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REVIEW

Forensic odontology: a comprehensive review of advances and applications in dental forensic medicine

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ABSTRACT

Forensic odontology is essential in medico-legal investigations, aiding in the identification of individuals, particularly in cases involving decomposed or severely damaged remains. Teeth, due to their durability and uniqueness, serve as critical forensic markers. This field also plays a role in age estimation, bite mark analysis, and legal disputes related to dental malpractice. Key forensic dental techniques include comparative dental analysis, radiographic imaging, and DNA extraction from teeth, which offer resistance to environmental degradation. Advanced methods such as aspartic acid racemization, radiocarbon dating, and histological analysis further enhance age estimation accuracy. Bite mark impressions, though controversial, remain relevant in forensic investigations. Additionally, forensic odontology collaborates with anthropology, botany, and entomology to strengthen identification processes. Technological advancements, including digital forensic tools, 3D imaging, and improved DNA analysis, have enhanced the precision of forensic dental identification. Bite mark analysis, while debated, benefits from computer-assisted comparisons. Forensic dentists are increasingly involved in legal cases, particularly in dental malpractice disputes, requiring specialized knowledge to assess liability and damages. Forensic odontology continues to evolve, integrating innovative technologies to improve accuracy and efficiency. Future research will focus on refining identification methods, utilizing AI-driven forensic analysis, and addressing ethical concerns related to DNA usage. Expanding forensic dental expertise in both clinical and legal contexts will be crucial for maintaining the discipline's role in forensic science.

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Introduction

Personal identification, whether of a living person or a corpse, is a crucial medico-legal procedure from both ethical and legal perspectives, often requiring multidisciplinary expertise.[1]

Personal identification means the restoration of the registry identity to a corpse that is not identifiable because it is decomposed, skeletonized, carbonized or severely traumatized. This is likely one of the primary tasks of a forensic dentist, as unidentified corpses are a reality in every country. In all cultures, restoring a name to a deceased individual holds fundamental moral, criminal, and civil significance.[2]

The identification of a living person is normally the responsibility of the police, although there are cases in which living people are unable, or have no interest, in revealing their identity (fugitives, infant substitutions, subjects who have lost their memory or are in a coma, illegal immigrants): it is in such cases, therefore, that a medical-legal intervention may be required.[3]

Generic identification refers to determining an individual's general characteristics, such as ethnicity, sex, age, and height (which can sometimes be inferred from the length of a single or a few skeletal remains using specific reference tables). It may also include identifying occupational markers, distinctive features (*e.g.*, moles, tattoos), and is typically conducted to narrow the field of identification before proceeding to specific identification. This method is also used to determine the age of young individuals and the sex of those with congenital malformations.[4]

Identification is instead defined as specific when it leads to the recognition of the individual, detecting his somatic characteristics to compare them with the data of the same individual previously reported.

It is performed on subjects, generally already registered in criminal records, who want to hide their true identity or even on individuals missing due to disasters, or affected by mental illnesses; it is also performed to recognize a corpse or to establish who body fragments, skeletal remains, somatic or biological imprints belong to. The signaling consists in the survey and notation of normal somatic characteristics (features) and abnormal ones (marks) for identification purposes.[5]

Teeth are among the most resilient structures in the human body. Their anatomical, pathological, and therapeutic characteristics are unique to each individual, making them an excellent tool for identification. Furthermore, they are the only peri-skeletal structures that can be directly observed in a living person.[6]

Some cultural modifications can then provide information about the geographical origin of a subject such as particular dental pigmentations, non-therapeutic modifications of anatomy, setting of stones or jewels in the dental enamel or covering of dental elements with non-therapeutic crowns.

Dental structures can provide valuable information for generic identification. When isolated teeth, bone fragments, skeletonized remains, or bodies in advanced decomposition or carbonization are found, it is often necessary to first determine the species, sex, ethnicity, and age before proceeding with personal identification.[1-6]

Additionally, certain lifestyle habits or occupations can leave permanent, distinctive marks on the teeth, aiding in the identification of a corpse (*e.g.*, wind instrument players, pipe smokers, carpenters, etc.).

Human remains

In the event that skeletonized human remains, decomposed or charred cadavers, or bodies that do not allow visual recognition are found, there arises a need – driven not only by judicial requirements but also by ethical and humanitarian concerns – to establish their identity, the time of death, and, if possible, the cause of death.

At the site of discovery, various specialists with specific expertise may be called upon to intervene, all coordinated by the coroner. These include:

- forensic archaeologists, who, in cases involving even partially buried remains, use proper excavation techniques to prevent damage from unsuitable tools, ensuring better preservation and collection of all findings associated with the remains and their discovery site;
- forensic anthropologists, particularly in the European context, who determine whether bone remains are human when dealing with incomplete or highly altered specimens. Morphological differences between species are not always immediately apparent; for example, metacarpal and metatarsal bones of some mammals (*e.g.*, bears, pigs) closely resemble human ones, and upper central incisors of oxen can be mistaken for human teeth. This interpretative challenge increases with bones in the growth phase, where epiphyses are still unfused. In such cases, microscopic techniques – especially histological analysis – are crucial, as the calcified structure remains largely intact against environmental degradation. DNA analysis, while useful, can be complicated by contamination, degradation, and the high affinity of DNA for hydroxyapatite (the primary component of bones and teeth), which can hinder extraction.

In Anglo-Saxon countries, forensic anthropologists are also frequently tasked with determining the ethnicity of the deceased based on skeletal remains;

• forensic botanists, who study plant material to pinpoint the exact location of a body's burial and estimate the time of burial by analyzing tree roots infiltrating the grave. Plants, pollen, and spores can provide valuable evidence about the location and timing of a crime. Forensic botany gained international recognition when it was used by the United Nations War Crimes Tribunal to investigate mass executions during the Yugoslav conflict. In those cases, victims were exhumed and reburied elsewhere to obscure evidence, but mineral and pollen analyses from the bodies helped determine the actual sites of their deaths;

• diatom researchers, who study diatoms (unicellular algae with silica skeletons that persist in decayed tissue) to assess not only the cause of death but also the location of drowning and the water currents the body traveled through. A notable example is the case of Countess Francesca Vacca Agusta, whose body was found in the sea near Bormes-les-Mimosas, France, on January 22nd, 2001 – 14 days and over 300 kilometers from where she fell from her villa in Portofino. Her autopsy showed no signs of drowning, confirming that she died from multiple traumatic injuries sustained in the fall. The diatom analysis of her tissues revealed species that did not match those at her recovery site but instead corresponded to those found along the route her body had drifted;

• forensic entomologists, who specialize in determining the time of death based on the life cycles of cadaveric insect species, particularly Diptera (flies), which follow well-defined reproductive patterns.

Among the professionals involved in such investigations, the forensic dentist plays a significant role.[7-12]

Dental structures are highly resistant to decomposition and heat. The human dentition, consisting of 52 teeth (20 deciduous and 32 permanent), exhibits countless variations in shape, size, and position. Along with pre-existing dental treatments, these characteristics allow for identification through ante-mortem and post-mortem data comparison. Individuals with extensive and complex dental work are often easier to identify due to the greater number of comparative elements.

Therefore, an expert must be present at the discovery site to distinguish and identify dental structures, fragments, and prosthetic devices, which can later be analyzed in the laboratory for personal identification.

During the examination, the forensic dentist must:

• locate and document all dental elements present;

• conduct a detailed photographic survey of the discovery site;

• preserve the integrity of remains using fixative sprays or other appropriate means before transport;

• provide an in-depth description of the dental findings, supported by digital photographs.

This process becomes particularly challenging in cases of mass disasters, such as plane crashes, train collisions, explosions, fires, or natural catastrophes, where multiple remains may be mixed.

Forensic guidelines recommend that, in addition to digital recordings and photographs, a total station be used during the investigation. This laser-based electronic measuring instrument records precise distances and angles, ensuring accurate documentation of each finding's location. Advanced total stations integrate GPS receivers and are controlled *via* specialized software to create a detailed spatial survey of all recovered objects.

Dental characteristics can also provide clues – though not definitive evidence – regarding ethnic origin. Certain traits are more commonly found in specific populations. For example:

• African American populations often exhibit a median interincisor diastema (Figure 1) and pronounced prognathism;

• mongoloid populations frequently present shovel-shaped incisors (Figure 2).

While these features alone are insufficient for conclusive ethnic identification, they complement the information obtained from the overall skeletal analysis.

Figure 1.—Medial interincisor diastema.

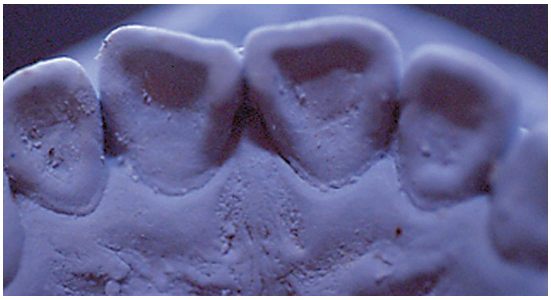

Figure 2.—Palatal view of the upper central incisors (plaster model). Note the perimeter of the tooth with a thicker thickness than the central part of the elements. This particular anatomical conformation called "shovel tooth" is more frequently found among Asian populations.

Sex

Sex determination in cadavers with partially preserved soft tissues is generally possible by identifying residual organs, as the genitals are among the last structures to decompose. Additionally, sex can be determined from blood stains by analyzing neutrophil granulocytes for the presence of Barr chromatin, indicative of XX chromosomes, or by staining the Y chromosome with quinacrine hydrochloride, a reaction that remains fluorescent for 4-6 weeks.

In skeletonized cadavers, sex determination relies on the analysis of sexual dimorphism in specific bone structures, particularly the pelvis and skull:

- the pelvis is the most reliable skeletal indicator of sex when observed in its entirety. In females, it has a broad, heart-shaped pelvic inlet, lower iliac bones, and a subpubic arch shaped like an inverted "U," whereas in males, the pelvis is narrower with a "V"-shaped subpubic arch;
- the skull also exhibits sexually dimorphic traits, including:
  - glabella – more pronounced in males;
  - supraorbital margin – sharp and thin in females, rounded in males;
  - zygomatic arch – ends just before the external auditory meatus in females; extends beyond it in males;
  - mastoid process – smaller and medially inclined in females; robust, larger, and more vertical in males;
  - nuchal crest – less prominent in females, well-defined in males;
  - mandible – more "V"-shaped in females, whereas in males it is squarer and more rounded.

Dental structures, by contrast, exhibit minimal sexual dimorphism. However, some studies suggest a sex-related difference in the crown-to-root ratio and a measurable variation in the mesiodistal diameters of the lower canines.

When genetic material is available, sex determination is definitive. DNA extraction from nucleated cells in the dental pulp, which is well-preserved within teeth, allows for identification of the Y chromosome. Additionally, biomolecular analysis can target intron 1 of the amelogenin gene, where:

- males exhibit both a 106 base pair (BP) and a 112 BP variant;
- females exhibit only the 106 BP variant.

Age

General estimation

The determination of biological age is highly relevant in forensic investigations, both for cadaver identification and for its legal implications in living individuals.

In criminal proceedings, accurately determining age is essential for assessing criminal liability and sentencing. For example:

- under 14 years old → not criminally liable (Art. 97 Penal Code);
- 14-18 years old → criminally liable only if capable of understanding and willing (Art. 98 Penal Code), with reduced penalties;
- 18-21 years old or over 70 years old → may

be eligible for suspended sentences (Art. 163 Penal Code);

• minors under 14 years old and individuals declared mentally incompetent cannot file a legal complaint themselves; a parent or guardian must do so (Art. 120 Penal Code).

In civil law, age determination is also crucial. Examples include:

• legal adulthood is reached at 18 years old, granting full legal capacity (Art. 2 Civil Code);

• minors in need of protection → The public authority can intervene in cases of moral or material risk (Art. 403 Civil Code);

• emancipation → minors may perform legal acts with the assistance of a guardian (Art. 394 Civil Code).

The penal code also criminalizes false identity declarations (Art. 494-498), including impersonation and misrepresentation of one's age. This is particularly relevant in cases involving undocumented or irregular immigrants, who may lack reliable identification documents.

To address age uncertainty, Presidential Decree No. 448/88 (Art. 8) states that if there is doubt about whether an accused individual is a minor, a forensic expert assessment must be conducted. If uncertainty remains, the presumed age should be the lower one, ensuring the application of juvenile legal protections.

Additionally, Art. 67 of the Code of Criminal Procedure requires the judicial authority to refer cases to the Juvenile Court if there is any reasonable suspicion that the accused is underage. This applies even if the defendant's legal majority is uncertain.

Forensic medical assessment of age

Forensic medical evaluation is often the only reliable method of determining age in the absence of official documentation. This assessment may be requested ex officio, by the public prosecutor, or by the defense attorney at any stage of legal proceedings (Articles 508 & 603, Code of Criminal Procedure). However, such assessments are typically ordered early in the investigation, as uncertainty about age usually arises during the initial police inquiry.

Since age determination can affect criminal liability, forensic evaluations follow the principle of in dubio pro reo, meaning that when in doubt, the legal system defaults to treating the individual as a minor.

The legal consequences of age determination vary:

• under 14 years old → not included in a "continuing offense" series, and serious offenses committed at this age cannot serve as the base for sentencing;

• 14-18 years old → crimes committed during this period are included in continuing offenses, provided the individual's mental capacity to understand and will was established.

Thus, forensic age assessment is critical for legal responsibility and sentencing.

Methods of age determination

The starting point for determining age is the beginning of independent life, which is legally and biologically marked by breathing at birth. However, age assessment is more challenging in adults and the elderly due to the fewer developmental markers available.

For both cadavers and living individuals, age is initially estimated based on observable somatic characteristics, including:

• general physical development (height, weight, skin appearance);

• primary and secondary sexual characteristics.

More precise forensic methods include:

• dental assessment, examining the eruption and wear of teeth;

• skeletal analysis, particularly using X-ray imaging to observe bone maturation and epiphyseal fusion.

Forensic dentists play a crucial role in age determination, as dental and skeletal features remain some of the most reliable biological indicators for estimating age, both in the deceased and in the living.

Age determination in corpses

In determining the age of a deceased individual, forensic experts use various methods in addition to those applied to living subjects. These techniques include:

• skull suture fusion analysis: the degree of cranial suture closure can provide an approximate indication of age, though it is not highly precise;

• histological analysis of Haversian canals: measuring the average diameter of these bone structures helps classify individuals into youthful, adult, or elderly age groups;

• microscopic examination of bone tissue:

• Kerley and Ubelaker's method[13] analyzes osteon formation in the femur, tibia, and fibula. With aging, the number of osteons and osteon fragments increases, while the number of non-Haversian canals and the percentage of lamellar bone decrease. This technique is particularly useful for estimating the age of elderly individuals;

• pubic symphysis morphology:

• the Todd method[14] categorizes pubic symphysis changes into ten age stages (18-19, 20-21, 22-24, 25-26, 27-30, 30-35, 35-39, 40-45, 45-49, and over 50 years);

• the Suchey/Brooks method[15] defines six age stages with increasing margin of error as the subject's age rises;

• ileo-sacral joint surface wear:

• the Lovejoy method[16] stages sacroiliac surface changes into eight age groups (20-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-59, and over 60 years);

• sternal rib surface wear:

• the Iscan/Loth method[17] examines wear patterns on the fourth rib, categorizing individuals into nine age groups (under 16, 17-19, 20-23, 24-28, 26-32, 33-42, 43-55, 55-64, and over 65 years);

• aspartic acid racemization (AAR) analysis:

• this technique measures the ratio of D-aspartic acid to L-aspartic acid in teeth, bones, and cartilage. Since D-aspartic acid levels increase with age (by approximately 0.1% per year), this method allows age estimation with an accuracy of ±3 years.[18] The initial application in 1976 (Helfman & Bada)[19] had a larger margin of error (±12 years);

• radiocarbon (Carbon-14) dating of tooth enamel:

• a recent study published in *Nature*[20-22] by researchers from Lawrence Livermore National Laboratory (LLNL, USA) and Karolinska Institutet (Sweden) demonstrated high accuracy in age determination by measuring radioactive Carbon-14 levels in dental enamel;

• the technique takes advantage of radiocarbon levels from atmospheric nuclear tests in the 1950s-60s, correlating tooth formation periods with known Carbon-14 atmospheric concentrations;

• accuracy: ±1.6 years;

• limitations: this method cannot be applied to individuals born before the 1940s, as their teeth developed before the nuclear test-induced spike in Carbon-14 levels.

Age determination in living individuals

It is important to emphasize that chronological age rarely corresponds to biological age. Each child reaches adulthood with different height and body proportions, yet, within the same ethnic group, they typically share similar growth cartilage development, bone maturation, secondary sexual characteristics, and dental eruption patterns, except in pathological cases.

Individual variability in growth is influenced by both genetic and environmental factors, including nutrition, disease, and socioeconomic conditions. These factors also contribute to ethnic differences in size, growth rate, and body proportions. Moreover, early or delayed maturation can be evaluated based on key developmental markers, such as the eruption of the first tooth or menarche in girls.

Sexual maturation

Sexual development is closely linked to overall maturation and is categorized into:

• primary sexual characteristics → essential for reproduction (*e.g.*, gonads and reproductive organs);

• secondary sexual characteristics → manifestations of puberty, including:

• males → voice deepening, body hair growth, testicular volume increase;

• females → breast development, widening hips.

Tanner's staging of sexual maturation

Tanner[23] classified sexual development into five stages, correlating them with different growth phases:

- A1 → pre-pubertal phase;
- A2 → early puberty;
- A3 → pubertal stage;
- A4 → advanced puberty;
- A5 → adult phase.

Skeletal maturation

Skeletal maturation is assessed by standardizing the typical morphological changes in bones during growth. The assumption is that, in healthy individuals, bone maturation follows a predictable sequence, ultimately leading to a near-uniform adult skeletal structure.

Methods of skeletal age determination

- Tanner's bone maturation method:[23]
  - uses X-rays of the hand to analyze 20 selected bones representative of overall skeletal maturation;
  - maturity indicators are assigned scores from 0 to 1000, with the total compared to reference populations of the same chronological age.
- Greulich and Pyle Atlas Method:[24, 25]
  - hand and wrist X-rays are compared with standardized images in an atlas;
  - margin of error: 7-13 months.

While X-rays remain the gold standard, radiation exposure poses potential risks, especially in young individuals who may require multiple assessments due to frequent legal detentions. Therefore, medical consent from a guardian or judge is required.

Legal considerations for forensic age determination

A 2006 ruling by the Supreme Court of Cassation, Criminal Section IV (Sentence No. 6284/2006) confirmed the legality of mandatory radiographic examinations for age assessment, establishing key principles:

- X-ray examinations fall under forensic inspections and can be ordered by a judge under Art. 245 of the Criminal Procedure Code;
- radiological techniques extend forensic examination beyond external appearances;
- minimal radiation exposure makes X-rays safe for the accused;
- X-rays are beneficial for community interests, prioritizing public safety over individual objections.

Key radiographic markers for age determination

Age estimation through hand X-rays involves evaluating:

- distance between ossification nuclei;
- relative position of ossification centers and phalangeal margins.

Chronological evolution of ossification centers

- 6 years → distal ulna epiphysis (both sexes);
- 6-9 years → ulna styloid process (earlier in females);
- 11 years (females) → thumb sesamoid bone;
- 11 years (males) → ulna styloid process;
- 13 years (females) → fusion of the first thumb phalanx epiphysis.

The pubertal growth spurt

The pubertal growth spurt occurs in all populations but varies in onset, duration, and intensity:

- African American populations → earlier onset than Caucasians;
- Asian (Mongoloid) populations → intermediate onset, later than African American but earlier than Caucasians;
- lower socioeconomic conditions → delayed onset but more intense growth spurts.

Sex differences in growth

- Height growth spurt:
  - Females → peak growth around age 12.
  - Males → peak growth around age 14.

Adolescent growth in females (≈3.5 years)

- Stage 1 → Onset of growth spurt, breast bud development, pelvic hair appears.
- Stage 2 → Peak growth rate, breast enlargement, darkening and spread of pubic hair, axillary hair appears.
- Stage 3 → Menarche (~1.5 years after Stage 2).

Adolescent growth in males (≈5 years)

- Stage 1 → "Fat peak" phase, with temporary female-like fat distribution (due to early estrogen secretion before testosterone production).

• Stage 2 → Fat redistribution, pubic hair appears.
• Stage 3 → Growth peak (~8–12 months later), axillary hair appears, facial hair above upper lip, muscle mass increases.
• Stage 4 → Final growth stage (~15–24 months later), facial hair extends to chin, adult pubic hair distribution.

Impact of puberty timing on final adult height

• Earlier puberty onset → Shorter final height.
• Later puberty onset → Taller final height.

Bone growth and hormonal influence

• Height increase is due to endochondral bone growth at long bone epiphyses.
• Sex hormones regulate this process in two phases:
  • Phase 1 → Stimulates cartilage growth, causing a growth peak;
  • phase 2 → Accelerates ossification, leading to growth plate closure and termination of height increase.

Mandibular *vs.* maxillary growth

• Mandibular growth accelerates during adolescence, surpassing maxillary growth.
• Growth peaks:
  • Females → ~12 years.
  • Males → ~15 years.
• Males experience a longer growth period, as they grow more slowly but for a longer duration than females.

Tanner: example in age determination by hand and wrist X-ray

The Tanner-Whitehouse (TW) method (Table I) is a way of assessing the bone age of children. There are several variations of this method, but all use a DP radiograph of the left hand and wrist to assess the relative maturity of the bones of the patient. The TW2 (Tanner-Whitehouse 2) methods:
• RUS (radius-ulna-short bones): 13 bones including the radius, ulna and short bones of the thumb, middle and little fingers;
• carpus: 7 carpal bones;
• 20-bones method: combines the two.
Each of the bones that is being evaluated is compared to a standard set of bones at different stages of maturation. A score is assigned to each bone based on maturation and sex of the patient.

Once all the bones have been scored, a total score is generated by adding all bone scores together and then plotted on a graph to determine how the bone age relates to the chronological age.

Table I.—*Tanner: example in age determination by hand and wrist X-ray.*

Bone	Stadium	Score
Radio	G	85
Ulna	D	30
I metacarpal	F	24
III metacarpal	F	17
V metacarpal	F	18
The next phalanges	F	24
III next phalanx	F	20
V next phalanx	F	19
III medial phalanx	F	20
V medial phalanx	E	14
Distal phalange	F	24
III distal phalanx	F	17
V distal phalanx	F	17
Big bone	G	85+149
Hooked	G	85+161
Pyramidal	F	36+80
Semilunar	G	46+106
Scaphoid	E	29+71
Trapeze	F	32+80
Trapezoid	F	31+77
Total		673+724
	Score	Bone age (years)
20 bones	673	8.4
Carpus	724	8.3

The relevance of DNA in forensic dentistry

DNA is an essential tool in forensic investigations due to its high individual variability, stability over a lifetime, and resistance to environmental degradation.[26] DNA can be extracted from any tissue or liquid containing nucleated cells. It is present not only in the cell nucleus but also in mitochondria.

Mitochondrial DNA (mtDNA) in forensic investigations

• Mitochondrial DNA (mtDNA) accounts for 5-10% of the genome and is valuable in forensic cases involving highly degraded samples with limited genetic material;

• the hypervariable regions (HV1 and HV2) of mtDNA exhibit polymorphic characteristics, making them useful for forensic identification;

• mtDNA is transmitted exclusively through the maternal lineage, as sperm mitochondria do not penetrate the egg during fertilization. This means that maternal relatives share identical mtDNA, enabling identification through comparison with maternal relatives, including siblings and the mother;

• mtDNA can be extracted from saliva traces on bite marks, cigarette butts, fingerprints, and teeth, even when other biological material is severely degraded.

Teeth as a source of DNA

Teeth provide an excellent natural protection for DNA due to their mineralized external structure, which shields the dental pulp from environmental damage. Even in cases of decomposition, water immersion, or fire exposure, dental DNA remains recoverable.

Among all teeth, molars are the most valuable for DNA extraction, as they contain the largest pulp volume and offer the best protection against degradation.[27, 28]

PCR and the role of DNA amplification in forensic dentistry

The use of DNA in forensics advanced significantly with the discovery of long-chain trace elements (LTRs) (400-1500 base pairs) and microsatellite loci (STRs) (100-350 base pairs).

• This progress was made possible by Polymerase Chain Reaction (PCR), introduced by Kary Banks Mullis in 1983, earning him the Nobel Prize in Chemistry in 1993;[27]

• PCR is a chemical amplification technique that exponentially replicates specific DNA sequences *in vitro*, enabling forensic scientists to work with extremely small or degraded DNA samples for identification purposes;

• STR amplification *via* PCR has revolutionized forensic identification, allowing tiny DNA traces to be analyzed and linked to individuals with high accuracy.

DNA extraction from teeth

To access dental DNA, forensic scientists use two primary techniques:

• sectioning the tooth (preferably a molar) longitudinally or transversely to access the pulp chamber for DNA extraction;

• crushing the entire tooth, which increases the DNA yield but is a non-conservative method.

Case study: DNA-based identification of a WWI soldier

A notable case of DNA analysis in forensic dentistry was reported in the *Italian Journal of Legal Medicine* by Caenazzo *et al*.[29]

• In 2000, skeletal remains were discovered near an Austrian trench with personal objects and historical documents suggesting they belonged to a Habsburg soldier killed in June 1916 by a grenade explosion;

• DNA was extracted from two teeth and a femur fragment;

• best results were obtained from the teeth, as the bone sample contained scarce and degraded DNA;

• identification was confirmed using DNA polymorphism analysis (SNPs) and comparison with the soldier's only living daughter, yielding a paternity probability of 97.47%.

DNA and privacy in light of recent regulatory innovations

The increased use of genetic material in criminal investigations appears imminent, raising both legal and ethical concerns regarding privacy and personal data protection.

On October 12th, 2006, the Council of Ministers introduced a bill entitled "Amendments to the Code of Criminal Procedure for Biological Sampling and Medical Examinations on Living Persons." This legislation aligns with the Prüm Treaty (signed on May 27th, 2005, by Austria, Belgium, France, Germany, Luxembourg, the Netherlands, and Spain), which promotes cross-border cooperation in combating terrorism, crime, and illegal migration. The bill grants judicial authorities the power to order forced collection of biological material (hair, saliva, etc.)

or medical tests for DNA identification, enabling genetic profile comparisons in criminal investigations.

Legal and constitutional considerations

Previously, the Constitutional Court (Sentence No. 238, July 9th, 1996) had ruled Article 224, Paragraph 2, of the Criminal Procedure Code unconstitutional, as it allowed judges to impose measures restricting personal freedom without clear legal provisions. This new legislative intervention aims to close that gap, ensuring that judicial authorities can compel biological sampling for forensic evidence and investigative purposes.

The law states that forced DNA collection may be ordered if absolutely essential for proving facts and applies to cases involving:

- crimes punishable by life imprisonment;
- non-negligent offenses with a maximum prison sentence exceeding three years.

Notably, Article 133 of the Criminal Procedure Code allows compulsory sampling even for individuals who are neither suspects nor accused. This means that victims or third parties may also be subjected to DNA collection, expert evaluations, or forensic assessments.

Destruction of biological samples and DNA data management

Under Article 6 of the decree, biological samples must be immediately destroyed upon judicial order, after DNA extraction and analysis. This destruction process must be:

- documented and recorded in official case files;
- executed by forensic experts or consultants.

However, critical questions remain regarding who will manage the national DNA database, how genetic data will be stored, and what security measures will protect this highly sensitive information.

Privacy concerns and international perspectives

The Italian Data Protection Authority has yet to issue an official stance on this matter. However, DNA is widely recognized as one of the most sensitive categories of personal data.

At the 27th International Conference of Data Protection and Privacy Authorities (Montreux, September 2005), attended by the Italian Data Protection Authority, the Montreux Declaration (Point 7) emphasized: "The rapid expansion of genetic knowledge makes human DNA the most sensitive personal data ever."

This highlights the urgent need for strict regulatory safeguards to balance forensic utility with individual privacy rights in the handling of genetic data.

Teething and its importance in forensic science

During embryonic development, the primitive oral cavity (stomodeum) forms early, giving rise to the dental lamina, a thickening of the ectoderm-derived lining epithelium. This structure invaginates into the underlying mesenchyme, marking the initial stage of tooth development at the future alveolar sites.

From intrauterine life until approximately 21 years of age (when the third molars fully mature), dental development serves as a reliable indicator of biological age, showing a strong correlation with chronological age.

In forensic investigations, the odontological assessment of a fetus can be performed by analyzing the degree of cusp mineralization in developing teeth. This can be observed through simple radiographic examination of the mandible, providing an accurate estimation of fetal age (Figure 3 and Figure 4).

Up to approximately 14 years of age, a simple evaluation of the dental eruption stages allows for age estimation with a margin of error that increases proportionally with age, reaching up to 36 months (Figure 5, 6).

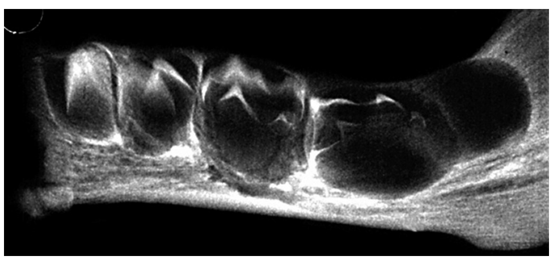

Figure 3.—X-ray of a fetal hemimandible. The cusps of the developing teeth can be seen.

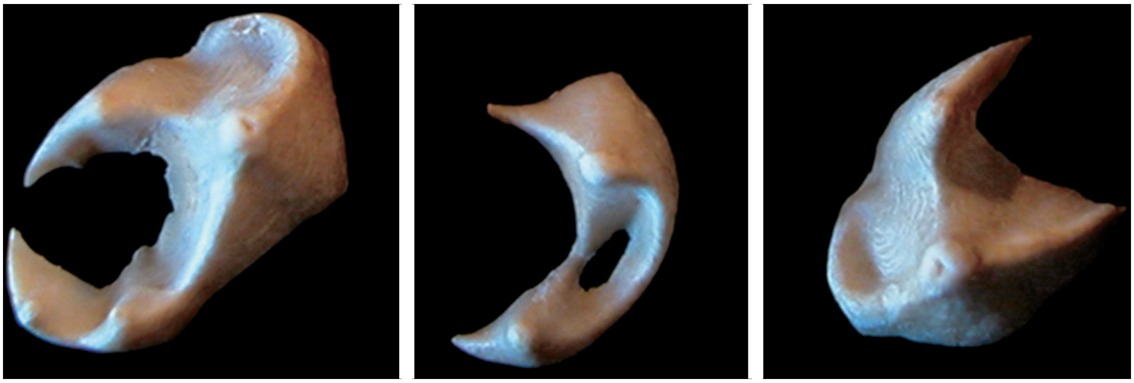

Figure 4.—Calcifying cusps taken from the mandible of a fetus.

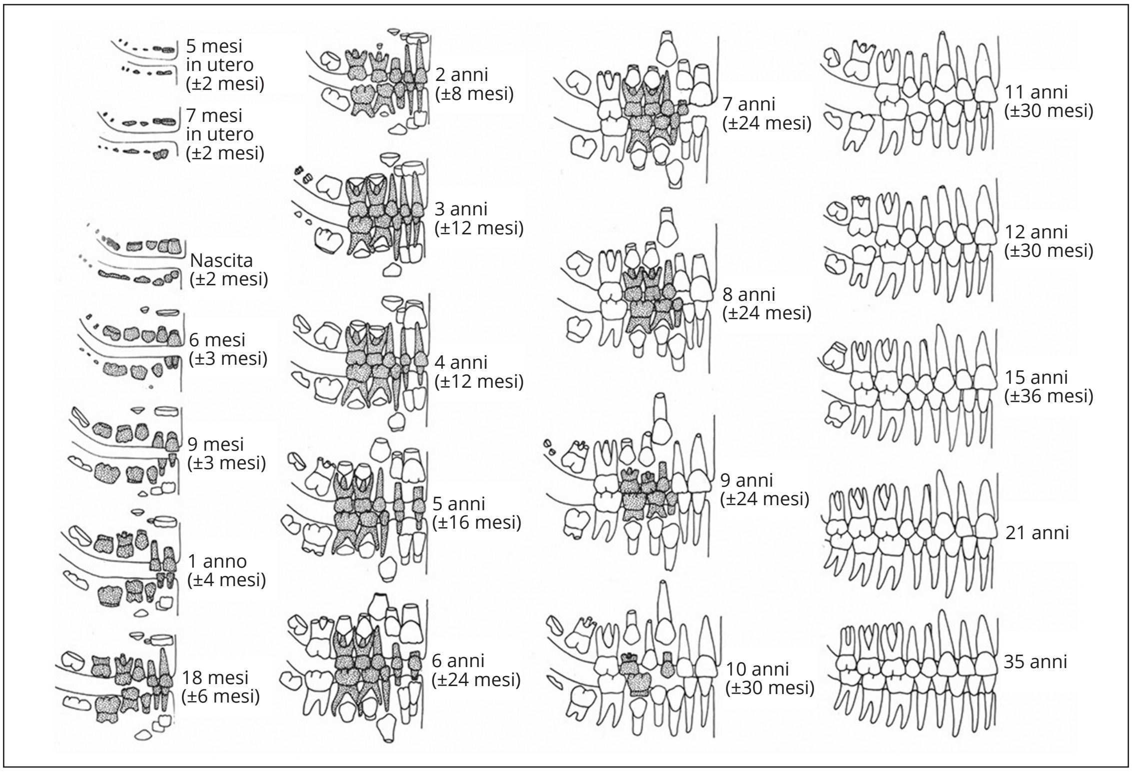

Figure 5.—Schematic representation of the phases of tooth exchange and eruption. By comparing the dentition of the sub-adult subject with the drawings shown here, it is possible to estimate the age with an error that increases with age.

Beyond this stage, the maturation of the third molars[30-32] serves as a key indicator for estimating biological age up to approximately 21 years (Figure 7).

Once dental maturation is complete, the progressive structural changes in teeth allow for age estimation even in adults.

For assessing the age of ancient populations, occlusal surface wear is commonly analyzed. Additionally, several continuous dental changes serve as valuable age markers, including:

- root transparency;
- root resorption;
- periodontal attachment migration;
- apposition of dentine and cementum.

These phenomena can be observed in intact

Figure 6.—Mandible of a subject of about 8 years of age. It is possible to observe mixed dentition (simultaneous presence of deciduous and permanent teeth).

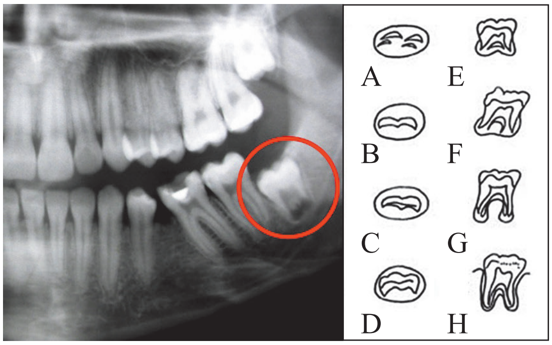

Figure 7.—Portion of panoramic radiograph highlighting a third molar and diagrams used to evaluate the maturation of this tooth.

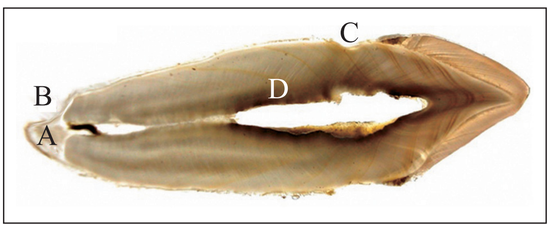

Figure 8.—Thin section of a canine. Some of the characteristics that allow an estimate of the age can be observed: A) the transparency of the root; B) root resorption; C) migration of the periodontal attachment; D) apposition of secondary dentine.

teeth or sectioned specimens, enabling a simple age estimation through systematic analysis and classification (Figure 8).

However, many of these characteristics are not solely dependent on age – they are significantly influenced by pathological conditions and lifestyle factors. Therefore, age estimation must always be complemented by a comprehensive dental examination of the entire oral cavity.[33]

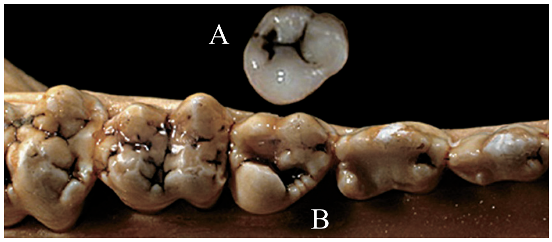

Figure 9.—The dental crown of a pig (B), given its similar shape to that of a human molar (A), could be interpreted as human.

As previously mentioned, more advanced methods for age estimation from dental structures are still in the experimental phase. These include:

- cementum annulation counting;
- quantification of stereoisomeric forms of aspartic acid in dentine;
- measurement of artificial radiocarbon levels in tooth enamel.

Additionally, knowledge of human dental anatomy allows for species identification when isolated and complete dental elements are found. In cases where morphology alone is inconclusive – such as porcine molars, which resemble human molars (Figure 9) – or when only dental fragments are available, species identification is performed through microscopic analysis of dental structures.

A cross-section of human enamel reveals the characteristic keyhole-shaped enamel prism pattern, which serves as a distinctive microscopic marker for human teeth (Figure 10).

Bite marks in forensic investigations

A forensically relevant bite mark is defined as a physical alteration or lesion that displays distinctive characteristics of the stomatognathic system responsible for causing it. Bite marks can be found on various substrates, including:

- objects or food present at a crime scene;
- human skin (on a living victim or a cadaver).

Their forensic significance lies in the ability to trace the individual who inflicted the bite, often providing key evidence in criminal investigations.

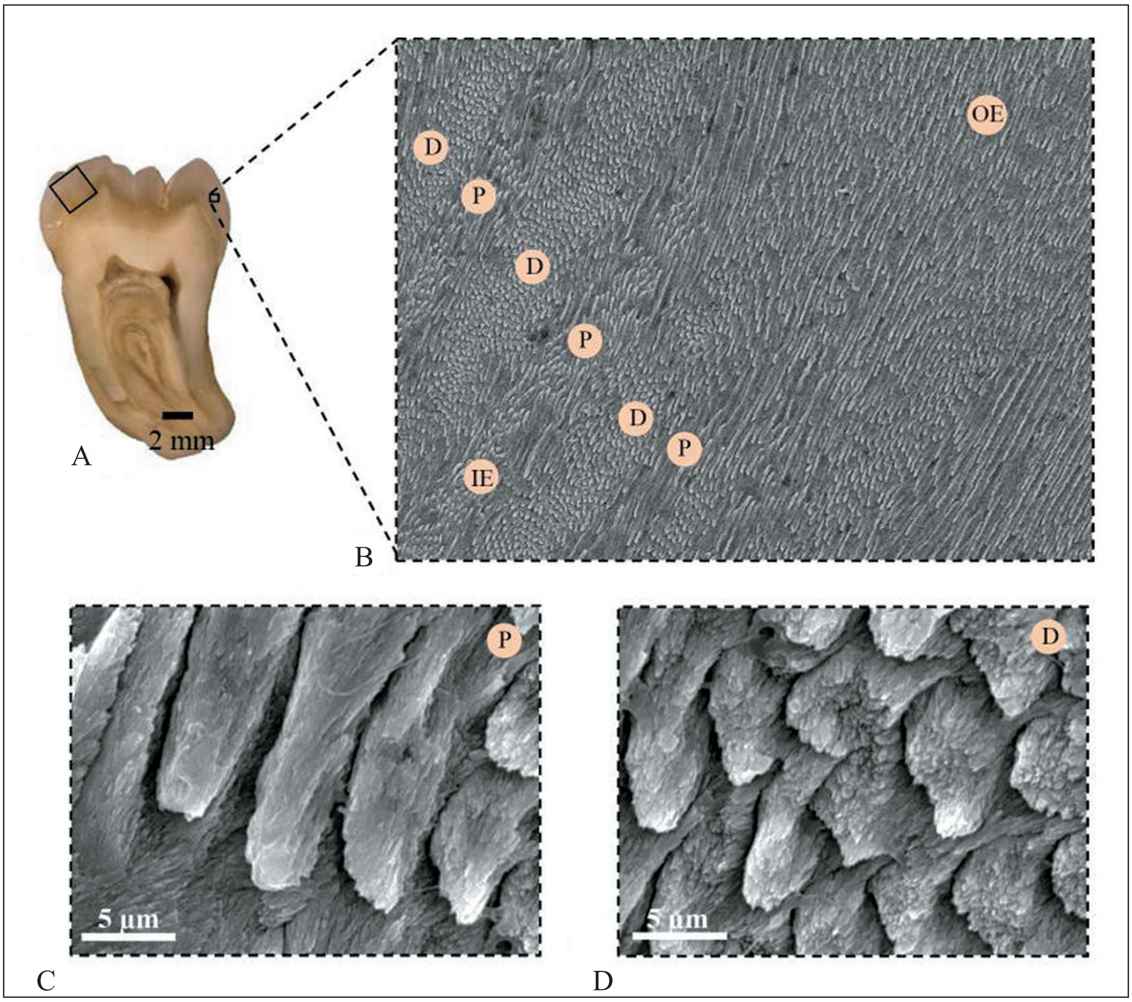

Figure 10.—The classic conformation of human enamel prisms as it appears under the microscopeoptical. A) A section of the tooth and any enamel insert. B) Distinction between the external (OE) and internal (IE) enamel. Prisms are predominantly oriented rectilinearly to the external enamel. Decussation, which is caused by crossing bundles between prisms is seen mostly in the internal regions of the cusp enamel and includes bundles of prisms known as parazone (letter p) and diazone (letter d) shown in (C) and (D), respectively.

Forensic analysis of bite marks

A bite mark can produce a relatively accurate impression of a suspect's dentition. By conducting comparative analysis between the bite mark and dental impressions taken from a suspect, forensic experts can establish evidentiary value in a criminal investigation.

Forensic dentists are frequently called upon to analyze and interpret bite injuries found on:
- cadavers;
- living individuals;
- impressionable materials at crime scenes.[34-41]

Teeth as instruments of aggression and defense

Teeth are often used as:
- weapons in an assault (inflicted by the attacker);
- defensive tools (when a victim bites in self-defense).

Bite wound analysis is critical for identifying the assailant and may also allow for DNA extraction.

- Saliva deposited in the bite may contain oral mucosal cells, providing material for nuclear DNA and mitochondrial DNA analysis.

Legal relevance of bite marks

Bite marks have both criminal and civil implications, including:

• child abuse cases → bite injuries are sometimes misreported as accidental trauma, but forensic identification of bite patterns can confirm abuse;

• HIV transmission cases → the HIV virus in saliva of infected individuals may pose a risk of transmission through biting.

Techniques for documenting bite marks

• Bite marks on impressionable materials → elastomeric molds can be used to create an accurate impression of bite marks on objects;

• bite marks on human skin → immediate documentation is essential due to natural changes:

  • in living individuals → healing may alter the lesion.

  • in cadavers → Post-mortem changes can distort the mark.

The typical bite lesion is elliptical, with:

• a central ecchymotic area;

• peripheral imprints of the teeth (Figure 11).

Step-by-step forensic protocol for bite marks on skin

• Swab the lesion to collect potential genetic material from the attacker;

• photograph the bite mark:

  • use a metric reference scale placed adjacent to the bite mark;

  • take images from multiple angles to avoid distortion.

A cast of the bite impression can be created using dental impression materials, such as polyvinyl siloxanes, to obtain a detailed three-dimensional record of the bite.

In cadavers, the removal and transillumination of the skin can enhance the visibility of lesion characteristics, making bite mark analysis more precise.

If the bite is located in a region accessible to the victim's mouth, a dental impression of the victim may be necessary to rule out self-inflicted injuries.

Some bite-related lesions may be difficult to definitively attribute to human teeth, particularly in the following scenarios:

• minimal force applied → only a rounded ecchymosis may appear, lacking distinctive dental characteristics;

• excessive force applied → the removal of tissue may obscure dental patterns;

Figure 11.—Classic appearance of a bite mark: the lesion is rounded, there is a central ecchymosis, and the marks left by the teeth can be seen around the perimeter.

Figure 12.—Bite marks on particularly curved surfaces, such as in this example, are difficult to compare with the suspects' teeth.

• multiple, overlapping, or partial impressions → complicates pattern recognition;

• bite marks on highly curved body areas → may lead to distortion and misinterpretation (Figure 12).

Certain tubular objects can leave skin lesions that may be mistakenly interpreted as bite marks. Therefore, forensic analysis must carefully differentiate true bite injuries from artifact-induced patterns.

Forensic dental examination of the suspect

A comprehensive dental examination is conducted on the suspect, documenting:

• dental formula;

• pathologies;

• coronal fractures;

• shape, position, and alignment anomalies.

It is highly recommended to take precise dental impressions using high-detail materials, ensuring the detection of even the smallest enamel infractions.

The first step in bite mark analysis is to determine whether the lesion was inflicted by human dentition. This involves identifying class characteristics, such as:

• morphology of the lesion;

• intercanine distance;

• number and positioning of teeth, consistent with human dentition.

The comparison between the bite mark and the suspect's teeth can be performed using:

• physical models → a dental cast of the suspect's teeth is compared directly with the lesion (Figure 13);

• reproductions of the lesion → molds or overlays are created for side-by-side analysis (Figure 14);

Figure 14.—Model of the suspect's teeth and reproduction of a victim's finger bearing a lesion complex attributable to a bite. The simulation allows studying the compatibility between lesions and teeth.

• computer-assisted analysis → 2D and 3D digital reconstructions enhance accuracy in comparing bite patterns and dental morphology.

Multidisciplinary considerations in forensic dentistry and medico-legal challenges

The evolution of dental practice and the increasing medico-legal challenges require a multidisciplinary approach that integrates biomechanical, biological, and preventive aspects. The survival of dental implants, particularly in postgraduate educational settings, is a critical factor in legal disputes, where failures may be linked to biomechanical issues or inadequate treatment planning.[42] Additionally, the safety and properties of ceramic biomaterials in prosthetics and implantology are key in minimizing complications and forensic investigations related to material failures.[43]

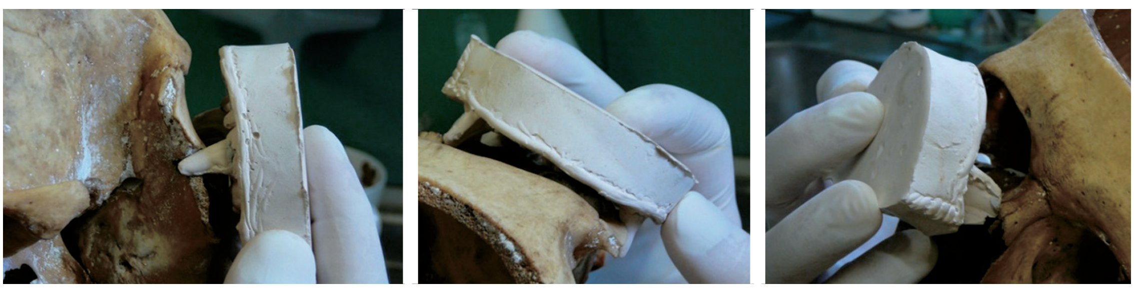

Figure 13.—Plaster model of a dog's dentition compared with some bone lesions found on a skull.

Forensic dentistry plays a crucial role in assessing medico-legal claims related to oral diseases, especially in cases of alleged misdiagnosis or delayed intervention. The awareness of HPV-related oropharyngeal cancer among dental students impacts preventive strategies such as vaccination and early diagnosis, which can have forensic implications when failure to inform patients leads to legal consequences.[44] Similarly, the forensic evaluation of periodontitis-related tooth loss is relevant in cases where systemic health deterioration, including cognitive impairment, is cited in legal proceedings.[45]

The forensic dimension of periodontal disease extends to systemic conditions such as cerebrovascular health, where stroke-related periodontal deterioration may raise questions about clinical negligence.[46] Legal disputes can also arise from the mismanagement of viral infections affecting the oral mucosa, such as primary herpetic gingivostomatitis, particularly in cases involving improper diagnosis or treatment. These scenarios highlight the growing intersection between forensic dentistry, liability claims, and healthcare litigation.

Conclusions: future perspectives for the forensic dentist

The demand for forensic dentists is expected to increase significantly due to:

- the rising number of dentist-patient legal disputes in recent years;
- the high percentage of unfavorable outcomes for dental professionals compared to other medical specializations;
- current regulations, which require a specific degree in Dentistry and Dental Prosthetics to practice as a dentist.

For several years, Dentistry has been taught as an independent discipline, separate from the degree course in Medicine and Surgery. Furthermore, Odontostomatology is no longer a specialization for medical doctors. As a result, in legal disputes involving dental malpractice, judicial authorities increasingly rely on expert consultants (CTUs) with specific forensic dental expertise. This trend follows the example of the USA and other countries, where postgraduate training in forensic dentistry has already been established.

It is also worth highlighting that the annual incidence of litigation for professional liability in dentistry is an increasing phenomenon, with a high prevalence in prosthetic and implantology-related disciplines. According to Montagna *et al.*,[47] an analysis of 1690 cases of professional liability in dentistry revealed that litigation is most prevalent in prosthodontics (30-70% of cases), followed by implantology (20-25%), endodontics (15-25%), oral surgery (5-20%), orthodontics (5-10%), restorative dentistry (0.5-5%), and periodontology (1-2%). Furthermore, in 95-98% of cases, the outcome of legal proceedings is unfavorable for the dental professional.

The same authors also reported that permanent biological damage (DBP) is estimated in 3-5% of cases, while temporary biological damage (DBT) results in an average of 20-40 days of temporary incapacity. The emerging expenses (SPE) for patients range between € 5000 and € 10,000, while requests for reimbursement of fees (RECO) vary between € 6000 and € 8000.

The study also highlights the predominance of recourse to ordinary justice rather than extrajudicial solutions, with a significant disproportion reflecting a tendency to prefer lengthy and costly legal proceedings. According to Montagna *et al.*,[47] this choice can be attributed to disproportionate compensation claims, patient retaliation motives, disputes over payments for treatments deemed inadequate, or the dentist's refusal to refund fees for improperly or unsuccessfully performed treatments.

Legislative reforms and expected impact on legal dispute resolution

A significant reduction in litigation resolution times is expected due to amendments to Article 696 of the Code of Civil Procedure (CPC). The introduction of Article 696-bis CPC provides an alternative dispute resolution mechanism, aiming to streamline legal proceedings between patients and dentists.

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Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions
All authors read and approved the final version of the manuscript.

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