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Biochemical method of age estimation by dentition

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Abstract

The human teeth function to mechanically break down items of food by cutting and crushing them in preparation for swallowing and digesting. Humans have four types of teeth: incisors, canines, premolars, and molars, which each have a specific function. The incisors cut the food, the canines tear the food and the molars and premolars crush the food. The roots of teeth are embedded in the maxilla (upper jaw) or the mandible (lower jaw) and are covered by gums. Teeth are made of multiple tissues of varying density and hardness. However, the combination of methods results in increased accuracy of the estimation. So, whenever possible, dental age estimation should be combined with skeletal and biochemical methods in order to achieve more accurate results. It means that in age estimation, as in all other fields of forensic science, multidisciplinary represents a clear benefit on the results.

Introduction

The functions of human teeth is to mechanically fragment the food particles by the process of crushing, chewing and swallowing them before leading them to alimentary canal for further digestion. The teeth of Humans are of four types: incisors, canines, premolars, and molars, each of them having a specific and distinctive function. The incisors are used for fragmenting the food, the canines for teasing or tearing the food particles, the molars and premolars for crush activity of the food particles. The gums in the oral cavity coverroots of teeth thatare entrenched in the maxilla (upper jaw) or the mandible (lower jaw). The composition of Teeth consists of tissues comprising of enamel, denting, crown etc., of different density and hardness.

Age estimation by Dentition is used in both living and dead with varying accuracies. Age estimation by densities finds a very important application in mass disasters along with identification and other cases involving application of Forensic science and medicolegal aspects. Required in dead body investigations; the criteria of dental age estimation also finds application in the living such as in criminal cases and immigration related issues. In every such case, age estimation by dentition serves as a reliable, scientific, quick and inexpensive tool.

Age estimation by dentition method assessments rely on developmental changes in the teeth throughout the life including the post formation changes. Hence, such methods, studying the tooth shaping and development, will be routinely applied on adult individuals, relying extensively on the examination and evaluation of incremental developmental changes seen in imaging modalities such as radiography, CT-scan or Magnetic resonance Imaging. Post formation changes in tooth methodology applied on adults also rely on conventional radiography along with microscopic or macroscopic evaluation of certain teeth post formation stages or changes.

The classified categories are: (1) morphohistological methods, (2) radiological methods, and (3) biochemical methods.

Gustafson's method [1]

Gustafson's method was the earliest technique employed for age estimation of adult over 21 years based on the physiological age changes like certain regressive changes or alterations in each of the dental tissues. This was developed by Gosta Gustafson in 1947 and 1950. Gustafson's method is mainly a histo-morphological study applied on single-rooted teeth. The changes as per gradation are:

- Attrition of the enamel (*A*)
- Secondary dentin deposit (*S*)
- Alteration/recession of periodontal ligament (*P*)
- Cementum apposition (*C*)
- Root resorption (*R*)
- Transparency/translucency of dentin (*T*).

Radiological methods

These methods are more noninvasive or destructive and hence became widely applicable in the dead as well as in the living individuals. Also they are used in archeological studies or some cases of medicolegal or judicial setups where tissue extraction from human remains become problematic. These nondestructive methods became popular because of their applicability in living and dead individuals as well as in

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archaeological studies or in certain judicial setups which prohibit tissue collection from human remains which become problematic. Routinely, the decrease in size of dental pulp cavity from secondary dentin deposition is used as an indicator for age estimation but estimation of root transparency criteria is the most reliable of all criteria.

Biochemical methods

The tooth parts such as enamel, dentin or cementum are nothing but metabolically stable protein structures. Rapid racemization of aminoacids in these proteinaceous structures forms the histochemical basis of age estimation. Aminoacid such as L-aspartic acid may undergoes racemization which is stored as D-aspartic acid in increasing amounts as age advances.[2]

Analyzing a study of Helfman and Bada, it was deduced that the calculated ratio of D/L enantiomers in aspartic acid from the enamel and coronal dentin could be appropriately applied to calculate the age of any stable protein from a long-lived mammal and thus the age of the subject or organism.[3] A study by Ohtani *et al.* proposed that dentin aspartic acid shows linear increase with age.[4]

Reflecting upon the study by Ritz *et al.*, where racemization method in dentinal biopsy specimens (1 mm × 1 mm) was obtained from the molars of living individuals, a close association was seen between the range of racemization of aspartic acid in biopsy specimens obtained from the dentin and age.[5]

Age estimation using biochemical methods are based on natural aging process inducing derangements or alterations in tissues and organs at various biochemical levels. Hence, as per these levels, age estimation methods are classified into chemical methods, that includes racemization of aspartic acid, lead accumulation, collagen cross-links, chemical composition of the teeth and analysis of advanced glycation end products (AGEs).Then, there are the Molecular methods which include telomere shortening analysis, rearrangements of sjTRECs, the mitocondrial mutations studies and epigenetic modifications which was highlighted in recent times.[6-8]

In living subjects, the L optical form is found among most of common amino acids. They are converted into their D form by racemization which results in protein alterations causing a change in their biological activities and chemical properties. The study or observations of these derangements in the protein structures can be correlated with the progressive or regressive changes of aging. Aspartic acid, on account of it undergoing fastest racemization, to D-Asp, and also commonly used amino acid in age estimation studies has an important place in such studies. So, therefore, racemization of aspartic acid has been applied to different tissues, exhibiting its accuracy in dentin, cementum, intervertebral discs, elastine and bone. Though this method is fairly accurate, (+/-3 years of error regarding chronological age), high temperature exposed bodies does not find a place in its application. [7-14]

The notion that the common contaminant like lead and its measurements of incremental accumulation in dentin has been accepted and studied for age determination. A documented error of 1.3 +4.8 years was found among the Kuwaiti population, as per a study of Al-Qattan and Elfawal.[15] The current results along with additional research are needed to supplement the fact that this technique can be employed in age estimation in forensic science.

The covalent bonds connecting collagen molecules in the Collagen matrix of cartilage, bone, dentin and other skeletal materials helps in stabilization. A part of these linkages is deoxypyridoline (DPD). This metric is studied and analysed in relation to age, when an error was documented in the estimate of +14.9 years. [16,17]

As teeth ages, there is a noticeable change in the chemical structure, especially, the dentin mineralization and the decrease in the dentinal tubules, thereby stimulating an increase in the root transparency with the age. Dentin has been a subject of study with respect to structural and biochemical changes in age estimation. However, though the error in estimation is low, several more studies are desired to conclude that it may have a practical use in forensic field.[18]

 Advanced glycation end products which present through the Maillard reaction between reduced sugars and amino groups of the proteins, inducing different modifications in those proteins. These compounds are seen in aging with increased severity and are hence correlated with age-related complications and diseases.Very fewer studies have been done for forensic purposes, relating to the accumulation of these compounds with age.

Telomeres present at the chromosomes, are shortened with each cell division, thereby hindering the proliferation of human cells and inducing early cell death. Shortening of telomeres as age advances is shown in various studies. This has prompted some researchers to study them in age determination. However, there is a increased error percentage with the mean error of this technique being about 10yrs, which is greater compared to other previously described techniques.

Lymphocytes are matured and developed in thymus. During this process, each immature T lymphocyte undergoes a process of somatic rearrangement of its receptor (TCR, T-cell receptor), to generate many copies or forms of TCR molecules. In 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, and hence they are analyzed with respect to age, or rather age related progressive decreasing of these products. However, as in the previous technique, the associated error is 10 years.

InMitochondrial theory of aging, mitochondrial DNA (mtDNA) is located closely to inner membrane of the mitochondria, thereby making it more vulnerable to damage from the free radicals released or derived from the electron transport chain. These phenomena inducesmtDNA mutations. The mitochondrial respiratory function decreases or gets deranged as oxidative damage of mtDNA is aggravated, resulting in an increased production of free radicals and consequently cellular degeneration which cannot be set right by the mitochondrial DNA.

Conclusion

Te study highlights the fact that various combinations of methods and techniques should be employed to increase the frequency of age estimation. Therefore, more frequently and routinely, dental age estimation should be done along with skeletal and biochemical methods, thereby increasing the accuracy of estimated results. To conclude, age estimation by dentition should be done in an inclusive fashion encompassing many disciplines as resorted to in many other forensic science fields so that results give a concise and precise benefit.

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