Forensic anthropology and molecular biology: independent or complementary sciences in forensic dentistry? An overview

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Abstract
Human identification is currently one of the most outstanding areas of Forensic Sciences. Forensic anthropology is a branch of the Forensic Sciences concerned with the application of general anthropological knowledge and methods to the process of law. *Postmortem* human identification is one of the major areas of study and research in Legal Medicine and Forensic Dentistry and both sciences work with the same type material and the human body in several conditions. Every human being has an identity in life, which should be recognized after death both for family consolation and for juridical purposes. Therefore, the aim of this literature review was to discuss and elaborate on the relationship between Forensic Anthropology and Molecular Biology in the expert practice of Forensic Dentistry. The use of DNA profile tests in Forensic Dentistry offers a new perspective in human identification. Molecular biology techniques should definitely be added to the constellation of investigative resources of the forensic sciences, providing a variety of tools to Forensic Anthropology practice in human identity research, especially when the usual identification methods fail due to the deleterious effects of heat, trauma or autolytic processes on the body or body remains.

Key Words:
Forensic dentistry; forensic anthropology; molecular biology

Introduction

Forensic anthropology is a branch of the Forensic Sciences concerned with the application of general anthropological knowledge and methods to the process of law. Its main goal is related to the medical-legal identity, defined as the aggregate of unique characteristics of persons, animals and objects. Taken together, positive or negative signs, marks and physical characteristics individualize the human beings, making possible to distinguish one person from another[1].

Molecular Biology has been recently applied in Forensic Anthropology. Until the 1980's, the science of identification of criminal cases was based only on serological analyses of protein polymorphism, blood groups and some genetic markers. Forensic examination of biological samples started in the beginning of the 20th century by application of the ABO blood group system in evidences related to crimes or human identification[2]. The discovery of the double-helix structure of DNA, in 1953, which is responsible for the genetic inheritance in human beings, caused a major impact and led to remarkable changes in nearly all fields of science. This knowledge has been the basis for development of techniques that allow characterizing each person's individuality based on the DNA sequence.

Three decades later, Jeffreys et al.[3] created radioactive molecular probes that could recognize certain highly sensitive regions of DNA and determine specific patterns of each individual, which were named DNA "fingerprints". The currently performed DNA profile tests are very reliable, being accepted as legal proofs in courts for investigation of paternity and human identification.

There are many acceptable methods for human identification, each one with its limitations. Fingerprints have historically been used to determine people's identity. However, in some situations (e.g. in fires, decomposing bodies or skeletonized bodies), fingerprints are easily destroyed. Identification of human remains using genetic tests has thus been recognized as an important and reliable resource of Forensic Anthropology. Hairbrush, toothbrush and other objects of personal use may contain material that can be used for comparison. Nevertheless, only the presence of hair or oral cells does not provide all elements necessary for identification. In the same way as for dactyloscopy, for DNA fingerprint molecular typing techniques to be of value in human identification or crime solving, it is also necessary to have a previous registration or available offspring, in addition to a sophisticated sample

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processing. Furthermore, although the anthropologic analysis can provide useful information about stature, race and gender, it does not determine someone's identity [4].

In this way, *postmortem* human identification is one of the most important areas of study in Forensic Dentistry, as it can work with the human body in several conditions (quartered, lacerated, carbonized, macerated, putrefied, in skeletonization process and skeletonizated), pursuing to establish human identity[5]. The advent of molecular biology has introduced new methods, equipments and perspectives to the contemporary Forensic Dentistry, though it still have some limitations.

According to Sweet[6], because every human being has an identity in life, there is a basic societal need for this identity to be recognized after death, both for family consolation and for juridical purposes. Human identification has a legal implication and is part of the process of investigation of death causes, since a responsible person should be indicated, even in a suicide case[7].

In this sense, the technical requirements of human identification techniques for any recommendation could be listed as follows[8]: uniqueness (identification elements should allow an accurate and clear distinction between the suspected and the others); immutability (the analyzed features should not suffer alteration with the course of time); perenniality (the analyzed features should be present throughout the life); practicability (safe and rapid procedure for collection of identification data in order to avoid creating constraint for the identified person and allowing a good safety and reliability degree); classification (it should allow comparison among collected data, in a synthetic and accurate way, quickly distinguishing a subject in a population); and reproducibility (capacity to reproduce the same technique under the same conditions, anywhere and at any time).

A critical analysis of these ideal conditions for human identity research shows that only few methods fulfill all these requisites, for example dactyloscopy, dental arch identification, genetic identification tests (except for identical twins who have the same genetic load). Other tests can be used, such as analysis of the palatal rugae pattern (a less used method that is simple to perform, but do not provide a clear classification); and biometric tests, which include patterns and comparison of iris, voice, retina, and signature.

As far as human identity is concerned, it is important to emphasize the difference between recognition and identification. While recognition can be understood as an empiric identification (by family, friends and/or colleagues) without scientific criteria, identification is established using well-established scientific knowledge and techniques[5,7-9]. There are two types of human identification: comparative and reconstructive. The first is based on *antemortem* evidence, allowing individual identification by medical and dental records. In the reconstructive process, it is not possible to use these data and it is necessary to make general identification by defining gender, age and race[10].

The purpose of this literature review is to discuss and elaborate on the role of Forensic Anthropology and Molecular Biology in human identification, as well their application in Forensic Dentistry.

The Legal Role of Forensic Dentistry in Human Identification

The legal competency of the dentist for procedure in cases of forensic expert identification is based on the Brazilian Federal Law no. 5081/66[11], which rules:

"Art. 6. It is the dentist competency:

I – The practice of all the acts pertinent to the Dentistry, resulting from the knowledge acquired in a regular undergraduate or postgraduate course;

(…)

IV – To proceed with forensic dentistry expert examination in Civil, Criminal, and Labor Court in administrative headquarters".

Furthermore, the Brazilian Federal Council of Dentistry by its Resolution No 63/2005[12] determines in Art. 64, the expert ability of Forensic Dentistry.

"Art. 64. The competency areas to the practice of the Forensic Dentistry expert include:

a) Human Identification;
b) Expert analysis in Civil, Criminal, and Labor Court;
c) Expert analysis in administrative area;
d) Expert analysis, evaluation and planning in labor hazard;
e) Forensic thanatology;
f) Elaboration of: 1) records, reports, opinions; 2) records and certificates;
g) Legal dentistry traumatology;
h) Forensic ballistic;
i) Logistics expert analysis in alive, dead, complete or fragmented persons;
j) Expert analysis in correlated vestiges, including blot or liquids from oral cavity or present in it;
l) Image examination with expert analysis purposes;
m) Deontology
n) Forensic dentistry orientation to the professional practice;
o) Image examination with legal dental purposes."

According to Sweet[6], the dentist has a key role in the search for human identity. This author has emphasized that oral records are currently used in three kinds of identification, two of which have been used for many decades and are characterized as the main responsibility of the forensic dentist. The first is dental identification and involves comparison between *antemortem* and *postmortem* records. The second refers to the reconstruction

of the *postmortem* dental profile, which is used in cases when the subject's or his/her offspring's identity are unknown. The third refers to the application of modern DNA profile techniques. In this way, the collaboration of Forensic Dentistry with Forensic Anthropology can be verified by human identification using body remains, such as skull, working by comparison with dental records, photographs and clinical records[7,13]. Moreover, Forensic Dentistry can act in the identification of victims of mass events such as natural disasters[14,15], bus accident involving body carbonization[16,17], plane crashes[18-20], conflagration[21], train accidents[22], military accidents and wars[23], in addition to the study of allelic frequency to be used in forensic investigation[24,25].

Odontological identification examination of a decedent is based on a systematic comparison of the antemortem and postmortem dental traits of the individual according to the analysis of dental records and supported by radiographs (periapical films, panoramic radiographs and cranial radiographs)[26].

Molecular Biology Applied to Human Identification: Activities in Forensic Dentistry.

DNA is responsible for storing all genetic information. It is found in the cell nucleus chromosomes (genomic DNA) and in the mitochondria (mitochondrial DNA).

The currently performed DNA profile tests are totally reliable, being accepted as legal proofs in courts for investigation of paternity and human identification[27]. It is possible to acquire DNA from practically any human tissue, like blood, oral smear, saliva, bone, tooth, tissue, organs, hair, semen, urine, among other biological materials[28]. The amount of extracted DNA, however, varies depending on the tissue[12].

In forensic investigations, DNA studies are usually done by the analysis of tandem regions of a determined base sequence. STRs (short tandem repeat) are the most used technique in forensic samples because they indicate a repetition sequence (from 2 to 9 base pairs), which make loci smaller than 300 base pairs. These repeated sequences are spread throughout the human genome and present a sufficient variety to be used in human identification tests, and allows a variability of choice in forensic identification[29].

In forensic investigations, the characterization of the biologic sample aims to limit or to reduce the number of subjects that could be the material origin in analysis, since the application of DNA technology provides a valid discriminatory capacity to reach the required limit for human identification[30]. This demonstrates the applicability of dental samples in several conditions. The environmental influence on the concentration, integrity and recovery of DNA extracted from dental pulps has been previously measured by Schwartz et al.[31]. The authors varied the pH (3.7 and 10.0), temperature (4°C/39.2°F, 25°C/77°F, 37°C/98.6°F and tooth incineration), humidity (20, 66 and 98%), type of the soil in which the teeth were buried (sand, potting soil, garden soil, submersion in water and burying outdoors) and periods of inhumation (one week to six months). It was determined that the environmental conditions examined did not affect the ability to obtain high-molecular-weight human DNA from dental pulp.

In order to verify heat resistance, Remualdo[32] evaluated the PCR amplification of DNA retrieved from teeth subjected to heat (200°C/392°F, 400°C/752°F, 500°C/932°F, and 600°C/1,112°F) during 60 minutes, testing 3 different DNA extraction methods (organic; ammonia acetate/isopropanol and silica). The authors concluded that amplification of all samples at all temperatures was possible using the ammonium/isopropanol acetate method, which gives a high credibility to the use of teeth in DNA-based forensic investigations, regardless of tooth conditions.

In order to evaluate the different dental tissues as DNA sources in forensic analyses, Malaver and Yunis[33] conducted a study in which 20 teeth were obtained from unidentified bodies buried in 1995 and exhumed in 2000, providing 45 DNA samples (5 from the pulp, 20 from dentin and 20 from cementum). The pulp produced the strongest PCR amplification signals, while dentin and cementum signals were very similar to each other.

There is a good possibility of using teeth as sources of DNA material, even in cases where DNA extraction and retrieval seem impossible. If there is a structure in the human body that can resist the most different environment conditions and still provide material for analysis, this structure is the tooth.

In addition to the teeth, molecular biology applied to Forensic Dentistry also permits the analysis of cells from oral smear and human saliva, which can be obtained in cases of physical violence, such as sexual crimes, murders, child abuse, where bite marks can be found on the skin[34]. Koh et al.[35] has reported that saliva is a very useful DNA source because it can be collected in a painless and non-invasive way and can be used even when stored in many different ways. The usefulness of saliva for bimolecular techniques is due to the fact that approximately 99% of its composition is water, having also leucocytes (25 to 650,000) and desquamated epithelial cells (6 to 600,000)[36]. The amount of saliva deposited on the skin is often very small in cases of bite impression, being necessary to use specific collection methods, which can recover the maximum amount of saliva and minimize any potential contaminations. Anzai-Kanto et al.[37] studied saliva obtained from volunteers was deposited on skin and recovered for DNA extraction and typing in order to evaluate its usefulness for practical case investigation and discuss the contribution of forensic dentistry to saliva DNA typing. It was verified that the double-swab technique was sensible and efficient for collection of saliva deposited on the skin and use for salivary DNA analysis in forensic cases.

Furthermore, Sweet et al.[38] used simulated situations of bite impressions in two experimental series. For such purpose, three 40 ìL saliva samples were deposited over 27 skin corpses (on 33 different sites) and three 100 ìL saliva samples were deposited over 5 skin corpses (on 12 different sites). Saliva was then collected by the double-swab techniques in periods of 5 minutes, 24 hours and 48 hours. It was observed a decrease in concentration within the first 24 hours and stability between 24 and 48 hours. Amplification was successful regardless of the time elapsed after saliva deposition. No cases of contamination were detected.

In a case of expert analysis, Sweet and Shutler[39] used DNA analysis by PCR of a saliva sample recovered from a bite mark found in a corpse that was recovered after approximately 5.5 hours submerged in a lake. Enough DNA from the bite impression was recovered to allow a genotypic contribution to identify the aggressor. The respective efficiency is attested by the fact that the saliva in contact with the intact skin is kept in stable conditions and can be recovered even 60 hours after its deposition[40]. However, it is not always possible to recover DNA from a bite impression because the sample may be subject to a series of modifications, such as contamination, degradation, and putrefaction, depending on the circumstances to which the body and/or object had been submited[41].

In this way, either or not associated to molecular biology techniques, forensic dentistry provides an effective cooperation to human identification with analysis of dental structures and/or saliva.

Forensic Anthropology and Molecular Biology: Independent or Complementary Sciences?

Molecular Biology is a helpful tool in forensic cases. Discussions involving the areas pertinent to forensic investigation are important to elucidate some issues. Legal Medicine has benefited from the unquestionable advance deriving from the study of human genome[42]. Therefore, a marked evolution has been observed in both civil and criminal identification, widening the scopes in this area and making it an important branch of Legal Medicine Haemogenetics.

In the expert analysis, the Cartesian method should be considered, i.e., from the simplest exams to the most complex ones, starting performance in Forensic Anthropology with the use of personal impressions, measurements, dental arch analysis, and, if necessary, application of modern biomolecular techniques, with the purpose of establishing identity. Nonetheless, the cost of genetic tests varies from region to region, as well as the need for formation of qualified human resources in this area.

The main restrictive exogenous factors that may hinder the retrieval of evidence from body remains, even with the use of biomolecular technology, and hence compromise human identification processes, are the burning elements, such as fire, heat and explosions. Furthermore, tissue deterioration, incorrect preservation and other external factors may also influence sample quality.

From all issues discussed hereby, what is clear in the relationship between Forensic Anthropology and molecular biology is that, even with the inestimable contribution of the anthropometric resources, their application does not allow a precise nomination of the individual because the sole analysis of bones gives an estimative of the specie, gender, race, age and stature, but not in an accurate manner. According to Campos[43], in the forensic expert analysis, when the skeleton is complete, the legal physician works, mainly, with the bones from the axial, limb and cingulum skeleton, while the legal dentist examines the teeth, bone from the neurocranium and viscerocranium or their fragments. As emphasized by Silva et al.[2], forensic dentists should incorporate these new technologies in their work because forensic expert analysis will never have totally predictable in results. Further studies in the fields of Forensic Anthropology and Molecular Biology are necessary in the search for new approaches in Forensic Dentistry.

It may be concluded that molecular biology techniques should definitely be added to the constellation of investigative resources of the forensic sciences, providing a variety of tools to Forensic Anthropology practice in human identity research.

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