microbiological changes in tooth pulp to assess post-mortem interval

Immediately after a person passes away, there is a cascade of changes that may be seen in their physiology and biochemistry. These changes are brought on by the cessation of blood circulation and the lack of regulatory systems. Autolytic enzymes are responsible for the earliest stages of decomposition of the deceased, whereas putrefying bacteria are responsible for the latter stages. These stages lead to degenerative alterations that may be comprehended both in terms of their structure and cellular composition. Research has been carried out to histomorphologically examine these degenerative alterations in the blood, skin, eyes, sebaceous gland, skeletal muscles, kidney, and liver. The findings of these studies contribute to the assessment of an individual's post-mortem interval (PMI). The dental pulp is securely enclosed within the pulp cavity, which is bounded by the hard tissues of the dentin, enamel, cementum, and jaws, providing it with exceptional protection compared to other soft tissues. The tooth pulp possesses a distinctive quality that sets it apart from other soft tissues. Consequently, it is devoid of any impurities and largely remains unharmed by external assaults. While other soft tissues degrade rapidly, tooth pulp can be preserved for a duration of four to five years, hence prolonging the timeframe in which postmortem interval (PMI) can be identified. Only a limited amount of study has focused on the delayed decomposition ability of tooth pulp tissue, which has been utilized as an indicator to estimate the period since death. An attempt has been made to identify the characteristic degenerative cellular and nuclear alterations in pulp tissue stained with hematoxylin and eosin (H & E) under a light microscope [31]. Zhihua et al stated that the histological scoring method evaluates not only new bone formation but also assesses newly formed cartilage, fibrous tissue, and the size of the remnant bone defect. We assessed the reliability of our new scoring method through Kappa analysis and interclass correlation analysis. The inclusion of these additional parameters enables differentiation between the hard callus and soft callus phases of healing, thereby providing more valuable insights into the impact of various tissue-engineering treatments on the healing process [32]. Mehendiratta et al stated that to identify the series of changes that occur during the putrefaction of dental pulp in a coastal environment, specifically in Southern India. An effort has been made to estimate the time since death by evaluating the duration for which dental pulp remains microscopically intact. Three distinct study setups were established sequentially at different times. In each configuration, 10 specimens of porcine jaws with teeth were interred in surface soil, while 10 specimens were placed in subsurface soil. Dental pulp was collected at 24-hour intervals to observe the various changes. All environmental parameters, including average daily rainfall, temperature, soil humidity, soil temperature, and soil pH, were documented. A series of morphological changes, including alterations in size, color, consistency, and odor, as well as a sequence of histological changes, were observed in both surface and subsurface samples. The dental pulp located in a coastal environment undergoes distinct morphological and histological changes that can be analyzed for up to 144 hours post-burial, after which the pulp no longer exists [33].

The study by Lipsa Bhuyan et al, examine tooth pulp's morphology and microscopic properties over two years after its death. Each of eight experimental groups had five removed teeth. The apical foramen was blocked with modeling wax. Groups 1–8 had dental pulp extracted after 24 hours, 48 hours, 72 hours, 1 month, 3 months, 6 months, 1 year, and 2 years. Standard histological processing and H & E staining were performed on pulp tissue. The slides were inspected under a light microscope. The pulp turned pink at 24 hours and pale pink at 72. The viscosity changed from soft to firm to jelly for 72 hours then dried and became friable after 2 years. Microscopic investigation showed rapid pulp constituent deterioration up to 72 hours, then a slowdown after one month with fewer histological changes. A 24-hour post-mortem pulp had smaller vacuolations that grew to cover the stroma in two years. The nuclei showed autolytic modifications throughout time. The odontoblastic layer lasted up to 6 months in some areas. Gram-positive staphylococci and streptococci were found for up to 2 years [34].

Conclusions

Dental and skeletal hard tissues are widely used in forensic investigations for their durability and capacity to preserve trauma evidence. Histological and morphological alterations in craniofacial bones and tooth pulp can differentiate antemortem, perimortem, and postmortem traumas, aiding in the estimation of postmortem interval and forensic identification in instances of advanced decomposition or heat damage, according to this narrative review. This review's results justify several considerations for caution. Most of the literature comprises observational studies employing various methodology, sample sizes, and postmortem techniques. In the absence of quantitative thresholds and histological criteria, these approaches become more challenging to duplicate and utilize for forensic purposes. Standardized histological protocols, expanded and more diverse populations, and AI-driven imaging and processing technologies ought to be employed to corroborate findings. These strategies may enhance the accuracy, objectivity, and admissibility of forensic trauma analysis in court. Forensic odontologists, anthropologists, and pathologists must collaborate when analyzing hard tissue injuries resulting from suspected abuse, catastrophic events, or unidentified human remains.

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