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TECHNIQUES FOR DETECTING TRAUMA IN ANCIENT HUMAN REMAINS

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ABSTRACT

Once being asked by a student what she considered the first evidence of civilization in ancient culture, Margaret Mead replied that the most significant evidence of a civilization would be a 'broken femur that has healed'. To her the healing and care given in ancient time was worth observing to identify civilization but for a forensic anthropologist the broken femur is an evidence enough to identify a trauma that may have occurred in the long gone past. Of all the prehistoric pathological conditions, traumas are the most commonly observed cause of death or the bearings on the human remains.

Bone being a connective tissue capable of healing/repairing over time is an appropriate indicator of a trauma. Traumas have been classified using various criteria and can be broadly categorized as accidental and intentional. They may be present as results of fractures, dislocation (luxation) and subluxation, dental trauma, deformation, mutilation, trephination, trauma resulting from intentional violence (organizational or structural), scalping, cannibalism and other forms of trophy taking violence against bodies, problems arising from pregnancy, infanticide, child abuse, elder abuse, intimate partner abuse; traumatic surgical interventions- amputation, trepanation, sincipital T mutilation, trauma to the skeleton through cultural modifications- cranial modifications, foot binding, waist training and dental modifications.

However, trauma specific protocols are being developed for reporting. The technological treatment given to the evidences (fossils) are more or less generalizable. These include primary techniques of age (C14 dating) and sex determination, reconstruction of the associated cultural and environmental conditions, photography, visual inspection, X-ray, MRI (magnetic resonance imaging), CT (computed tomography), microscopic observation, scanning electron microscopy, BSE-SEM imaging (backscattered electron imaging in scanning electron microscopy), endoscopic observations, biochemical analysis of bone, identification of environmental and postmortem organic growths and other decomposition agents degrading the sample, ancient DNA (aDNA), epigenetics, etc.

Keywords: *Paleopathology, Trauma, Techniques, Forensic Anthropology.*

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INTRODUCTION

Once being asked by a student what she considered the first evidence of civilization in ancient culture, Margaret Mead replied that the most significant evidence of a civilization would be a 'broken femur that has healed'. To her the healing and care given in ancient time was worth observing to identify civilization but for a forensic anthropologist the broken femur is an evidence enough to identify a trauma that may have occurred in the long gone past. Of all the prehistoric pathological conditions, traumas are the most commonly observed cause of death or the bearings on the human remains.

Trauma: Definition and Classification

The literal meaning of trauma is 'a wound' which in context of the human skeletal remains refers to any change in the natural morphology of the bone before or during death.

Lovell (1997) opines that Trauma may be defined many ways but conventionally is understood to refer to an injury to living tissue that is caused by a force or mechanism extrinsic to the body. Trauma is defined as an injury to body tissues and organs subsequent to the transfer of energy from the environment (Robertson, 1992).

According to Hamblen et al. (2007) the term trauma is used in its broadest sense to any event that results in partial or complete discontinuity of a bone. These events can be accidental and intentional injury cosmetic or therapeutic practices affecting bones and pathological conditions that can increase the vulnerability of bone to biochemical stress (Rodfern and Roberts, 2019).

The prevalence and location of trauma vary depending upon the factors like civilizational condition of the population, ethnicity, age and gender, (Buhr, 1959) (Ogden, Ganey, and Ogden, 1996) (Kimmerle, 2008) (Wedel, 2014), socio-cultural and environmental, (Hamilton, 1853) (DeSouza, 1973) (Iqbal, 1974), economic, geographic, political, and physiological conditions including diseases (Rodfern and Roberts, 2019).

Various types of trauma affecting the skeleton include fractures. Dislocations, subluxation, dental trauma, trephination, scalping, problems arising from pregnancy, traumatic surgical interventions like amputation, trepanation, Sincipital T mutilation, trauma to the skeleton through culture modification-cranial modifications, foot binding, waist training and dental modifications. To facilitate data-sharing and comparability with clinical datasets, the International Classification of Diseases published by the World Health Organization can also be used when recording trauma (Rodfern and Roberts, 2019).

THE TECHNIQUES

1. Physical Observation

1.1 Description of the abnormal bone: After finding a bone in a dig the abnormalities parted through biogeographical agents are examined. If the abnormal area observed on the bone is a smooth with regular margins and devoid of any exposed cortex then the bone is labeled to have some pathological condition.

A careful observation of the specimen involves describing the bones abnormality which is based on standard terminology (Ortner, 1991, 2003, 2011, 2012; Buikstra, 1994) as suggested by the '*Nomina Anatomica*' and its successor '*Terminologica Anatomica*' approved by the International Anatomical Nomenclature Committee, 1989 (Committee, 1989).

1.2 Process recognition: Process recognition includes defining the mechanism of the abnormal bone, changes and variations in size and shape, and destruction of the natural morphology. The pathological variations of abnormal bone size, shape, formation, and destruction varyingly span several biologic phenomena: cellular and vascular disturbances, innervations /biomechanical disease, trauma/repair, errors in growth, metabolic diseases, inflammatory processes, and neoplastic disorders. (Rodfern and Roberts, 2019).

The process of description and process identification in paleopathology builds toward the final goal—the differential diagnosis of abnormal skeletal tissue which involves:

1. gathering information in the description of the pathological condition
2. identification of possible etiology and pathogenic conditions
3. comparison of the abnormal bone with known disease patterns and deciphering differences. (Rodfern and Roberts, 2019).

1.3 Differential diagnosis: Differential diagnosis refers to inferring the pathology (trauma) on the basis of the description of the sample being examined and the mechanism identified for the causation of the abnormality.The results of differential diagnosis are presented using the '*Istanbul Protocol Manual on the Effective Investigation and Documentation of Torture and Other Cruel, Inhuman or Degrading Treatment or Punishment (2004)*'.

2. Histomorphometry

Bone Remodelling: Bone formation involves the non-coordinated functioning of the bone cell forming cells known as *osteoblasts* and bone absorbing cells known as *osteoclasts* guided by the Osteocytes connected through canaliculi.

Once being formed the bone is said to be modelled and requires subsequent repairs and growth. This process is known as remodeling which occurs at different sites in a bone. (Stout, *et al.,* 2019).

When a *trauma* occurs in a bone the osteocytes arrange teams of osteoblasts and osteoclasts and send them to the trauma site. The teams clump together and form the *basic multicellular unit* or BMC (Sims, 2014). The BMCs travel to the trauma site and perform remodeling. BMCs along with the channelized osteocytes from the *Osteons* or the *Haversian system*. (Stout, *et al*., 2019).

Resorptive bays (Howship's lacunae), distinguished by their scalloped border are evidence of the initiation (activation) of a BMU, and the amount of bone that was resorbed. Osteons (Haversian systems) represent forming and completed BMUs. An algorithm is available that employs these static histological features to estimate bone remodelling rates without the use of in vivo tissue time markers (Frost and Wu, 1967; Wu, *et al.,* 1970) (Stout and Paine, 1994). The Algorithm recommends the use of rib for estimating the age of the skeleton from which the number of osteons is calculated. Visible evidence of bone remodeling is represented by osteon population density (OPD), comprised of the sum of numbers of complete osteons (N.On) and fragmentary osteons (N.On.Fg), those partially removed by subsequent remodeling events, and number of resorptive bays (N.Rs. Vd), all divided by the section area (Sa.Ar)

$$OPD = \frac{[N.On + N.On.Fg + N.Rs.Vd]}{Sa.Ar^{-1}}$$

Where OPD= osteon population density

N.On= numbers of complete osteons

N.On.Fg= number of fragmentary osteons

N.Rs.Vd= number of resorptive bays

Sa.Ar= section area (Stout, et al., 2019)

These observations provide with Bone Remodelling Rates and related parameters like Osteon Population Density, Cortical Area, Osteon Area resulting into Activation rate of BMCs and Bone Formation Rates (Stout, et al., 2019).

3. Microscopy

The changes in the bone affected with trauma are observed using microscopy to assess the remodeling conditions. Sample preparation of the specimen is required for microscopic observation.

3.1 Light microscopy: Light microscopy visualises osteolytic lacunae, radiating canaliculi and cortical pores along with cement wall demarcating the secondary osteons. However, the histology of dry archaeological bone may be visualized using the same techniques of bright field or polarized light microscopy. Sample preparation mirrors that of modern bone tissue. Transmitted light microscopy requires ground or microtome-cut thin sections, while reflected light microscopy involves polishing the bone surface (Stout, et al., 2019).

3.2 Scanning electron microscopy: Scanning electron microscopes visualise the osteocytes lacunae along with those occupying mineralized osteocytes (Bell, *et al.,* 2008).

3.3 Transmission electron microscopy: Apoptosis resulting from the activity of osteoclasts are visualised using transmission electron microscope (Bell, *et al*., 2008).

3.4 Confocal laser scanning microscopy: Confocal laser scanning microscope (CLSM) utilizes precise laser beam to generate 3D images of the bone tissue. It is a type of fluorescence microscopy that uses a precise laser and a pinhole to block out-of-focus light. This precision produces two-dimensional images with higher contrast and better lateral and axial resolution than traditional wide-field epi-fluorescence microscopy. Images at successive two-dimensional depths can be stacked into a three-dimensional image (Maggiano *et al.,* 2009) CLSM has been advanced as a technique for visualizing and quantifying the three-dimensional structures of cortical pores, osteocyte lacunae and canaliculi, and micro cracks in archaeological bone (Papageorgopoulou *et al.,* 2006, 2007, 2010) CLSM can also identify tetracycline labelling in well-preserved archeological bone, following osteons and lamellae through three-dimensional space. (Maggiano *et al*., 2006, 2009).

4. Radiography

Radiological examinations can be complementary to the macroscopic analyses of bones but are indispensable when dealing with mummified remains. Radiography and CT scanning of bones should be performed whenever possible, both to help in the diagnosis of the disease and for a permanent documentation of the remains.

Radiography of a specific lesion (trauma) demonstrates two features:

1. involvement of the bone tissue in areas not observed externally and
2. patterns of bone density in and around the area affected by trauma.

4.1 X Ray: Radiographic techniques use X-rays to visualize the internal structures of the object. X-rays are electromagnetic waves able to penetrate materials. They interact with the atoms of the material and can be absorbed or scattered. The X-rays are said to be attenuated with a reduction in X ray intensity. Their absorption depends on the density, thickness, and atomic number of the material. The X-rays that pass through the examined object have a pattern of intensity that reflects the absorption characteristics of the object, and this pattern is recorded to form an image.

4.2 Radiogrammetry: Radiogrammetry quantifies the cortical bone in tubular bones and can be measured on a plain radiograph (usually by computing the ratio between the total width of diaphysis and the medullary cavity thickness, not unlike measurement for calculating body mass) (Ives and Brickley, 2004). It is used for measuring the cortical thickness of a bone. It provides age related bone loss data. For trauma studies the quantity of cortical bone accumulated during growth is used as an indicator of stress and direction of movement of the bone.

4.3 Bone densitometry: Bone densitometry, initially used as photo densitometry, used aluminum step wedge as a standard and estimated the bone density using an optical densitometer, which was later replaced by photons. Dual-energy X-ray absorptiometry (DEXA) is currently used. The trauma area is exposed to the X-rays and the density of the bone is known. The data is helpful in determining and comparing the bone densities among skeletal populations. (Agarwal, 2008; Beauchesne and Agarwal, 2017).

4.4 Computed Tomography (CT): For evidences having preserved soft tissues which can't be probed using invasive techniques, computer-aided tomography (CT) is done. CT is advantages in paleopathology of trauma as it provides access to skeletal structures which can't be observed otherwise. The sliced images of skull and pelvis obtained from CT are easy to study. The first CT scan was performed on an Egyptian mummy in 1979 (Harwood-Nash, 1979).

5. Biomolecular Techniques

The molecular techniques for studying trauma in skeletal remains include-

5.1 Palaeomicrobiology of human pathogens: Palaeomicrobiology, the study of the antiquity and molecular evolution of pathogens, most usually involves the study of DNA from modern pathogens rather than ancient DNA. However, it is likely that, as work on large samples of skeletons becomes more common and techniques for amplifying and studying ancient DNA improve, the study of pathogen DNA from ancient skeletons will begin to make a significant contribution to the understanding of the evolution and spread of microbial human pathogens. Palaeomicrobiology of human pathogens- the microorganismic remains found on the skeletal remains are useful in establishing the pathogenic activity. (Pinhasi and Mays, 2008)

5.2 Immunological Detection of Carbohydrates and Proteins: Antigen, antibodies, Immuno-enzymatic, protein specific molecular techniques have been applied to detect the presence of parasitic organisms particularly in mummies. Bone can protect proteins from degradation and it is possible to isolate extracellular matrix proteins, including collagen and immunoglobulin G molecules, from archaeological material (Schmidt-Schultz and Schultz, 2004).

Molecules are separated by one-dimensional polyacrylamide gel electrophoresis and identified by Western blot analysis using antibodies linked to an enzyme- or fluorescent-detection system, or silver stained. Two-dimensional gel separation increases sensitivity. Kolman et al. (1999) examined 200-year-old tissue for the causative organism of syphilis, Treponema pallidum. High-performance liquid chromatography (HPLC) was used to isolate immunoglobulin from femoral bone tissue, which was identified using ELISA against treponemal antigen, although this did not distinguish between subspecies. (Pinhasi and Mays, 2008).

5.3 Ancient DNA (aDNA): Ancient DNA (aDNA) can be helpful in identifying the evolutionary pattern of any morphological feature. In a particular case, the aDNA can be helpful in underlining the adaptation to environment and may be indicative of pathological conditions during the life span till death. The technology employs attaching the extracted aDNA to a primer and regrowing it using PCR technique. The DNA sequence thus obtained is compared with the known databases and possible pathology is identified. (Pinhasi and Mays, 2008).

CONCLUSION

Trauma is the most frequent pathological condition, and hence requires a wide array of techniques for its diagnosis and etiology. Based on the above review we can conclude that the impacts of traumas can be assessed in skeletal remains by utilizing the techniques of physical observation, histomorphology, microscopy, radiography, and biomolecular sciences.

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