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Application of Molecular Biology in Forensic Dentistry

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Abstract

Forensic expertise methodology normally used in different criminal investigation and forensic medicine field such as blood type, anthropologic analysis and forensic dentistry (dental records, X-rays, bite marks, among others) solved and will continue solving many crimes. Those methods will continue estimating age of several people. Nevertheless, since the development of genetics and molecular biology there were an increase in number and quality of solved cases. The present work points out the importance to associate certain forensic biology areas to traditional investigation methods in human identification, especially with forensic dentistry. It also shows that in some situations, teeth are an important source to genetic analysis and molecular studies. After a scientific literature review it was concluded that it is mandatory that those in forensic investigations acquire knowledge in forensic genetics in order to apply with traditional investigation techniques, this fact would produce an increase of information to Justice.

Key Words: Forensic dentistry; Genetic analysis; Molecular biology; Victim identification.

Introduction

Forensic dentistry can be defined as the field that aims to research psychics, physics, chemical and biological alterations that may occur to the living or dead as well to a person's skeleton and even human parts or evidences, also it researches human lesions that may happen partially or totally, reversible or irreversible. Forensic dentistry researches are usually associated to the Dental Surgeon field of action and it may consist of analysis, expertise and evaluation of dentistry related episodes, but it also can be extended to other areas if it is necessary in order to find the truth within Justice and Administration interests.

Therefore, Dental Surgeon acting as a forensic expert helps to solve judicial matters in several Lawsuit areas such as civil and criminal which are the ones most commons. In the former, patients usually argue dentist clinical conduct that possibly resulted in a malpractice case and therefore capable of receiving money to compensate moral or material damage[1].

On the other hand clinicians may also sue patients in civil area especially when the issue involves charging for professional work. While civil matters are related to financial and material reasons, criminal cases deal with applying punishment either preventive or repressive. The most common studied area is to establish relationship between the action and lesions that may have been caused by dental treatment. Others studied areas are related to oral maxilla facial trauma caused by different sources such as mechanical,

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physical and chemical and dental analysis used for human identification.[2]

Several proper and specific individual characteristics may be used in human identification, among the identification methods; finger printing is the most used one whenever the observed soft tissue is in good conditions.[3] When the body is an advanced stage of decomposition, burned or only bones, anthropologic and forensic dentistry techniques are powerful tools, showing trustable results.[4]

Nevertheless, Genetics great development in the 80's allowed innovations to medical field as well to forensic sciences due to discovery of specific mini satellite regions of human genome which had the so called DNA (deoxyribonucleic acid) "digital impressions". Its analysis led to human individuality information.[5] From that point, genetics research dealing with DNA polymorphism has had a great development and this human identification methodology relies upon three important steps: DNA isolation or extraction, amplification of specific DNA regions using PCR technique (Polymerase Chain Reaction) and molecule profiles analysis.[7]

Human individuality identification tests using DNA are based upon polymorphism of several mini and micro satellite loci. This polymorphism in autosomal chromosomes (not sexual chromosomes) is considered a great individuality indicator.[7] First, in order to do the amplification phase, highly specific probes were developed which allow simultaneous scanning in several loci of the sample. After the material amplification using PCR technique, they are analyzed in agarose or polyacrilamid gel or in automatic sequencer. Depending on the quality and quantity of the obtained sample, analysis can be done from nuclear DNA or mitochondrial (mtDNA), both shows advantages and limitations.[8]

Computer aid in several laboratories steps associated to biotechnology and forensic mathematics increase the reliability of examination to determine sex, age estimation, parenthood and human identity. Therefore it became mandatory that forensic experts in several areas of criminal investigation, forensic medical and dental would associate classical investigation techniques to molecular biology analysis and DNA examination in order to achieve more reliable, objective and specific results facing complex cases.

Expertise examination must show a multidisciplinary approach; therefore the present paper presents a scientific literature review aiming to point out the importance of forensic genetics used along with traditional investigation methods in human identification, mainly with forensic dentistry. It also intends to show the forensic relevance of tooth as a source of material to genetic and molecular studies.

Molecular biology studies

Nowadays, forensic anthropology has molecular biology as a powerful allied, mainly in species, family background, sex and age investigation. Since that, determination of species using bone, tooth, hair or organic fluid samples was done

searching for exclusive human molecular indicators.[9] Those indicators are polymorphic and easily detectable in the population. They can refer to a gene, a restrict site or any other DNA sequence which presents different allelic version to that locus. The mechanisms that explain polymorphism can be single changes in nucleotides (substitution) such as SNP (Single Nucleotide Polymorphism), VNTR (variable number of tandem repeats) and STR (short tandem repeats) and the insertion or exclusion of DNA sequences (Indels), such as Alu insertions.[10] It is important to add that some indicators are specific to a determined population, this happens because the difference frequency between two big populations is more than 50% (such as Europeans and Africans). Those indicators are called PSAs, more recently named ancestor indicators (AIMs).[11]

In order to determine sex or gender it is possible to associate qualitative and quantitative anthropologic analysis of cranium, pelvis and other bones with

cytogenesis examination (Barr corpuscle presence in female population), molecule examination (indicators in Y chromosomes) and mainly analyzing the responsible gene for amelogenin which is the most found protein in the dental enamel. The amelogenin gene is found in chromosome X and Y and the different size and pattern of both genes are used as reliable indicators to determine sex, even when there is little DNA quantity. [12-13] Meyer et al.[14]using old bones aging 4000 and 7000 years compared the morphologic sex with amelogenin analysis results from bone and tooth materials, they found an amplification index of this gene greater than 90%. In another research, Faerman et al.[15] determined sex using amelogenin analysis extracted from bones and teeth in 18 from 22 bodies. Those bodies had aged from 200 to 8000 years.

Concerning age estimative, classical methods are performed using chronologic eruption, tooth mineralization and bone formation centers analysis. Those methods show satisfactory precision in youngsters. [16-17] Nevertheless, when the subject to have the age estimated is an adult or elder person, the preference methods are suture bonding associated to regressive tooth changes related to Gustafson[18] technique, but the range of results may deviate a lot. In order to increase age estimative precision of decomposed bodies such as bones several researches have been developed trying to mainly the racemic mixture of aspartic acid (D/L) from tooth tissues along with one chronological age. It is well known that aspartic acid, particularly in its destrogen form (D), linearly increases along aging. [19-22] Others biochemical indicators such as gelatinase A and glutamic acid from dentine, are also being researched to be use in age estimative cases. [23-24]

Human identification using DNA

Genetic material can be obtained from several biological sources such as body fluids (blood and saliva), soft tissues (muscles and viscera) and mineralized tissues (bones and teeth). There are advantages and disadvantages for each source, those aspects are related to how the source is available, how it degrades and extraction technique, among others.

When dealing with living suspects in parenthood test (father, mother and son), genetic material is preferably obtained from either blood (leukocyte) or buccal mucosa. Trevilatto et al.[25] collected buccal mucosa epithelium samples from 83 individuals, they wrote that this particular method shows the advantage to be more feasible to be done specially when the suspect refuses to cooperate with blood or when dealing with children. The amount of DNA obtained to verify the gender in amelogenin locus of X chromosome was satisfactory in most cases.

In the other hand, if it is necessary to verify genetic relation involving pot-mortem material, time from death and corpse condition are relevant factors to choose which method of DNA extraction to be used. In a recent death case blood, viscera and soft tissue are the first materials choice, but as time goes by those sources become inappropriate, leading to mineral tissues sources: bones and teeth. Bones are an important source to obtain genetic material since they are inner the body and because their mineral condition, which cortical protects the medullar part from external factors and microorganism that may degrade the DNA. [26] In mass disaster situations, Andelinovic et al.[27] presented that DNA analysis from bone and tooth material allowed identify 109 victims of 12 year war in the former Yugoslavia. Traditional identification methods would not give such good results. DNA analysis from bones and teeth would also guide anthropologic studies to identify population migration origin in ancient times. [28-29]

In exhumation cases, there are some procedures that must be observed to collect sample in order to minimize its contamination. Melki et al.[30] studied 10 exhumations for genetic relationship purposes. The authors presented a procedure protocol to grant the origin and the choice of the best bone material to be examined.

Even though Sample integrity is a fundamental factor in DNA extraction, studies with degraded biological material showed to be possible to analyze genetic material when it is fragmented (200 to 1200 pair bases). [31-33]

Teeth as genetic material source

Teeth also are a good source to obtain genetic material. This is true mainly because their great tissue resistance (enamel, dentin, cementum and pulp) against external injurers. [34-35]

Malaver et al.[36] extracted DNA obtained from dentin and cementum of 20 corpses that had been buried for at least 5 years. Pulp tissue is a loose connective tissue and it degrades easily when compared to others dental tissues. Pfeiffer et al.[37] studied the environment influence in DNA degradation in teeth that were kept underneath soil. They observed that a tooth with opened pulp exposed to external agents showed a significant degradation in 18 weeks, but 20 sound teeth that had been buried for one year showed preserved genetic material, allowing mtDNA amplification in all examined samples (20 teeth).

Dental pulp is protected by tooth structures and therefore can present better condition than others soft tissue for DNA extraction. Lessing et al.[38] showed that pulp can be source of DNA in teeth that had been kept or obtained in different conditions such as teeth that had been extracted when the person was alive and after death and kept in room temperature for respectively 12 and 6 months.

Amelogenin can be also studied from dental material that had been through adverse situations. Murakami et al.[39] showed that a person's sex can be determined using dental pulp kept in room temperature for 22 years, teeth kept in sea water for one to four weeks and buried teeth for one, four and eight weeks.

There are several techniques to obtain dental material to extract DNA such as tooth grinding or crushing, tooth horizontal sectioning, pulp extirpation by tooth irrigation and sectioning and nitrogen liquid cryogenic pulverization. [40-41]

Some legal precautions must be followed when dealing with dental material as source to obtain DNA since the process destroys the material used in the examination. Those precautions involve proper teeth identification, describing all characteristics and if it is possible, taking pictures and X-rays in the original positions when they are removed from dental arch. Those precautions are done to preserve dental characteristics of evidences in order to prove their genuine value when doubts are raised concerning their origin or the results of the person identity.

After the dental recording and filing phase and before the handling of samples, it is necessary to decontaminate dental surface, Sodium Hypochlorite is substance most used, but it is necessary to have a optimization among concentration, time and applying method of this substance. [42]

Multi disciplinary approach in human identification

Person identification is necessary in several law situations and the most appropriate identification methodology is related to collected samples such as: person is living or dead; recent or past death; the corpse is complete, in pieces or decomposed. After being properly collected, suspected samples are compared to materials which origin is previously known or proven, in other words, standard material that can have biological nature or records (medical, dental or photographical).

Nowadays, some identification cases urge for a multi disciplinary approach, this happens either because of lack or absence of standard material to be compared or because of the presence of more than one type of evidence to be examined.

For instance, in criminal investigations the person's identity can be achieved by digital impressions left in the crime scene3. But if the collected material does not show quality or quantity of enough characteristics to be compared, genetic profile analysis can be

performed trying to get biological material from suspect digital material. [43]

Forensics investigations developed a lot from the moment that they had genetic examination associated to traditional methods. Not too long ago, sexual violence cases were mostly restricted to spermatozoid research in the collected sample, blood type or other blood tests which results were not accurate. When genetic material became possible to be extracted from spermatozoid, hair bulb, fetal material the capacity to include or exclude a rape or sexual violent attempt suspect was a real possibility. This did not depend on the presence of genetic material in the examined sample. [44-45]

In forensic dentistry, DNA examination can be used together with traditional techniques showing great results to identify destroyed or advanced decomposed stage corpses. [46] Besides that, DNA analysis obtained from oral mucosa cells has great importance in dental impressions, also known as bite marks. Normally, the primary investigation approach of this type of evidence is related to the analysis of dental characteristics left in the victim or in the object. [47-48]

Nevertheless, when those marks do not give a conclusive result, biological material collection from the place where bite was applied is extremely important to find the identity of the person who was responsible for the mark. Among the techniques to obtain DNA from human skin there is the double swab technique which consists of applying a swab with sterilized distil water followed by another dry swab on the bite mark. This technique showed good results to obtain biological material to be researched. [49-50] In the Borgula et al.[51] experimental research was demonstrated that is possible to analyze the genotype of specific bacteria found in the oral cavity (Streptococcus) of individuals as an alternative when it is not possible to obtain the DNA of the one who caused the bite mark.

In more complex cases such as presented by Bilge et al.[52] a multi field approach investigation was necessary to identify a corpse whose head was found approximately 6 months after the body was found. Anthropologic techniques, forensic dentistry, computer superposition (face/cranium) and DNA investigation were used. Sex was determined by cranium characteristics and as well by amelogenin analysis.

Age was estimated using longitudinal divided crown measurements and computer superposition showed a positive identification between victim facial structures and the found head. DNA was extracted from dental pulp, bones, muscle tissues and compared to genetic profile of the victim's presumed daughter and wife. Fatherhood indication was verified in 11 examined loci. In another case, Sweet et al.[53] presented an identification of human parts from a woman that had been disappeared for 3 years. Investigations showed that the presumed victim had 3 smear cell laminas in the laboratory files. DNA was extracted and compared to genetic profile obtained from the dental sample of the found corpse. The result was positive, showing coincidence in 8 of the 8 examined loci, including amelogenin.

In the identification of charred bodies, the great resistance of mineralized tissues allows the victims to be identified not only by DNA extracted from bone material, [54] but also from dental material. Sweet et al.[55] showed an identification of a homicide victim that had been charred with fuel. DNA was obtained from dental pulp extracted from intra osseous third molar. Yamada et al.[56] presented another identification case of a charred body whose head was found 4 months after the body. The relation between head and body was positively proven according to DNA obtained from muscle tissue and DNA from dentin. Facing an overwhelming result presented in the forensic literature and from the even more reliable techniques, DNA examination and Molecular Biology analysis became an essential tool to help or solve investigation matters that had been considered irresolvable in crime investigation and forensic medicine. Therefore it is mandatory that those in forensic investigations acquire knowledge about forensic genetics in order to apply with traditional investigation techniques.

This association would result in a greater increase of Justice and society would be the most beneficiate.

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