

## Age Assessment in Forensic Cases: Anthropological, Odontological and Biochemical Methods for Age Estimation in the Dead

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### ABSTRACT

One of the main goals of forensic science is determining the age of an individual to perform the biological profile. This is important in criminal cases and mass disaster scenarios, where the skeletons are often incomplete, which makes difficult their identification. The knowledge and application of anthropological methods and new biochemical approaches is essential to correct age determination, leading to the identification of an individual. Forensic anthropology and odontology methods estimate age through the macroscopic changes in bones and teeth due to growth and development in subadult individuals, and degenerative changes in adults. When growth has ceased, age estimation in adults is basically based on the degenerative changes of bone and teeth, and is generally less precise than in subadults. Due to the accuracy of the age estimate decreases as the age of the individual increases, other methods have been developed for estimating the age of the adult individual based on changes at the biochemical level due to the physiological process of aging. Biochemical methods are accurate, however, they have some limitations, so its use in combination with anthropological methods can be very useful for accurately estimating age.

### KEY WORDS

Age Estimation; Biological Profile; Hman Identification.

### INTRODUCTION

#### Anthropological and Odontological Methods for age estimation

Traditionally, forensic anthropology and odontology methods estimate age through the macroscopic changes in bones and teeth due to growth and development in subadult individuals, and degenerative changes in adults. Selection of methods to be employed in age estimation depends upon the materials available for examination, their condition and the age category of the individual [1].

According to the age group to which the individual belongs, different methods of age estimation can be used. In this way, dental development could be used in fetal individuals [2-4], the presence of ossification nuclei [5], or long bones development [6]. In the case of children, dental development can be used [4, 7], the presence of ossification centers and the fusion of the epiphyses [3,8], and development of hand and

wrist bones [9]. Dental development has been demonstrated to reflect chronological age more accurately than osteological development. Dental development appears to be under stronger genetic control, while osteological development is more influenced by environmental factors such as biomechanics, physiological stress and nutrition [1]. So that, in cases of age estimation of immature individuals (fetal, neonatal, infant, child and adolescent) a special attention on dental age estimation methods should be considered.

In adolescents and young adults the most relevant age indicators are the development of the third molar [10], development of hand and wrist bones [9,11], spheno-occipital fusion [6,12] (Scheuer and Black, 2000; Madeline and Elster, 1995), and fusion of the sternal end of the clavicle [13, 14]; It is advisable to use as many indicators as possible, to obtain better results.

As in the case of children, adolescents and young adults (up to about 20-25years of age) skeletal indicators of age are based on the individual's growth and development; but when growth ends and the development of the individual is completed, age indicators are based on degenerative changes in the skeletal system.

There are different indicators of bone age in the adult as the pubic symphysis [15, 16], auricular surface of the coxae [17], the acetabulum surface [18], the sternal end of the ribs [19-21], as well as degenerative changes in teeth [22-24].

Also, at a microscopic level an estimate of age can be achieved by the number of osteons [25-27].

All these mentioned methods based on different skeletal indicators have certain limitations that must be taken into account when applying them in forensic cases. Suchey-Brooks method [15] as well as Lamendin method [24] show greater accuracy in individuals between 20 and 40 years old. While the Isçan method [19-21] is more reliable for individuals over 60 years old. The Lovejoy method based on auricular region changes and his method based on the closure of cranial sutures [17, 28] have shown inter- and intraindividual variation. Also, the microscopic method based on the number of osteones present in the bone [25-27] is harder to implement than the observational methods mentioned. Table 1 resumes the different skeletal indicators for age assessment.

**Table 1:** Different indicators of skeletal age for different age groups [15-28]

When growth has ceased, age estimation in adults is basically based on the degenerative changes of bone and teeth, which can be affected by pathology.

Age range	Analyzed feature
FETUSES	Dental development Presence of ossification nuclei Long bone development
NEWBORN	Dental development Presence of ossification nuclei Diaphyseal fusion
CHILDREN	Dental development Presence of ossification nuclei Epiphyseal fusion Bone dimensions Hand and wrist bone development
ADOLESCENTS	Dental development Epiphyseal fusion Hand and wrist bone development
TRANSITION	Third molar development Epiphyseal fusion Hand and wrist bone development Spheno-occipital basilar synchondrosis fusion Clavicle sternal end fusion
ADULTS	Pubic symphysis Auricular surface changes Acetabular surface changes Sternal end of ribs

Therefore, the estimation of age in adult individuals is generally less precise than in subadults [29]. Because of that reason, most researches suggest that

when determining age in adult individuals, assessing multiple age indicators provides more accurate results than using a single indicator [1]. So that, it is always recommended to use as many indicators as possible in order to achieve the most accurate results.

Age estimation accuracy decreases as the age of the individual increases, other methods have been developed for estimating the age of the adult individual. These newer methods are based on changes at the biochemical level due to the physiological process of aging [30].

### Biochemical methods for age estimation

Biochemical methods for determining age are based on the natural process of aging, which induces alterations in tissues and organs at different biochemical levels [30]. According to these levels, methods for determining age are divided into chemical methods, including racemization of aspartic acid, lead accumulation, collagen cross-links, chemical composition of the teeth and analysis of advanced glycation end products (AGEs). Molecular methods include the analyses of telomere shortening, sjTRECs rearrangements, the study of mitochondrial mutations and more recently epigenetic modifications [31, 32].

In living organisms, the most common amino acids have the L optical form. The racemization converts these amino acids into their D form, producing conformational alterations in proteins, which affect their biological activities and their chemical properties [33, 34]. These alterations in proteins can be correlated to the progressive changes associated with aging [35]. Aspartic acid has the fastest racemization range, becoming D-Asp, and being the most widely used amino acid in age estimation studies. Hence, aspartic acid racemization has been applied to different tissues, showing its accuracy in dentin [36, 37], cementum [36], intervertebral discs [38], elastine [39] and bone [40]. Despite the accuracy of this method (+/-3 years of error regarding chronological age), it can not be applied to bodies exposed to high temperatures.

Since lead is one of the most common contaminants, measurement of its accumulation in dentin has been studied for the determination of age. The study of Al-Qattan and Elfawal [41] found an error of 1.3 + 4.8 years in the Kuwaiti population. Despite this result, more research is needed in this line to be able to assure that this technique can be applied to determine the age in forensic science.

Collagen matrix of cartilage, bone, dentin and other skeletal materials is stabilized through covalent bonds between collagen molecules [42]. A component of these linkages is deoxyypyridoline

(DPD). This component has been studied in relation to age, obtaining an error in the estimate of +14.9 years [43].

The chemical structure of the teeth changes with aging, particularly, the mineralization of the dentin and the reduction of the dentinal tubules, inducing an increase of the transparency of the root with the age. Structural and biochemical changes in dentin have been studied by several researchers in relation to age [5, 44], although the error in the estimation is not very high, additional studies are required to determine its possible application in the forensic context.

Advanced glycation endproducts occur through the Maillard reaction between reduced sugars and amino groups of the proteins, which induce different modifications in those proteins. These compounds accumulate with age and have been correlated with complications derived from aging and associated diseases [44]. Few studies have been developed for forensic purposes, but they relate the accumulation of these compounds with age [45, 46]. Also, because of the color change caused by these compounds, this change has been related to age in some tissues [47].

Telomeres are at the end of the chromosomes, with each cell division, these terminations are shortened, limiting the proliferation of human cells and inducing senescence, differentiation or cell death. Different studies show that during the aging process telomeres are shortened [48, 49]. Because of this some researchers have studied them to determine the age [50, 51, 52], however, the mean error of this technique is about 10 years, being greater than in other techniques previously described.

During lymphocyte development in the thymus each immature T lymphocyte undergoes somatic rearrangement of its receptor (TCR, T-cell receptor), to generate a variety of TCR molecules. During this process, the DNA sequences of this TCR are eliminated and circularized in what are called "signal joint TCR excision circles, sjTRECs". These products do not replicate in cell division, for this reason they have been analyzed in relation to age [53, 54, 55], progressive decreasing of these products with the age. Like the prior technique, the associated error is 10 years.

According to mitochondrial theory of aging, mitochondrial DNA (mtDNA) is near the inner membrane of the mitochondria, which makes it more susceptible to damage generated by the free radicals released by the electron transport chain. This produces mtDNA mutations. An increase in the oxidative damage of mtDNA induces a deterioration in the mitochondrial respiratory function, producing more free radicals and consequently, an accumulation of lesions that can not be repaired in mitochondrial DNA [56]. Different studies have shown the

correlation between mitochondrial mutations and age in different tissues [56-60]. However, further research is required in this line to determine the level of accuracy of this method.

Recently the correlation between methylation levels and age has been established in blood samples and dentin, being for blood samples the associated error of 3.75 years, with respect to chronological age and 4.86 error, with respect to chronological age for dentine [31]. These results are encouraging for its possible application in forensic science, although this research is still starting, it requires the analysis of more methylation markers to increase the efficacy and accuracy of this method.

Biochemical methods available for age estimation with its standard errors are presented in table 2.

**Table 2:** Biochemical methods available for age estimation with its standard errors [30-52].

Type of Method	Technique	Standard error
CHEMICAL METHODS	Aspartic Acid Racemization	+/-3 years
	Lead accumulation	+/-4.8 years
	Collagen cross-links	+/-14.9 years
	Teeth chemical composition	+/-5 years
	AGEs	+/-13.7 years
MOLECULAR METHODS	Telomer shortening	+/-9.8 years
	sjTREC rearrangements	+/-10.47 years
	Mitochondrial mutations	variable
	Epigenetic modifications	+/-3.75 years

### CONCLUSIONS

In this article, anthropological and biochemical methods for the correct determination of age have been discussed. Biochemical methods seem to be more accurate for this purpose, particularly the racemization of aspartic acid and more recently the epigenetic modifications, however, they have some limitations, so they could be useful in combination with other anthropological techniques. The selection and application of one technique or another depends on the forensic context and the availability of the human remains.

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