Different Methods Establishing Time Since Death From Skeletal Remains

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Abstract: Estimation of time of death is an intrinsic part of the medico-legal investigation. The forensic anthropologist requires an innate knowledge of the human skeleton, which includes various anatomical planes of reference, the features of skeletal tissue at various levels, internal features of the bone to estimate the time since death. According to various researches ages of skeletons are determined by various methods like DNA profiling, anthropometry. Various changes are observed at decomposing phases which include putrefaction, mummification, and many others. After the skeletonization, various challenges have been faced by researchers. Hence postmortem changes were examined using microradiography, electron microscopy and UV fluorescence examination of the cross section of bones, chemical methods like radiocarbon dating provides information to decode the time of death of skeletal remains. This review gives knowledge about the use of gross examination of bone, physical, chemical methods carried out on the bone for determining the time since death. [Chawala A Natl J Integr Res Med, 2019; 10(4):78-82]

Key Words: Anthropology, bone, death, time, skeletonization

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Introduction: Time since death also known as postmortem interval is the time that has elapsed since the death of a living being. To estimate the time since death plays an important role in the justice delivery system. Various methods are used to estimate the time since death [1]. The gold standard method used for estimation of time since death is the nomogram method based on a linear model of body cooling.

To reduce error rate of the nomogram method, a method was developed based on electrical and mechanical excitability of skeletal muscle, excitability of the iris, rigor mortis, and postmortem lividity [2]. A decomposition formula for calculating the post-mortem interval (PMI) of human remains has been sought, but it has remained ambiguous due to the factors associated with human decomposition. The aim is to come close and be able to include all the taphonomic parameters which influence decomposition [3].

Forensic osteology is a sub-specialty of forensic medicine which deals with examination and assessment of human bones, the assessment includes both- the identification of victim's characteristics and cause and manner of death from skeleton [4]. The determination of the postmortem interval is an important contributing factor in the identification of the skeletal remains. However, there are various challenges, because postmortem decomposition depends on the environmental conditions such as climate, nature of the soil, effect of insects or animals [5-7]. After death, many changes take place in the body due to physical, metabolic, autolysis, physiochemical and biochemical process. These changes occur in a definite manner until the body disintegrates. The measurement of these changes along with time is used for estimating time since death.

Method : Methods for estimation of the time since death are

- Gross examining the bone
- Physical test on bone
- Chemical test on bone

Gross examining the bone [10]: The gross examination of the bone was done by naked eyes and with the use of a simple microscope. The resultant differences between the recent and old bones are described below in table1

Table 1: Gross differences between the new and old bones

New bones	Old Bones
Have soft tissues attached to it especially to joint sites It is estimated that all the soft tissues are removed within an interval of 3-10 years But this period is highly dependent on the climatic conditions.[11]	No soft tissues attached
Periosteum is visible	No periosteum visible
Emit a putrid smell	No definite smell

The bones would be soft, moist and greasy to touch	The bone will hard, dry and not greasy to touch

*(*The definite period to differentiate the new and old bones could not be determined as in gross examining the bone there are higher chances of inter-observer and intra-observer variations*)

Physical test- Procedure [12]**:** Freshly sawn cross-sections of bones were examined in the dark by using ultra-violet light and the presence, intensity and distribution of fluorescence was noted

Result:
The resultant differences between the recent bones and old bones based on the various physical criteria are described below in table 2

Table no. 2: differences between the recent and older bones based on the physical criteria

CRITERIA	New bones	Old Bones
Sawing	The bones become light due to loss of organic matter and collagenous stroma, the outer cortex and the zone around the marrow cavity will show a sandwich effect	The bones are hard and uniform through the whole thickness
Examination under UV light	Fluorescence is observed with a silvery blue tint across the whole cut surface	The outer rim and rim around the marrow cavity will cease fluorescence due to loss of organic matter estimated around 100 to 150 years
Specific gravity	2	1.2 for fossil bones 1.7 for forensically significant bones

Chemical test on bone[12]: Various methods were used in order to determine the time since death from bone chemically. All the methods used bones in different forms

The various test described are:

- Nitrogen test
- Benzidine test
- Citrate acid concentrations
- Presence of amino acids
- Iodophenol test
- Nile test
- Effervescence test

Most commonly used tests giving accurate results are nitrogen tests and benzidine tests

Nitrogen test-procedure: The Kjeldahl method was used, using a combined digestion/distillation flask in conjunction with ordinary laboratory glassware. Representative cross-sectional bone fragments of the order of 1 gm. were used.
The procedure for the Kjeldahl test is described below in figure 1 and 2

Figure1: Procedure of Kjeldahl test

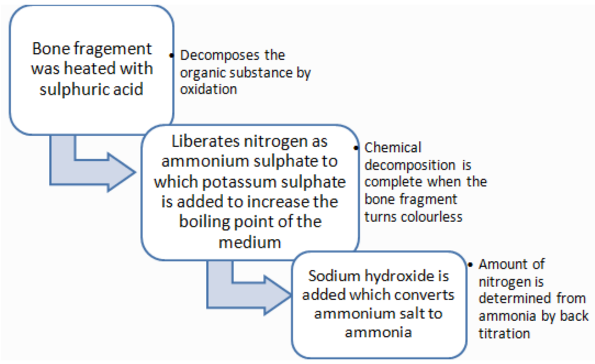
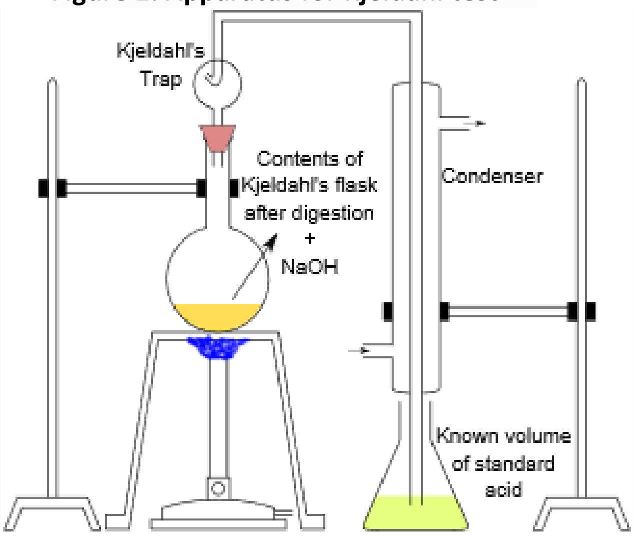


Figure 2: Apparatus for Kjeldahl test [17]



The results showing the difference between the recent and older bones are described in table 3.

BENZIDINE TEST- procedure [10,12]: Freshly prepared benzidine-acetic acid peroxide solution was added, to a small quantity of the powder (prepared during sawing the bone using electric saw), all glass-ware used are benzidine-negative. Result describing the difference between the bones is shown in table 4

Table 3: the resultant difference between the bones based on the Kjeldahl test

Age	% of nitrogen
0-2 Years	4.0-4.4%
200-700 Years(Approx)	2.1-3.8%
800-1700 Years(Approx)	1.1-2.5%

Table 4: The difference between the bones based on the benzidine test

Age	Benzidine test
0-2 Years	POSITIVE
200-700 Years(Approx)	NEGATIVE
800-1700 Years(Approx)	NEGATIVE

Citrate acid concentrations[13]: If the concentration of citric acid in bones was found more than 0.140% in the bones, the time since death was estimated to be more than 50 years.

Presence of amino acids[10.13]: Fresh bones showed about 15 amino acids present. Bones ages more than 50 years showed an absence of proline and hydroxyproline.

Other tests like iodophenol and Nile tests, effervescence test showed very little forensic significance in the estimation of time of death[12]. The Formula for estimating the time since death

According to Vass (2011), PMI estimation can be calculated for surface decomposition and burial decomposition contents. The following formula outlines the calculation for surface contexts in which there access to oxygen. This would not be relevant for confined spaces above the ground. This formula is designed for a scenario in the pre-skeletonization stage [14]

PMI(aerobic)= {1285 ×decoposition÷100} ÷ {0.0103 × temperature × humidity}

Where: 1285 i.e. ADD (accumulated degree days) constant can be calculated
ADD=10(0.002*TBS*TBS+1.81) + 388.16
(TBS- Total Body Surface)

Figure 3 describes the TBS score to be estimated in the formula

Figure 3 shows the Total Body Surface score to be estimated to calculate ADD[10]

Descriptions
Fresh, no discoloration
Pink-white appearance with skin slippage and some hair loss
Gray to green discoloration; some flesh is still relatively fresh
Discoloration and/or brownish shades, particularly at edges; drying of nose, ears, and lips
Purging of decomposition fluids out of eyes, ears, nose, and mouth; some bloating of neck and face may be present
Brown to black discoloration of flesh
Caving in of the flesh and tissues of eyes and throat
Moist decomposition with bone exposure less than one half that of the area being scored
Mummification with bone exposure less than one half that of the area being scored
Bone exposure of more than half of the area being scored with greasy substances and decomposed tissue
Bone exposure of more than half of the area being scored with desiccated or mummified tissue
Bones largely dry but retain some grease
Dry bone

Source: Megyesi et al., *J Forensic Sci* 50, 2005; pp. 618–626.

Decomposition: Figure 4 describes the percentage of decomposition to be estimated in the formula to calculate the PMI under aerobic conditions. 0.0103 is a constant that represents an empirically determined measure of the effect of moisture on decomposition rates.

Temperature is the value in degrees Celsius (^{0}C) of either the average temperature measured at the site of crime on the day the corpse was discovered or the the average temperature over a period of time.

Humidity is a value between 1 and 100, representing either the average humidity at the site on the day the corpse was discovered or the average humidity over a period of time

The above-mentioned formula can be applied only under special conditions and specifically under aerobic conditions. The tables describe the scores to be estimated in their respected formulas

Figure 4: The % of decomposition to be estimated in the PMI$_{(aerobic)}$formula[10]

Stage	Decomposition range under warm conditions	Decomposition range under cold conditions
Fresh	1–10%	1–20%
Bloat	11–35%	21–45%
Decay	36–85%	46–85%
Dry	86–100%	86–100%

Discussion:: Identification of an individual is a very important goal in forensic science. The use of conventional methods such as dactylography (the study of fingerprints), DNA profiling, cheiloscopy, handprints, footprints, etc. cannot be used when the body undergoes skeletonization. The last phase is marked by either complete skeleton and the subsequent breakdown of the skeletal elements.

A wide literature exists especially on the chemical methods of estimating the time since death from bones and is still increasing. However, most of the methods have never gained much practical relevance as they were not precise to the number of years, reliable.

Most methods proposed for estimating the postmortem interval are of academic interest since they describe the postmortem changes based on various environmental conditions. The methods used for estimating the time since death are not only different in nature but their scientific value, the underlying scientific background and the amount of validation also differ greatly. Some methods for estimation of time since death describe the quantitative measurements with mathematical description of a time change have been done, taking into account influencing factors quantitatively and clear data on the precision of estimating the time since death with proof of the precision on independent case material are available, and other methods are based on a subjective grading of postmortem changes[15].

Hence methods are being carried out based on just empirical data instead of statistically evaluated reference values.

Conclusion: When at a crime scene various fields of forensic science come together in order to come to a conclusion for estimating the time since death. A forensic anthropologist can assist in the identification of disfigured deceased individuals where bodies are decomposed, burnt, mutilated or otherwise unrecognizable, as might happen in a plane crash. Forensic anthropologists are also involved in the investigation and documentation of genocide and mass graves. Along with forensic pathologists, forensic odontologist, forensic anthropologists commonly testify in court as expert witnesses. Various methods like gross examination of the bone, physical tests and chemical tests on the bones have proved to be useful in estimating the time since death but quantifying the value of years is still in progress and thus the formula for dating the bone has proved to the 'thumb rule' for estimating a range for the estimation.

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